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Cinderella story: PI4P goes from precursor to key signaling molecule

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Abbreviations

AP-1, adaptor protein-1; Arf1, ADP-ribosylation factor 1; AtCBL, *Arabidopsis thaliana* calcineurin B-like; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; BARS, brefeldin A-dependent ADP-ribosylation substrate; CFP, cyan fluorescent protein; COF, CERT-OSBP-FAPP; COP, coat protein; CPY, carboxypeptidase Y; CtBP, C-terminal binding protein; DAG, diacylglycerol; Dvl, Dishevelled; EGFR, epidermal growth factor receptor; EH, Eps15 homology; EHD-1, Eps15 homology domain-1; EpsinR, Epsin-related; ER, endoplasmic reticulum; ERES, ER exit sites; ERGIC, ER-Golgi intermediate complex; ERK, extracellular signal-regulated kinase; F-actin, filamentous actin; FAPP, four-phosphate adaptor protein; FGF, fibroblast growth factor; FKBP12, FK506 binding protein 12; FRB, FKBP12- and rapamycin-binding protein; Frq, Frequenin; Fz, Frizzled; GBA, β-glucocerebrosidase; GBF1, Golgi brefeldin A-resistant factor 1; GEF, guanine nucleotide exchange factor; GFP, green fluorescent protein; GGA, Golgi-localized, gamma-ear-containing, Arf-binding; GOLPH3, Golgi phosphoprotein 3; GPCR, G-protein coupled receptor; HCV, hepatitis C virus; Hh, Hedgehog; INPP5E, inositol polyphosphate-5-phosphatase E; IP_3, inositol 1,4,5-trisphosphate; LAMP1, lysosomal-associated membrane protein 1; LCV, Legionella-containing vacuole; LIMP-2, lysosomal integral membrane protein type 2; LRP, low density lipoprotein receptor-related protein; MAPK, mitogen-activated protein kinase; MEF, mouse embryonic fibroblast; MVB, multivesicular body; NES, nuclear export signal; NLS, nuclear localization signal; ORP, OSBP-related protein; OSBP, oxysterol binding protein; PAO, phenylarsine oxide; PDGF, platelet-derived growth factor; PGC, post-Golgi carrier; PH, pleckstrin homology; PI, phosphatidylinositol; PI3K, phosphatidylinositol 3-kinase; PI4K, phosphatidylinositol 4-kinase; PI4P, phosphatidylinositol 4-phosphate; PI5P, phosphatidylinositol 5-phosphate; PIP,

phosphatidylinositol phosphate; PIP5K, phosphatidylinositol 4-phosphate 5-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PITP, phosphatidylinositol transfer protein; PKC, protein kinase C; PKD, protein kinase D; PLC, phospholipase C; PM, plasma membrane; RNAi, RNA interference; siRNA, short interfering RNA; SNARE, soluble n-ethylmaleimide sensitive factor adaptor protein receptor; STIM1, stromal interacting molecule 1; tER, transitional ER; TGN, trans-Golgi network; TIRF, total internal reflection fluorescence; TRP, transient receptor potential; ts, temperature-sensitive; VAP, vesicle-associated protein; VHS, Vps27-Hrs-STAM; VLDL, very low density lipoprotein; WASH, WASP and SCAR homologue; WHAMM, WASP homologue associated with actin, membranes, and microtubules; Wm, wortmannin; WNK1, With no lysine 1; YFP, yellow fluorescent protein

Abstract

Phosphatidylinositol lipids are signaling molecules involved in nearly all aspects of cellular regulation. Production of phosphatidylinositol 4-phosphate (PI4P) has long been recognized as one of the first steps in generating poly-phosphatidylinositol phosphates involved in actin organization, cell migration, and signal transduction. However, progress over the last decade has brought to light independent roles of cellular PI4P in membrane trafficking and lipid homeostasis. Here, we describe recent advances that reveal the breadth of processes regulated by PI4P, the spectrum of PI4P effectors, and the mechanisms of spatiotemporal control of PI4P that coordinate crosstalk among cellular signaling pathways.

Phosphoinositides

Eukaryotic cells are compartmentalized into organelles that engage in specialized functions. These subcellular functions are coordinated by signaling proteins and phospholipids such as phosphatidylinositol (PI; Fig. 1A) and its derivatives. PI lipids are minor membrane components containing a cytosolic *myo*-inositol head group amenable to phosphorylation at the D-3, D-4 and D-5 positions to produce seven different PI phosphates (PIPs or phosphoinositides). Specific PIPs are enriched on particular organelles, contributing to their identity and function. In addition, the ability of PIPs to be rapidly interconverted through the action of lipid kinases and phosphatases means that PIPs can be precisely regulated in space and time. Moreover, PIPs can relay changes in membrane status by recruitment of cellular effectors that recognize newly synthesized PIPs, or by activation of signaling pathways that utilize PIPs as substrates.

Generation of PI 4-phosphate (PI4P) by PI 4-kinases (PI4Ks) is the first reaction in forming PI 4,5-bisphosphate (PIP_2) and PI 3,4,5-trisphosphate (PIP_3), two phosphoinositides that

participate in well-studied signaling pathways, making PI4P a critical precursor in the PIP pathway (Fig. 1B). Cleavage of PIP_2 by phospholipase C (PLC) produces the second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), initiating downstream signal transduction cascades through protein kinase C (PKC), thereby controlling growth factor signaling, cytokine induction, neurotransmitter release, muscle contraction and other responses. Phosphorylation of PIP_2 by class I PI 3-kinases (PI3Ks) produces PIP_3, which activates Akt and other signaling proteins to control cell proliferation and survival.

Because of the importance of PIP_2- and PIP_3-dependent signaling pathways in animal cells, PI4P has mainly been regarded as essential only for its role as a precursor to these PIPs. However, the discovery of conserved roles for PI4P across plants, yeast and mammals suggests that the non-precursor, signaling roles of PI4P are ancient and fundamental. For this reason, it has been proposed that PI4P became essential in its own right, with PIP_2 and PIP_3 acquiring specialized roles (Delage et al., 2012a). This idea partly stems from the observations that PI4P is the most abundant cellular PIP (Lemmon, 2008). Indeed, PIP_2 is 10 to 100 fold less abundant and PI4P 5-kinases (PIP5Ks) are much less active in plant cells compared to animal cells. Also, class I PI3Ks are absent from yeast and plants. Finally, the abundance and distribution of PI4P vary less than for PIP_2 and PIP_3 across species (Delage et al., 2012a) (Fig. 2).

Importantly, studies of PI4Ks and the PI4P phosphatase Sac1, as well as tools to visualize and perturb cellular PI4P levels, have revealed prominent roles for PI4P in a wide range of basic cellular processes, most notably in membrane trafficking, sphingolipid metabolism and regulation of the cytoskeleton, as described below.

PI-4 Kinases

In the late 1960s-80s, biochemical studies identified PI4K activity in membrane fractions from animal tissues (Harwood and Hawthorne, 1969; Cooper and Hawthorne, 1976; Lefebvre et al., 1976; Behar-Bannelier and Murray, 1980; Collins and Wells, 1983). It became apparent that there were two types of PI4K activity based on the repertoire of inhibitors to which they were sensitive, leading to characterization of type II and type III PI4Ks. Cloning of mammalian PI4Ks led to identification of two type II PI4Ks approximately 55 kDa in size, PI4KIIα and PI4KIIβ (Barylko et al., 2001; Minogue et al., 2001; Balla et al., 2002; Wei et al., 2002), and two type III PI4Ks, PI4KIIIα (230 kDa), and PI4KIIIβ (92 kDa) (Nakagawa et al., 1996a; Nakagawa et al., 1996b). The type II PI4Ks are inhibited by adenosine, micromolar calcium, submillimolar phenylarsine oxide (PAO) and the 4C5G monoclonal antibody, whereas type III PI4K activity is inhibited by low amounts of PAO, with PI4KIIIα being more sensitive (IC_{50}=1-5 μM) than PI4KIIIβ (IC_{50}=30 μM), and PI3K inhibitors such as wortmannin (Wm) and LY294002 (Endemann et al., 1991; Balla and Balla, 2006). Cloning of PI4Ks from the budding yeast *Saccharomyces cerevisiae* revealed three conserved PI4Ks – the PI4KIIIα Stt4, the PI4KIIIβ Pik1, and a single type II PI4K, Lsb6 (Flanagan et al., 1993; Garcia-Bustos et al., 1994; Yoshida et al., 1994; Han et al., 2002). These same three enzymes are also found in the fruit fly *Drosophila melanogaster*.

In addition to their different biochemical properties, PI4Ks affect discrete pools of cellular PI4P and have distinct cellular functions. In yeast, Stt4 and Pik1 play essential, non-overlapping roles in the cell (Audhya et al., 2000). Stt4 localizes to cortical patches at the plasma membrane (PM) and regulates actin organization as well as cell wall integrity. In contrast, Pik1, which functions in secretion, localizes to the Golgi and nucleus. In mammalian cells and flies,

PI4KIIIα controls a PM pool of PI4P (Balla et al., 2008; Tan et al., submitted). Mammalian PI4KIIIα localizes to the endoplasmic reticulum (ER), dynamically to the PM (Nakatsu et al., 2012), and has also been detected at the Golgi, nucleolus, multivesicular body (MVB), and outer mitochondrial membrane (Wong et al., 1997; Balla et al., 2000; Kakuk et al., 2006) (Fig. 3A). As with yeast Pik1, mammalian PI4KIIIβ primarily localizes to the Golgi and nucleus, and is required for anterograde trafficking (Godi et al., 1999; Weisz et al., 2000; de Graaf et al., 2002). PI4KIIIβ has also been reported on ER, outer mitochondrial membranes (Balla et al., 2000) and, recently, on lysosomes (Sridhar et al., 2013) (Fig. 3B). The *Drosophila* PI4KIIIβ Fwd localizes to the Golgi, where it is required for male germ cell cytokinesis (Brill et al., 2000; Polevoy et al., 2009). Interestingly, unlike yeast Pik1, *Drosophila* Fwd is non-essential (Brill et al., 2000), suggesting it carries out a redundant function with another PI4K.

The type II PI4Ks examined so far appear to be dispensable for viability in yeast, flies, and mice, yet carry out specific cellular functions. Yeast Lsb6 regulates endosome motility in a kinase-independent manner (Chang et al., 2005). In contrast, catalytic activity of *Drosophila* PI4KII is required for sorting of secretory granule and endosomal cargo in the larval salivary gland (Burgess et al., 2012). *PI4KIIα* mutant mice are viable, yet develop late onset neurodegenerative disease (Simons et al., 2009). In mammalian tissue culture cells, PI4KIIα is required for endosomal sorting (Craige et al., 2008; Jovic et al., 2012; Mossinger et al., 2012) and its catalytic activity is needed for post-Golgi trafficking at the *trans*-Golgi network (TGN) (Wang et al., 2003; Wang et al., 2007). In contrast, mammalian PI4KIIβ is found on endosomal populations that translocate to the PM upon Rac signaling (Balla et al., 2002; Wei et al., 2002).

Challenges of PI4P Biology

Common methods to visualize cellular PI4P include antibody detection and expression of fluorescent proteins fused to PI4P-binding domains (Varnai and Balla, 2008; Hammond et al., 2009). However, determining which pools of PI4P are produced by individual PI4Ks has been difficult due to the lack of fluorescent probes that bind only PI4P, and the lack of complete and isoform-specific PI4K pharmacological inhibitors. Pleckstrin homology (PH) domains from oxysterol binding protein (OSBP) and four-phosphate adaptor proteins (FAPPs) are frequently used to detect PI4P, but they also bind ADP-ribosylation factor 1 (Arf1). At high expression levels, these probes titrate the lipid away from its normal function. Existing enzyme inhibitors do not distinguish between type II PI4Ks and, at the concentration of PAO that is specific for PI4KIIIα, only 80% of its activity is reduced (Balla et al., 2008). Additionally, the essential functions of PI4Ks mean that treatment with short interfering RNA (siRNA) either eliminates cells entirely or only moderately affects PI4P levels because of incomplete knockdown in surviving cells.

Nevertheless, cell biologists have found clever ways to overcome these obstacles in order to detect and perturb PI4P. Chief among these is a method to acutely deplete a specific phosphoinositide on a membrane of interest without caveats associated with enzyme inhibition or knockdown (Fili et al., 2006; Heo et al., 2006; Varnai et al., 2006). FRB (FK506 binding protein 12 [FKBP12]- and rapamycin-binding protein) is tethered to a transmembrane protein on the membrane of interest. Recruitment of a PIP phosphatase fused to FKBP12 to this site is controlled by acute rapamycin-induced heterodimerization of FRB and FKBP12. This technique has been used successfully to deplete PI4P on the PM and Golgi by targeted recruitment of a PI 4-phosphatase domain from Sac1 (Szentpetery et al., 2010; Hammond et al., 2012; Salcedo-

Sicilia et al., 2013). Importantly, this technique is effective in identifying the subcellular location where PI4P is important, but does not identify the PI4K responsible for its production. Other efforts to disrupt specific pools of PI4P include the pursuit of new drugs to inhibit specific PI4Ks, which resulted in identification of PIK93, a panspecific PI3K p110 subunit inhibitor that also selectively targets PI4KIIIβ over PI4KIIIα (Knight et al., 2006). The search for compounds with anti-viral activity has led to the discovery of isoform-specific PI4K inhibitors such as AL-9 (for PI4KIIIα) and T-00127 HEV1 and GW5074 (for PI4KIIIβ) (Arita et al., 2011; Vaillancourt et al., 2012). In addition, combinatorial evidence gathered from multiple inhibitors can provide valuable information. For example, a pool of PI4P that is dispersed by the application of Wm and <10 μM PAO, but not PIK93, is likely to be regulated by PI4KIIIα (Balla et al., 2008). Lastly, optimization and modification of earlier methods has helped overcome inconsistencies seen with PI4P antibody staining (Hammond et al., 2009), and revealed a previously undetected cyan fluorescent protein (CFP)-FAPP-PH signal at the PM (Wuttke et al., 2010). Our current understanding of the many cellular roles for PI4P is a direct result of these advances (Tables 1 and 2).

In this review, we present an overview of this current understanding of PI4P biology and the evidence from which it was derived. In the first half, we review the molecular roles and physiological goals that are accomplished with PI4P signaling. In the second half, we examine the mechanisms that fine-tune the production of PI4P in these contexts. We also emphasize the current gaps in our knowledge and suggest worthwhile directions to fuel the next chapter of discovery.

Cellular Roles of PI4P

Signal Transduction

Shortly after the discovery of phosphoinositides, it was observed that ligand stimulation can induce cellular phosphoinositide metabolism. Radiolabeled ATP and inositol were incorporated into the phosphoinositide lipid pool after incubation of guinea pig brain cortex slices with acetylcholine (Hokin and Hokin, 1955; Hokin and Hokin, 1958), a result termed 'the phospholipid effect'. Through a series of agonist stimulation experiments where specific phosphoinositides were monitored (Berridge, 1983), the idea of a 'phosphoinositide cycle' emerged: ligand receptors and GTP stimulate hydrolysis of PIP_2 by PLC, and the resulting second messenger products are recycled to regenerate PI and PIP_2 (Akhtar and Abdel-Latif, 1984; Dunlop and Larkins, 1986). Receptors that stimulate PLC include receptor tyrosine kinases and G-protein coupled receptors (GPCRs) such as rhodopsin, chemokine and ATP purinergic receptors, angiotensin II receptor, and the muscarinic acetylcholine receptor. Because PI4P is much more abundant than PI 5-phosphate (PI5P) (Lemmon, 2008), PIP_2 is likely generated through phosphorylation of PI4P rather than PI5P. Support for the idea that PI4P is the major precursor of PIP_2 comes from experiments in insulin-secreting MIN6 β-cells, which show PIP_2 levels closely following those of PI4P (Wuttke et al., 2010). Cells were permeabilized with *Staphylococcus aureus* α-toxin, which produces small pores, allowing depletion and re-addition of ATP to inhibit and activate PI4Ks. Using OSBP-PH-CFP to follow PI4P and the PIP_2-binding PH domain of phospholipase C δ (PLCδ-PH) fused to yellow fluorescent protein (YFP) to monitor PIP_2, re-addition of low concentrations of ATP induced the appearance of PM PI4P without a concomitant generation of PM PIP_2 until higher concentrations of ATP were added. Inhibition of PI4K activity with 200 μM LY294002 then decreased PI4P, with PIP_2 levels

following after a 25 second delay. This suggests that the majority of PM PIP_2 undergoes substantial turnover which requires prior formation of PI4P, and that this route of PIP_2 synthesis is more likely than others (Wuttke et al., 2010), such as synthesis from PI5P, trafficking of PIP_2 from intracellular membranes or dephosphorylation of PIP_3.

PI4P is thought to play a critical role in PLC signaling because of its function as a PIP_2 precursor. Indeed, PI4K activity is required for a sustained PLC response under signaling conditions (Creba et al., 1983; Balla et al., 2005). Continued second messenger production leads to prolonged binding of IP_3 to ER-localized IP_3 receptors, releasing intracellular calcium stores and promoting sustained PKC activity, which is also stimulated by DAG. PLC activation also leads to opening of transient receptor potential (TRP) cation channels, which aids in signal amplification (Montell, 2012). Activated PKC initiates downstream signal transduction pathways, including those propagated by MAPK/ERK.

With some exceptions, PI4KIIIα is generally thought to produce the PI4P precursor for PIP_2 that is consumed by PLC. Indeed, in mammalian cells, PI4KIIIα is needed to replenish PIP_2 during continued PLC, PKC and MAPK signaling (Balla et al., 2008; An et al., 2011; Hammond et al., 2012). In *Arabidopsis thaliana*, PI4KIIIβ1/2 contributes to PLC signaling induced by cold exposure, although the role of PI4KIIIα, which is essential, could not be directly assessed (Delage et al., 2012b). This role for PI4KIIIβ is surprising, given that it is not thought to contribute substantially to PM PIP_2. However, it is unclear whether plant PLC uses PI4P as a substrate, in which case production of PIP_2 would not be required. Yeast Stt4 is essential for PKC and MAPK signaling, although this is not mediated by PLC. Instead, Stt4-dependent production of PI4P, and subsequent production of PIP_2 by the PIP5K Mss4, recruits protein effectors that stimulate PKC (Audhya and Emr, 2002). The Rho guanine nucleotide exchange

factor (GEF) Rom2 is recruited to the PM in part by its PIP_2-binding PH domain. PM localized Rom2 activates Rho1, which in turn stimulates PKC in a MAPK pathway known as the 'cell wall integrity pathway.' Stt4 and Mss4 are also required for recruitment of the Ste5 scaffolding protein that induces MAPK signaling and cell polarization in response to mating pheromone (Garrenton et al., 2010).

PI4P has also been implicated in PLC signaling as a direct substrate. Mammalian PLC has been shown to cleave PI4P *in vitro* (Wilson et al., 1984; Smrcka et al., 1991; Seifert et al., 2004), but until recently this has not been demonstrated in cells. Activation of the G_q-coupled muscarinic receptor in tsA-201 (human embryonal kidney) cells leads to PIP_2 depletion and a rise in DAG and IP_3 levels (Falkenburger et al., 2013). Expression of a 5-phosphatase effectively depleted PIP_2, and thus strongly inhibited IP_3 formation by PLC during receptor activation. However, the level of DAG remained unchanged even with 80% depletion of PIP_2, suggesting that PLC may cleave PI4P to produce DAG and IP_2. Computational modeling using observed time courses and empirical rate constants supported this scenario. Indeed, when the authors attempted to model a scenario in which PLC does not cleave PI4P, no adjustment of parameters could reproduce the experimental data. In addition, strong evidence for PLCε cleavage of perinuclear PI4P comes from neonatal rat ventricular myocytes (Zhang et al., 2013). A perinuclear Golgi population of PI4P is depleted, and DAG signal is increased, when this specific PLC is activated. This effect is blocked by PLCε siRNA or disruption of the interaction of PLCε with muscle-specific A kinase anchoring protein, which anchors it to the nuclear envelope. The decrease in PI4P is unlikely to result from its conversion to PIP_2 because fluorescent markers such as GFP-PLCδ-PH did not detect perinuclear PIP_2 and because targeting of a 5-phosphatase to the Golgi did not restore PI4P levels following PLCε activation at the

nuclear envelope. In contrast, targeting of Sac1 to the Golgi during PLCε stimulation prevents nuclear protein kinase D (PKD) activation, an event that requires DAG production. Thus, in some contexts, PI4P is a PLC substrate, although how widespread this is remains to be seen.

In animal cells, PI4P also contributes to other signaling pathways. A PI4KIIα-dependent surge in PI4P and PIP_2 levels is required for aggregation and phosphorylation of the Wnt3a co-receptor Lrp5/6 and for β-catenin stabilization during canonical Wnt signaling (Pan et al., 2008; see below). Hedgehog (Hh) signaling in *Drosophila* requires PI4P for translocation of the GPCR Smoothened to the PM (Yavari et al., 2010). In addition, PI4KIIIα is required for expression of target genes in the Hippo signaling pathway and for apical localization of the upstream signaling component Merlin (Yan et al., 2011). However, exactly how PI4P contributes to these pathways is currently unclear.

A potential role for PI4KIIIα in generating the pool of PI4P that feeds into PIP_3 signaling has not been widely explored. A study in zebrafish showed that *PI4KIIIα* knockdown by morpholino injection produced a pectoral fin development defect similar to that caused by treatment with a PI3K inhibitor (Ma et al., 2009). In addition, *PI4KIIIα* morphants showed reduced expression of the PI3K-Akt target genes *fgf10* and *mkp3*, which are required for FGF signaling in limb bud development. Studies of PI4KIIα and PI4IIIβ in COS-7 cells suggested that kinase activity was dispensable for PI3K signaling and Akt phosphorylation (Chu et al., 2010). However, PI4KIIIα was not tested, raising the possibility that this enzyme has a conserved role in PIP_3 signaling.

ER and Golgi Trafficking

In addition to its role in PIP_2- and PIP_3-dependent cell signaling, a major function for PI4P lies in

its ability to recruit cytosolic signaling molecules that contain PI4P-binding motifs (D'Angelo et al., 2008; Vicinanza et al., 2008). Often, these proteins also require the presence of a coincident protein. Many membrane trafficking components have been identified as PI4P effectors, implicating PI4P as a key executor of events at multiple stages of trafficking. Indeed, depletion of PI4P by rapamycin recruitment of Sac1 to the Golgi virtually eliminated trafficking of cargo from the TGN to the PM and to endosomes (Szentpetery et al., 2010).

Bud formation and cargo sorting

Vesicle formation begins with extrusion of donor membrane to generate a bud. Bud formation is achieved either through a clathrin-mediated process, through processes dependent on other coatamer complexes, or through coat-independent pathways. At the ER and Golgi, PI4P plays a role in examples from each of these categories.

Clathrin adaptors enriched at the TGN include the tetrameric adaptor protein-1 (AP-1) complex, the Epsin-related (EpsinR) protein, and the Golgi-localized, gamma-ear-containing, Arf-binding (GGA) family of proteins. These adaptors have clathrin and specific cargo recognition sites to promote formation of cargo-containing clathrin-coated vesicles. In addition, these adaptors all contain PI4P- and Arf1-binding motifs (Ren et al., 2013). AP-1 binds PI4P *in vitro*, likely through the γ-adaptin subunit (Wang et al., 2003; Heldwein et al., 2004). Kinase activity of PI4KIIα is required for AP-1 recruitment and for TGN to PM transport in mammalian cells (Wang et al., 2003). However, in *Drosophila*, PI4KII is dispensable for AP-1 and EpsinR localization (Burgess et al., 2012). In yeast, modulation of PI4P levels by Pik1 exerts control over AP-1 localization (Daboussi et al., 2012). EpsinR binds PI4P through its ENTH domain; aids in the assembly of AP-1 and soluble n-ethylmaleimide sensitive factor adaptor protein

receptor (SNARE) proteins into clathrin-coated vesicles (Hirst et al., 2003; Mills et al., 2003; Hirst et al., 2004; Miller et al., 2007); and regulates retrograde trafficking of clathrin-coated vesicles to the TGN independently of AP-1 (Saint-Pol et al., 2004).

GGA proteins are recruited to the TGN by Arf1, the Arf1 GEF Golgi brefeldin A-resistant factor 1 (GBF1), a ubiquitin sorting signal, and PI4P (Lefrancois and McCormick, 2007; Wang et al., 2007), although each GGA varies in its reliance on each of these factors (Boman et al., 2002; Shiba et al., 2004; Wang et al., 2007). At the TGN, GGAs sort proteins through interaction with acidic-cluster dileucine sequences found in cytosolic tails of cargoes such as the mannose-6-phosphate receptor (Shiba et al., 2002). GGA proteins also promote trafficking of ubiquitinated cargoes to the endosomal pathway, including the yeast general amino acid transporter Gap1 and the human glucose transporter GLUT4 (Scott et al., 2004; Lamb et al., 2010). In mammalian cells, GGA Golgi localization depends on PI4KIIα (Wang et al., 2007), and acute enzymatic depletion of Golgi PI4P leads to rapid dissociation of clathrin, GGA1 and GGA2, but curiously not GGA3, from the Golgi (Szentpetery et al., 2010). Knockdown of PI4KIIIα diminishes GGA3 presence at the Golgi (Dumaresq-Doiron et al., 2010). However, this is likely due to an indirect effect on GBF1 localization and Arf1 activation rather than a direct effect on PI4P-dependent recruitment of GGA to the Golgi (see below).

In addition to binding PI4P, yeast GGAs control Golgi PI4P to mediate two waves of clathrin adaptor assembly. Using live imaging of clathrin adaptors fused to fluorescent proteins, appearance and disappearance of Gga2 puncta preceded AP-1 assembly and disassembly on the same TGN membrane by approximately 10 sec (Daboussi et al., 2012). Decreasing PI4P levels using a *pik1* temperature-sensitive (ts) (*pik1^{ts}*) mutant or increasing PI4P by overexpression of Pik1 had no effect on Gga2 bud formation but lengthened or shortened the time between GGA

and AP-1 assembly, respectively, suggesting that the level of Golgi PI4P dictates timing of progression from GGA- to AP-1-dependent trafficking. Proper timing is important because a shortened window between GGA and AP-1 adaptor assembly leads to TGN-endosome trafficking defects. Interestingly, adaptor progression is regulated by binding and recruitment of Pik1 to the VHS domain of Gga2, leading to local production of PI4P. In accordance with this, localization of Pik1 and the PI4P sensor Osh1-PH peak at the TGN approximately 5 seconds after the Gga2 peak, and depletion of GGA proteins delays Golgi association of Pik1, PI4P, and AP-1.

Trafficking to and from the ER and Golgi is mediated by the conserved cytoplasmic coat protein (COP) complexes I and II. The COPII coat mediates budding of vesicles from the ER at specific locations termed ER exit sites (ERES), or transitional ER (tER), and begins with recruitment of the small GTPase Sar1 by its GEF Sec12. Activated Sar1 induces membrane curvature by insertion of a hydrophobic helix (Lee et al., 2005), and recruits the first coat of proteins, the Sec23-24 concave heterodimer, to further deform the membrane. Sec24 recognizes ER sorting signals while Sec23 recruits the Sec13-31 heterotetramer which forms a cage to stabilize the bud and promote fission (Bhattacharya et al., 2012). Additional contacts between the core COPII components and Sec16 are also important for ERES stabilization and fission (Yorimitsu and Sato, 2012).

Sar1 stimulation of PI4P production at ERES is necessary for nucleation of COPII coats (Blumental-Perry et al., 2006). However, it is unclear which PI4K is responsible for regulating this process, or how multiple PI4K activities would be coordinated at these sites. In ER fractions from normal rat kidney cells, Sar1 induced a membrane-associated PI4K activity that is insensitive to Wm. Such an activity is more likely to represent a type II PI4K (Blumental-Perry

et al., 2006). Despite this, knockdown of *PI4KIIIα* significantly reduced ERES formation in HeLa cells (Farhan et al., 2008). This discrepancy may reflect cell-type specificity. Interestingly, PI4P does not appear to be required for COPII budding in yeast (Lorente-Rodriguez and Barlowe, 2011), which may indicate that specific ERES are not used in yeast. In animal cells, specialized exit sites may facilitate clustering of specific cargo, lipids and proteins required for vesicle formation, or to spatially target released vesicles to the ER-Golgi intermediate complex (ERGIC), a compartment not present in yeast (Zanetti et al., 2012).

In yeast and mammalian cells, the COPI complex traffics vesicles from the *cis*-Golgi back to ER or ERGIC membranes, and mediates retrograde transport within Golgi cisternae (Emr et al., 2009). Yeast COPI mutants show defects that resemble a block in anterograde trafficking, but this effect is restricted to specific cargo proteins and can be suppressed by overexpression of vesicle-associated SNAREs (v-SNAREs) that are known to be recycled (Gaynor et al., 1998). Hence, the block in anterograde trafficking may be due to defects in retrieving COPII machinery for recycled use at the ER. COPI-dependent tubule formation at the Golgi appears to be important for intra-Golgi anterograde transport (Yang et al., 2011). Assembly of COPI-coated vesicles involves simultaneous binding of COPI to activated Arf1 (Yu et al., 2012), GBF1 (Deng et al., 2009) and to dimers of the Golgi resident transmembrane proteins p23 and p24 (Popoff et al., 2011).

Recently, the ER GTPase Rab1, PI4KIIIα, and PI4P were proposed to function together to recruit GBF1 to Golgi membranes (Dumaresq-Doiron et al., 2010). Activated Rab1b binds directly to GBF1 and promotes GBF1 and COPI localization to the Golgi (Monetta et al., 2007). Rab1a also recruits GBF1, and constitutively active Rab1a can co-immunoprecipitate PI4KIIIα and increase the amount of the GFP-FAPP1-PH PI4P marker at the Golgi (Dumaresq-Doiron et

al., 2010). PI4KIIIα colocalizes significantly with GBF1. Inhibition of PI4KIIIα with Wm or PAO greatly reduces Golgi localization of GBF1 and GGA3, without affecting GBF1 or GGA3 protein levels or Golgi morphology. Knockdown of PI4KIIIα has a slightly stronger effect on GBF1 localization, which may be due to dispersal of the *cis*-Golgi, as seen by staining for the marker gigantin. PI4P may affect COPI assembly through recruitment of GBF1 via a polybasic domain (Dumaresq-Doiron et al., 2010), or through recruitment of the PI4P-binding protein Golgi phosphoprotein 3 (GOLPH3), a COPI-binding partner (Tu et al., 2008; Tu et al., 2012). Importantly, none of these effects were seen in cells treated with PI4KIIIβ siRNA. Thus, PI4KIIIα may promote COPI assembly in response to Rab1 stimulation. This unexpected role for PI4KIIIα may explain its long-recognized localization to the ER in mammalian cells. It will be interesting to test this model by examining COPI localization and function in PI4KIIIα-depleted cells, the effect of Rab1 on PI4K activity, and whether GBF1 is a bona fide PI4P effector.

Additional evidence for involvement of PI4P in COPI trafficking comes from the observation that knockdown of PI transfer protein (PITP), which catalyzes non-vesicular transport of PI from the ER to the *cis*-Golgi, reduces overall PI4P levels and specifically affects COPI-mediated retrograde transport of the KDEL receptor, but not anterograde trafficking from the TGN in HeLa cells (Carvou et al., 2010). This is consistent with the idea that a unique pool of PI4P in the *cis*-Golgi is dedicated to retrograde trafficking. This pool may be generated specifically by PI4KIIIα because (1) *PI4KIIIα* affects COPI assembly proteins (Rab1 and GBF1), but does not impair overall levels of Golgi PI4P (Balla et al., 2005), and (2) despite its effect on GBF1 and GGA localization to the TGN (Dumaresq-Doiron et al., 2010), *PI4KIIIα* is not thought to regulate anterograde trafficking. Lack of an effect of *PI4KIIIα* on TGN trafficking

could be explained by the presence of PI4KIIIβ and PI4KIIα, which may help recruit sufficient populations of GGAs and other clathrin adaptors. Indeed, AP-1 distribution is normal in PI4KIIIα knockdown cells (Dumaresq-Doiron et al., 2010). Thus, the ER and *cis*-Golgi may be sensitive to PI4P produced by PI4KIIIα.

Relatively little is known about formation of clathrin-independent vesicles at the TGN. The Arf1-binding glycolipid transfer protein FAPP2 generates PI4P-dependent membrane tubules *in vitro*, and has been suggested to mediate clathrin-independent trafficking (Cao et al., 2009; Valente et al., 2012). *In vivo*, FAPP2 regulates apical cargo transport in polarized and non-polarized cells (Godi et al., 2004; Vieira et al., 2005). Whether the lipid transfer activity of FAPP2 is connected to its role in membrane trafficking is not clear. However, it is possible that FAPP2-mediated flipping of glucosylceramide lipids to the inner leaflet of the TGN creates asymmetry across the membrane, leading to curvature (De Matteis and Luini, 2008). Alternatively, glycosphingolipid self-organization into liquid-ordered domains may facilitate sorting of lipid raft-containing carriers (Surma et al., 2012). FAPP2 may also initiate budding and tubulation through insertion of a hydrophobic wedge into the bilayer after it forms extensive contacts with PI4P (Lenoir et al., 2010).

While PI4P is clearly important for recruitment of proteins that initiate vesicle formation, it is also important for sorting of cargo destined for these vesicles, as evidenced by missorting of proteins under reduced PI4K activity (Burgess et al., 2012; Jovic et al., 2012). This makes sense, given that many adaptors localize through multiple low-affinity interactions with PI4P, ARF-1 and cargo recognition sequences that act synergistically to target them to the correct membranes and increase cargo-binding affinity (Wang et al., 2007). Thus, the role of PI4P in sorting is part and parcel with adaptor recruitment.

Vesicle formation and structural integrity

Filamentous (F-) actin facilitates vesicle trafficking by powering endosome motility and by remodeling the cell cortex during endo- and exocytosis (Lanzetti, 2007). For example, the F-actin regulator WASP homologue associated with actin, membranes, and microtubules (WHAMM) (WASP homologue associated with actin, membranes, and microtubules) interacts with microtubules or Arp2/3 to promote membrane tubulation or elongation, respectively, thereby regulating Golgi structure and anterograde trafficking (Campellone et al., 2008; Shen et al., 2012). However, by and large, the role of F-actin in Golgi and post-Golgi trafficking has not been well defined. Only recently has Golgi PI4P been linked to actin and to myosin motors that pull newly formed vesicles away from the TGN. The highly conserved PI4P effector GOLPH3 (Vps74 in budding yeast) binds PI4P with high affinity and specificity through its 34 kDa Golgi phosphoprotein (GPP34) domain (Dippold et al., 2009; Wood et al., 2009). In HeLa cells, GOLPH3 simultaneously binds PI4P and the unconventional myosin Myo18A, providing the tensile force necessary to separate vesicles from TGN. Interestingly, the vertebrate-specific GOLPH3 paralog GOLPH3L also binds PI4P, but counters forces exerted by GOLPH3 (Ng et al., 2013). The action of both proteins needs to be regulated, as unchecked activity of either GOLPH3 or GOLPH3L impedes anterograde secretion and disrupts Golgi morphology. Indeed, GOLPH3L knockdown leads to excessive stretching and vesicle formation by GOLPH3 as well as Golgi fragmentation. In contrast, GOLPH3 knockdown leads to Golgi compaction and loss of its characteristic ribbon-like appearance. Thus, in mammalian cells, Golgi architecture depends on the balance between GOLPH3 and GOLPH3L. This may be particularly important in secretory tissues, where GOLPH3L is highly expressed. The mechanism by which GOLPH3L inhibits GOLPH3 is currently unknown. Moreover, it is unclear whether GOLPH3 is regulated in

an analogous manner in flies and yeast, which lack both GOLPH3L and the ribbon-like Golgi morphology found in vertebrate cells.

In budding yeast, Pik1 is needed for proper localization of the GOLPH3 homologue Vps74, suggesting that PI4KIIIβ may regulate GOLPH3/GOLPH3L in mammalian cells. Indeed, PI4KIIIβ plays a critical role in regulating Golgi structure and function, since loss of this enzyme leads to Golgi fragmentation in a number of systems (Godi et al., 1999; Strahl et al., 2005; Polevoy et al., 2009; Daboussi et al., 2012). Golgi structure also relies on a continuous network of spectrin proteins that form a scaffold to shape and support the cell. PI4P and Arf1 jointly recruit βIII spectrin to the TGN, where it maintains the Golgi's ribbon-like appearance and facilitates secretory trafficking (Salcedo-Sicilia et al., 2013). Knockdown of βIII spectrin or depletion of PI4P through rapamycin-mediated recruitment of FKBP12-Sac1 to the Golgi results in Golgi fragmentation. Thus, PI4P is essential for structural integrity of the Golgi complex.

Vesicle Fission

Since PI4P is important for multiple steps leading to generation of a post-Golgi vesicle, adding vesicle fission to its repertoire of functions would be an intuitive way to link all of the events in this process. Indeed, at the PM, PIP_2 recruits the clathrin adaptor AP-2, actin remodeling factors, and the GTPase dynamin to promote endocytosis and vesicle scission (Rohde et al., 2002; Yarar et al., 2007; Ramachandran, 2011). However, there is currently little evidence for involvement of PI4P in procurement of fission machinery for coated vesicles at the Golgi. Although dynamin and dynamin-like proteins have been implicated in scission of clathrin-coated carriers at the TGN, they have been reported to bind PIP_2, not PI4P (Bonekamp et al., 2010; Weller et al., 2010). COPI and COPII vesicles, which require PI4P for their formation, do not require dynamin

(Campelo and Malhotra, 2012). Interestingly, PI3Kδ kinase activity is required in macrophages for recruitment of dynamin 2 and release of cytokine carriers from the TGN (Low et al., 2010), suggesting that PIP_3, rather than PI4P, regulates fission of at least one class of vesicles at the Golgi.

A role for PI4P, however, has been reported in fission of both lysosomal vesicles (Sridhar et al., 2013) and coat-independent vesicles termed post-Golgi carriers (PGCs) that traffic from the TGN to the basolateral cell surface (Valente et al., 2012). Formation of PGCs requires the brefeldin A-dependent ADP-ribosylation substrate (BARS) protein, also known as C-terminal binding protein (CtBP) 1-short, a cytosolic protein originally identified for its ability to regulate Golgi tubulation and fragmentation during mitosis (Corda et al., 2006). Other trafficking steps that do not require dynamin, such as COPI-mediated retrograde traffic and fluid-phase endocytosis, similarly utilize BARS (Bonazzi et al., 2005; Yang et al., 2005).

Evidence suggests that PI4P is involved in BARS-mediated PGC fission. For example, in rat brain cells, BARS co-purifies with 14-3-3γ, a member of a PI4KIIIβ-interacting family of scaffolding proteins (Valente et al., 2012). In COS-7 cells, BARS also pulls down PI4KIIIβ itself, along with its activating kinase PKD. PKD-mediated phosphorylation of PI4KIIIβ triggers binding of 14-3-3γ, resulting in sustained PI4P generation (Hausser et al., 2006). The discovery that 14-3-3γ, PI4KIIIβ and PKD form a complex with BARS suggests that PI4KIIIβ is stabilized and activated at sites of vesicle fission. With no predicted role for BARS as an energy-coupled molecular motor, it is unclear how it is able to sever tubules growing from the TGN. However, since BARS acts as a scaffold for lipid-modifying enzymes such as PI4KIIIβ and PLD (Haga et al., 2009), it may help create a local lipid environment that is amenable to fission (Haga et al., 2009; Valente et al., 2012). In addition, PKD phosphorylates the PI4P effectors CERT (ceramide

transfer protein) and OSBP (a sterol transferase) to remove them from the Golgi (Olayioye and Hausser, 2012). Although it seems contradictory that PKD-stimulated production of PI4P recruits these effectors only to have them phosphorylated and removed, this may represent a negative feedback loop to turn off PKD action, since CERT generates DAG, a PKD activator. This may also represent a mechanism to ensure optimal PI4P, ceramide and sterol levels for fission, or to eliminate proteins that sequester PI4P. With regard to the latter possibility, dephosphorylation of PIP_2 to PI4P facilitates dynamin-mediated fission of endocytic vesicles in COS-7 cells (Chang-Ileto et al., 2011). This is thought to induce dynamin disassembly from the membrane, although it is also possible that electrostatic properties of concentrated PI4P are more amenable to the final stages of membrane separation.

Vesicle fusion

After liberation of vesicles from one organelle, they are transported to and fuse with target membranes at a different site. Vesicles are first tethered by landmark proteins such as the exocyst components Sec3 and Exo70 at the plasma membrane (He and Guo, 2009), or by vesicular mediators such as the TRAPPI complex (Sztul and Lupashin, 2006) at the *cis*-Golgi. This is followed by fusion through assembly of a *trans* complex of SNARE proteins derived from vesicular and target membranes. Recently, PI4P was shown to be dispensable for vesicle budding and tethering of COPII vesicles, but critical for SNARE complex formation in fusion with the *cis*-Golgi during ER-to-Golgi trafficking (Lorente-Rodriguez and Barlowe, 2011). In *in vitro* assays, pre-treatment of acceptor membranes with Sac1 prevented fusion of transport vesicles. Similarly, sequestration of Golgi PI4P with the PH domain of FAPP1 *in vitro* or depletion of PI4P using a *pik1* mutant *in vivo* prevented transfer of radiolabeled cargo from ER-derived

COPII vesicles to Golgi acceptor membranes. Since overexpression of vesicle and *cis*-Golgi SNARE proteins can suppress these fusion defects, it was postulated that PI4P may interact directly with SNARE machinery.

PI4P Gradient in Golgi trafficking and function

Although GOLPH3 is a PI4P effector at the TGN, evidence from yeast suggests a reciprocal arrangement whereby GOLPH3/Vps74 also regulates PI4P levels at the Golgi. PI4P is enriched in the TGN, whereas Vps74 localizes primarily to early Golgi compartments (Schmitz et al., 2008) and Sac1 resides at the ER and Golgi (Whitters et al., 1993). Interestingly, *vps74* and *sac1* interact genetically and physically, and deletion of either gene results in greater colocalization of the PI4P marker FAPP-PH with the *medial*-Golgi marker Aur1 (Wood et al., 2012). Bimolecular fluorescence complementation experiments showed that Vps74 and Sac1 interact *in vivo* at the *medial*-Golgi, suggesting that Vps74 detects PI4P and recruits Sac1 to this compartment, thereby depleting PI4P in the early Golgi. Sac1 also localizes to the *cis*-Golgi in mammalian cells where it maintains a clear distinction between early and late compartments (Cheong et al., 2010). Here, knockdown of *sac1* resulted in increased GFP-FAPP-PH-positive structures, mislocalization of *medial*-Golgi glycosylation enzymes to intracellular and cell surface membranes, and alterations in *N*- and *O*-linked glycosylation patterns. Thus, expansion of PI4P-rich domains in the Golgi leads to aberrant entrance of resident Golgi proteins into the secretory pathway. The authors of this study proposed that ectopic PI4P in early Golgi compartments may recruit trafficking machinery normally assembled at the TGN. Therefore, Sac1 serves a conserved role in confining PI4P enrichment to the TGN, which ensures proper Golgi organization and function.

A local requirement for high levels of PI4P suggests that PI4P-dependent functions in the

TGN would be more sensitive to PI4P disruption than those at the *cis*-Golgi. Evidence for this idea comes from examination of yeast carboxypeptidase Y (CPY), which is transported and modified through the secretory pathway and can be monitored along the way by differences in molecular weight at the ER, Golgi and vacuole. *pik1*^{ts} mutants kept at the non-permissive temperature for a total of 25 minutes delayed transport of CPY only from the TGN to the vacuole (Audhya et al., 2000). In contrast, a temperature shift of 57 minutes led to a complete block of PI4P-dependent transfer of CPY from the ER to the *cis*-Golgi without significantly affecting Golgi structure or function (Lorente-Rodriguez and Barlowe, 2011). Collectively, these data indicate that a gradient of Golgi PI4P is integral to cisternae identity and function.

Non-vesicular transport and lipid metabolism

PI4P regulates lipid homeostasis through a number of PI4P effectors that have lipid binding and/or transferase activity that mobilizes substrates for biogenesis of complex modified lipids such as sphingomyelin and glycosphingolipids (Graham and Burd, 2011). These effectors include the COF family proteins CERT, OSBP and OSBP-related proteins (ORPs), and FAPP2, which transfer ceramide, oxysterol, and glucosylceramide, respectively (D'Angelo et al., 2008). CERT-mediated movement of ceramide from ER to Golgi requires PI4KIIIβ (Toth et al., 2006). OSBP stimulates CERT-dependent sphingomyelin synthesis through an unidentified mechanism that requires PI4P generated by PI4KIIα (Perry and Ridgway, 2006; Banerji et al., 2010). Importantly, CERT and FAPP2 transfer ceramide and glucosylceramide to the appropriate Golgi leaflet for sphingolipid biosynthesis in response to PI4P binding (Yamaji et al., 2008).

CERT and OSBP are thought to promote ER-Golgi contact sites through interaction with ER integral VAP (vesicle-associated protein) proteins via their FFAT (two phenylalanines

followed by acidic tract) motifs, and through association with Golgi PI4P and Arf1 via their PH (De Matteis et al., 2007) or ORD domains (Li et al., 2002). ER-Golgi contact sites may allow non-vesicular lipid transfer between the two organelles. OSBP, yeast ORPs (Osh proteins), and several human ORPs transfer sterols between liposomes *in vitro* (Raychaudhuri et al., 2006; Ngo and Ridgway, 2009; Schulz et al., 2009; Du et al., 2011). Osh proteins lacking PH domains and FFAT motifs have been proposed to mediate sterol transfer by acting as diffusible sterol carriers (de Saint-Jean et al., 2011). Although Osh ORD domains alone can tether membranes (Schulz et al., 2009), this is somewhat controversial (de Saint-Jean et al., 2011), indicating that the mechanism of sterol transfer remains to be further defined.

PI4P also modulates the activity of other lipid transfer proteins. The yeast integral membrane ATPase Drs2 requires Pik1-generated PI4P to stimulate its flippase activity, which transfers phosphatidylserine and phosphatidylethanolamine from the luminal to cytosolic leaflet of the TGN and endosomes (Natarajan et al., 2009; Jacquot et al., 2012). Localization of OSBP and CERT is also dependent on Nir2 (Peretti et al., 2008), a VAP-binding PI/phosphatidylcholine transfer protein that moves PI from the ER to the Golgi, providing substrate for PI4Ks. Indeed, knockdown of VAP proteins reduces Golgi PI4P and sphingomyelin synthesis.

Unlike most PI4P effectors, Osh4 decreases PI4P levels and inhibits secretion (Li et al., 2002; Fairn et al., 2007; LeBlanc and McMaster, 2010; Mousley et al., 2012). Indeed, sterol binding by Osh4 is required to keep PI4P levels in check (Stefan et al., 2011). Some insight was shed on this relationship with the demonstration that, *in vitro*, Osh4 can mediate rapid exchange of PI4P for ergosterol, transferring these lipids in opposite directions between liposomes (de Saint-Jean et al., 2011). Hence, *in vivo*, Osh4 may transfer PI4P from the Golgi to the ER,

exchanging it for ergosterol in the process. Interestingly, the authors note that Pik1 and Sac1 would maintain this directionality of transfer by producing a gradient of PI4P that is high at the Golgi and low at the ER. Consequently, Osh4 enhances a sterol gradient that is low at the ER, where it is synthesized, and high at the TGN. This gradient may help drive anterograde membrane trafficking, as sterols and sphingolipids are selectively enriched in secretory vesicles that bud from the TGN (Klemm et al., 2009). Depletion of other ORPs in HeLa cells or in *C. elegans* leads to sorting and trafficking defects in the endo-lysosomal system (Kobuna et al., 2010; Du et al., 2011), and overexpression of Osh4 induces autophagy (LeBlanc and McMaster, 2010; Mousley et al., 2012). However, it remains to be seen whether functions of these ORPs are regulated by PI4P.

Consistent with Osh4 mediating exchange of PI4P for sterols, sterol binding promotes dissociation of Osh4 from TGN and endosomes (Mousley et al., 2012). An Osh4 sterol-binding mutant showed increased membrane association in fractionation studies and, when fused to GFP, appeared more punctate than wild-type Osh4-GFP, which is both cytosolic and punctate. Enhanced recruitment of Osh4 to TGN and endosomes impairs secretory trafficking as well as endocytic trafficking to the vacuole, suggesting that sterol deficiency, or an inability to sense sterol, induces a brake on PI4P-dependent trafficking. Interestingly, Osh4 sterol-binding mutants fail to traffic amino acid permeases to the PM, which leads to disruption of the general amino acid control pathway governing amino acid homeostasis, and cell cycle arrest. Thus, the sterol regulatory functions of Osh4 are intimately related to PI4P-dependent trafficking and cell physiology.

Trafficking at Endosomes and Lysosomes

PI4P is detected on membranes of the endo-lysosomal system and is required for various steps of endosomal trafficking. PI4P and PIP_2 are found on tubules emanating from the endocytic recycling compartment, a compartment containing endocytosed material destined for return to the PM (Jovic et al., 2009). PI4P, but not PIP_2, is instrumental in tubule localization of Eps15 homology (EH) domain-1 (EHD-1), a protein known to regulate recycling of transmembrane cargo internalized by clathrin-dependent and -independent endocytosis. Indeed, mutation of a key lysine in the EH domain of EHD-1 that reduces binding to PI4P (K483E) renders it unable to associate with tubules and delays recycling (Jovic et al., 2009). PI4P may also regulate recycling endosomes through Rab11. PI4KIIIβ binds and recruits Rab11, a regulator of recycling endosomes, to the Golgi (de Graaf et al., 2004). Although Fwd/PI4KIIIβ kinase activity is dispensable for Rab11 binding, it is required for full rescue of spermatocyte cytokinesis, presumably because of the need for PI4P in restoring full secretory function (Polevoy et al., 2009).

Whereas PI4KIIIβ is localized predominantly at the Golgi, PI4KIIα has been detected at the PM, early and late endosomes, synaptic vesicles, immature secretory granules, as well as at the TGN, where it produces PI4P required for recruitment of the clathrin adaptors AP-1 and GGA1-3 (Fig. 3C; Wang et al., 2003; Wang et al., 2007). While it remains unclear how the pools of PI4P generated by PI4KIIIβ and PI4KIIα are partitioned at the Golgi such that each enzyme recruits specific PI4P effectors, the two PI4Ks clearly play separate, sequential roles at this organelle. This was shown recently for the trafficking of the lysosomal hydrolase β-glucocerebrosidase (GBA) and its sorting receptor lysosomal integral membrane protein type 2 (LIMP-2) (Jovic et al., 2012). Inhibition of PI4KIIIβ prevented exit of this cargo from the Golgi, a block that was mirrored by acute depletion of Golgi PI4P. In contrast, knockdown of PI4KIIα

resulted in accumulation of LIMP-2 in late endosomes and increased missorting of GBA to the extracellular medium. This latter effect was reversed by simultaneous inhibition of PI4KIIIβ, consistent with it acting at an earlier step. Thus, GBA/LIMP-2 transport relies on both PI4KIIIβ and PI4KIIα, albeit at different stages of trafficking.

Studies in *Drosophila*, mice, and human cell lines have also given insight to a role for PI4KIIα at endosomes. In the *Drosophila* larval salivary gland, loss of PI4KII leads to mislocalization of secretory granule cargo to late endosomes as well as accumulation of the lysosomal enzyme sorting receptor (Lerp) in this compartment (Burgess et al., 2012). *PI4KII* mutants exhibit aberrant Retromer dynamics, indicating that PI4KII may regulate retrieval of cargo or membranes from late endosomes to the TGN. Interestingly, PI4KII localizes to tubules emanating from late endosomes. Although kinase-dead PI4KII also localizes to late endosomes in a wild-type background, catalytic activity is required for tubule formation, pointing to a key role for PI4P in this trafficking step. In HEK293 cells, PI4KIIα kinase activity is needed for assembly of clathrin adaptor AP-3 onto late endosomes. In addition, PI4KIIα, which is palmitoylated, acts as membrane cargo via a canonical dileucine AP-3 sorting motif (Craige et al., 2008). Both catalytic activity and the dileucine motif are required for PI4KIIα localization to late endosomes as well as rescue of *PI4KIIα* knockdown endosomal phenotypes, indicating mutual regulation between PI4KIIα and AP-3. Moreover, biogenesis of lysosome-related organelle complex 1 (BLOC-1) mediates PI4KIIα-AP-3 interaction, and all three are needed for proper trafficking of the lysosomal-associated membrane protein 1 (LAMP1) receptor in HEK293 cells (Salazar et al., 2009). In mouse primary cortical neurons, AP-3 and BLOC-1 traffic synaptic vesicles carrying PI4KIIα from the cell body to the neurite tips (Larimore et al., 2011). Additionally, when PI4KIIα is not bound to AP-3, it can interact with BLOC-1 and the WASP

and SCAR homologue (WASH), an F-actin nucleation-promoting factor specifically associated with endosomes (Ryder et al., 2013). Knockdown of WASH produces long tubules containing PI4KIIα that emanate from normal size endosomes, whereas knockdown of the BLOC-1 subunit *pallidin* results in enlarged endosomes with no tubules. This suggests BLOC-1 may act downstream of PI4KIIα-AP-3 interaction to sort PI4KIIα into tubules, and that WASH may regulate scission of PI4KIIα-containing carriers. PI4KIIα may indirectly recruit WASH, given that the WASH subunit Fam21 binds PI4P *in vitro* (Jia et al., 2010; Ryder et al., 2013).

An unexpected role for PI4KIIIβ at the lysosome was recently uncovered using siRNA in cultured cells. Small fractions of PI4KIIIβ and PI4P were detected at the lysosome, and the presence of wild-type, catalytically active PI4KIIIβ was required to prevent abnormal tubulation of this organelle as well as efflux of missorted resident lysosomal proteins such as LAMP1 into these tubules (Sridhar et al., 2013). This effect was unrelated to the role of PI4KIIIβ in the Golgi, as disruption of Golgi function by brefeldin A or nocodazole showed no effect on LAMP1 dynamics. These data suggest PI4KIIIβ normally facilitates cargo sorting and fission of lysosomal vesicles, preventing tubule formation. Increased association of clathrin and the clathrin adaptor AP-2 with lysosomes was observed in the absence of PI4KIIIβ, leading to the suggestion that PI4KIIIβ may prevent ectopic recruitment of these proteins. Interestingly, this report followed another that identified PIP5K and PIP_2 as being necessary for tubule formation to create *de novo* lysosomes from autolysosomes following starvation-induced autophagy (Rong et al., 2012). Since tubules form in PI4KIIIβ and PIP5K doubly-depleted cells, Sridhar *et al*. propose that PIP5K is not necessary for vesicle or tubule formation *per se*, but that in special circumstances, such as those surrounding regeneration of lysosomes, PIP5K is required to deplete PI4P through its conversion to PIP_2 in order to inhibit vesicle fission and favor bulk

efflux through tubulation. However, important questions remain, such as what drives tubule formation in the absence of PI4P, and whether PI4P and PIP_2 are coordinately regulated to recruit AP-2 and scission machinery.

PI4P and Rabs Confer Compartment Identity

Because PI4P is found in multiple intracellular membranes, additional factors must be required to recruit organelle-specific effectors. In addition to simultaneous detection of Arf1 by PI4P effectors such as FAPPs (Godi et al., 2004), Rab family proteins can help determine distribution of PI4P effectors. Similar to organelle-specific distribution of phosphoinositides, the secretory and endosomal systems are decorated with compartment-specific Rabs. Together, phosphoinositides and Rabs define compartment identity, and synergistically recruit downstream effectors to perform compartment- and stage-specific functions. This is important because proper trafficking requires sequential events, including disassembly of fission machinery, assembly of fusion machinery, and cargo-specific events such as protein processing. Evidence suggests PIPs and Rabs participate in a finely tuned assembly line for moving cargo through sequential steps in the secretory pathway. This “Rab cascade” unfolds through Rab-mediated recruitment of the GEF that activates the subsequent Rab, and in some cases, the GAP to inactivate the Rab that defines the current compartment (Mizuno-Yamasaki et al., 2010; Jean and Kiger, 2012).

PI4P has been shown to play an integral role in this identity switch. Sec2, the yeast GEF that activates the Rab protein Sec4/Rab8 on secretory vesicles, is involved in both budding from the TGN and recruitment of the exocyst component Sec15 for docking at the plasma membrane. Interestingly, the level of PI4P on the membrane to which Sec2 is bound determines which role it plays at a given point during secretion (Mizuno-Yamasaki et al., 2010). Sec2 is recruited to the

trans-Golgi by binding to a yeast Rab11 ortholog (either Ypt31 or Ypt32) and PI4P via its Ypt31/32 binding site and three polybasic patches, respectively. During budding, the exocyst machinery is prevented from prematurely assembling because binding to Ypt32 occludes the Sec15-binding site on Sec2, and because PI4P itself inhibits Sec2-Sec15 interaction. Indeed, *in vitro* assays show that PI4P inhibits this association in a dose-dependent manner. However, once secretory vesicles are formed, Sec15 is able to outcompete Ypt32 for Sec2 binding. Since the PI4P probe mCherry-FAPP-PH colocalizes with Sec2 only at the Golgi and not at vesicular sites, it is proposed that low levels of PI4P on secretory vesicles allow assembly of docking proteins at the expense of budding machinery. Similarly, it has been proposed that Sac1 decreases PI4P on forming exocytic vesicles (Alfaro et al., 2011). Thus, PI4P regulates Sec2 binding partners and the switch in identity from TGN to secretory membrane. Whether other factors lead to a drop in PI4P, *i.e.*, a sorting out of PI4P at the Golgi or enzymatic depletion on vesicles, remains to be determined.

Although reduction in PI4P levels is necessary for progression along the secretory pathway, another report in yeast demonstrates that PI4P also plays an important role on secretory vesicles, and that modulating its levels can affect compartment identity and function (Santiago-Tirado et al., 2011). The myosin V motor Myo2 transports Golgi membranes and secretory vesicles along actin cables into the nascent bud. Myo2 is recruited by Rabs of the late Golgi (Sec7), the *trans*-Golgi (Ypt31/32), and secretory vesicles (Sec4). GFP-FAPP-PH and mCherry-Osh2-PH colocalize with Sec7 and Ypt31, respectively, and mCherry-Osh2-PH colocalizes with Sec4-containing vesicles that accumulate at the bud tip and neck of small budded cells. Sec4 and PI4P did not overlap in wild-type mother cells, but a small degree of overlap was seen in mother cells from *myo2* transport mutants, which accumulate secretory vesicles. This suggests that PI4P

concentration is high on Golgi membranes and low on smaller Sec4-positive secretory vesicles, such that secretory vesicle PI4P is difficult to visualize without vesicle aggregation. These results are consistent with the hypothesis of Mizuno-Yamasaki *et al*. (2010) that a drop in PI4P levels occurs during the transition from Golgi to secretory vesicle. Sec4 polarization and Myo2 transport of secretory organelles depends on PI4P (Santiago-Tirado et al., 2011). Increasing Golgi PI4P by Pik1 overexpression or *sac1* deletion results in recruitment of Myo2 mutant proteins unable to bind either Ypt31/32 or Sec4, rescuing growth and transport defects. Likewise, fusion of mutant Myo2 proteins with a FAPP-PH domain also rescues Rab-binding *myo2* mutants, indicating that a bridge between Myo2 and PI4P is necessary for transport. This is thought to occur via an as yet unidentified factor. Hence, under normal conditions, PI4P and a Rab synergistically recruit Myo2, with PI4P acting as a general marker for secretory membranes, and Rabs further defining specific secretory stages. However, upon disruption of Myo2-Rab association, enhancement of Myo2-PI4P interaction can compensate, showcasing the regulatory power of modulating PI4P.

Plasma membrane effectors

While much has been learned about Golgi PI4P, roles for PI4P at the PM are only beginning to be revealed. This is because PIP_2 and PIP_3 are also found on this membrane, and traditional genetic or biochemical techniques to disrupt PI4P made it difficult to assess whether resulting phenotypes were due to loss of PI4P, or to loss of its downstream metabolic derivatives. With the advent of technologies to detect and manipulate specific pools of lipids (Heo et al., 2006; Varnai and Balla, 2008; Clark et al., 2011; van den Bogaart et al., 2011), new data suggest that despite its many essential roles at the Golgi, the majority of cellular PI4P is on the PM (Hammond et al.,

2009).

The classical view is that PM PI4P allows for rapid replenishment of PIP_2 after acute signaling events. PI4KIIIα appears to be the isoform that generates this pool of PI4P, as pharmacological inhibition with Wm or 10 μM PAO abolished replenishment of both PM PI4P and PIP_2 after hormone-activated PLC-coupled signaling in COS-7 cells (Balla et al., 2008; Hammond et al., 2012). Knockdown of the enzyme gave a less robust effect, presumably because of incomplete silencing. Further, in permeabilized β-cells, parallel changes in PIP_2 levels in response to decreases or increases in PI4P depend on a type III PI4K (Wuttke et al., 2010).

Independent roles for PM PI4P are beginning to be defined, although the extent to which PI4P influences PIP_2 and PIP_3 levels remain unknown. New data suggest that, in some cells, PI4P and PIP_2 may be less intimately coupled than previously thought. Stimulation of β-cells through G_q-protein-coupled receptors leads to opposite changes in PM PI4P and PIP_2 levels: PIP_2 decreases as expected after activation of PLC; however, PM PI4P levels increase, suggesting that the two lipids may be independently regulated in this cell type (Wuttke et al., 2010). More strikingly, in COS-7 cells, acute depletion of all PM PI4P by rapamycin recruitment of the Sac1 phosphatase domain to the PM had no effect on PIP_2 resynthesis after PLC activation (Hammond et al., 2012). Since previous results on PI4KIIIα inhibition and knockdown differ from those obtained through acute PI4P depletion, this suggests that PI4KIIIα produces a small but essential pool of PI4P at specific PM sites that is not susceptible to acute PI4P depletion. For example, PI4KIIIα and its associated proteins may protect this pool through steric hindrance (see below). Alternatively, PI4KIIIα may replenish a pool of PI4P that is used immediately for PIP_2 resynthesis. Indeed, kinetic studies suggest PIP_2 replenishment occurs so quickly that both PI4K and PIP5K activity need to be stimulated to satisfy the observed concentration response and time

course (Falkenburger et al., 2010; Falkenburger et al., 2013). Additionally, PIPs may be replenished from other membranes. On this front, it is noteworthy that Golgi-specific depletion of PI4P moderately affects PM PIP_2 replenishment, suggesting that Golgi PI4P can contribute to PM PIP_2 (Szentpetery et al., 2010). In either case, although PI4KIIIα appears to play a critical role in PIP_2 replenishment after signaling, it is unclear whether it has a major role in regulating steady state PIP_2 levels in mammalian cells. While inhibition of PI4KIIIα in COS-7 cells under normal growth conditions did decrease steady state PM PI4P, it had little to no effect on steady state PIP_2, suggesting that PIP_2 normally experiences low turnover in these cells, or that it is made from a distinct or redundant pool of PI4P (Balla et al., 2008; Hammond et al., 2009).

On the other hand, genetic ablation of *PI4KIIIα* in yeast and in animal models suggests that this pool of PI4P is tightly linked to the functions of PM PIP_2 and PM identity (Audhya and Emr, 2002; Murray et al., 2012; Nakatsu et al., 2012). Indeed, in *Drosophila*, *PI4KIIIα* mutant female germ cells exhibit defects similar to those depleted for PIP_2 (Tan et al., in press.), and mouse embryonic fibroblasts (MEFs) mutant for *PI4KIIIα* upregulate expression of the PIP_2-generating enzyme PIP5K as a compensatory mechanism. Perhaps this reflects differential requirements for PI4KIIIα-dependent synthesis of PM PI4P in different cell types or in cells within living tissues.

Although previous studies identified few specific functions for PM PI4P aside from its role as a precursor to PIP_2, Hammond *et al*. (2012) found that PI4P makes a substantial contribution to the total negative charge that defines the inner leaflet of the PM. Seven membrane-targeting protein domains fused to GFP were monitored before and after dynamic depletion of PM PI4P or PIP_2. Rapamycin was used to recruit the phosphatase domains of Sac1 and inositol polyphosphate-5-phosphatase E (INPP5E) to deplete PI4P and PIP_2, respectively. Of

those proteins that were predicted to localize via non-specific polyanionic lipid interaction through a polybasic domain, elimination of both PM PI4P and PIP_2 was required to abolish PM localization, whereas depletion of either lipid alone had minimal effect. For example, while stimulation of the heat and capsaicin-activated transient receptor potential vanilloid 1 (TRPV1) cation channel was previously associated with binding to PIP_2, activation was achieved in the presence of either PM PI4P or PIP_2, with channel activity being inhibited only when both lipids were absent. However, not all cation channels operate on a general requirement for polyanionic lipids, as activation of the menthol-activated transient receptor potential melatastatin 8 (TRPM8) channel specifically required PIP_2. Therefore, PM PI4P functions in processes that require a general polyanionic lipid pool. With this in mind, it is possible that PI4P fulfillment of this function is especially important when PIP_2 is rapidly consumed, which may explain why PI4P levels increase during PLC signaling in some cells (Wuttke et al., 2010), although this would not be the case in cell types where PI4P and PIP_2 react in parallel (Balla et al., 2008). Whether PI4P specifically targets any protein for PM localization or activation remains to be seen. With new methods for perturbing membrane-specific pools of PI4P, analysis of lipids in sub-membrane microdomains (van den Bogaart et al., 2011), and imaging of PM dynamics (Wuttke et al., 2010; Nakatsu et al., 2012), it is an exciting time for this avenue of research.

Dynamic Regulation of PI4P Signaling: Mechanisms of Spatiotemporal Control

Thus far, we have discussed steady-state roles for PI4P in replenishing other signaling phosphoinositides, and in recruiting organelle-specific effector proteins, for which little phosphoinositide turnover is apparently required. However, the ability of phosphoinositides to be

rapidly phosphorylated and dephosphorylated makes them ideal candidates to regulate dynamic processes in time and space. Therefore, it is not surprising that physiological cues requiring rapid cellular responses, such as nutrient availability and cell signaling, invoke pathways that regulate PI4P.

Response to Nutrients

An early report describes modulation of PI4K activity in response to nutrients. Secretion of insulin granules by pancreatic β cells in response to increased glucose concentration requires type III PI4K activity, and is stimulated by injection of PI4P (Olsen et al., 2003). Given the relatively low concentration of the pharmacological inhibitor PAO required to inhibit insulin exocytosis (Balla and Balla, 2006), the bulk of this PI4K activity is likely provided by PI4KIIIα. Indeed, PI4Ks were proposed to act as metabolic sensors because reduced levels of cellular ADP, which mimic what occurs upon glucose stimulation, correspond with increased PI4K activity. Indeed, addition of ATP to permeabilized β cells stimulates PI4P production in a dose-dependent manner, consistent with a mechanism for activating PI4Ks by increasing substrate availability (Wuttke et al., 2010). In these cells, stimulation with glucose also provoked an increase in PM PI4P that was dependent on increased cytoplasmic Ca^{2+}, although the exact mechanism by which energy status stimulates PI4Ks is still unclear.

Another example of regulation of PI4P signaling in response to nutrients involves Sac1. In yeast and mammalian cells, the largely ER-localized Sac1 reversibly localizes to the Golgi during periods of glucose deprivation (Faulhammer et al., 2007), or when cell growth is slowed in late log phase yeast cultures (Faulhammer et al., 2005). The benefit of this relocation during starvation appears to be twofold. First, ER-localized Sac1 is required for biosynthesis of

oligosaccharides for *N*-glycosylation. Removal of Sac1 from the ER when nutrients are scarce limits the use of these resources and slows passage of glycosylated proteins through the secretory pathway. Second, Sac1-dependent depletion of Golgi PI4P halts global secretion, thereby conserving cellular material. Nutrient status also appears to regulate Pik1, since it dissociates from the Golgi during glucose starvation, coinciding with Sac1 relocation to the Golgi and a decrease in Golgi PI4P (Faulhammer et al., 2007). In late log phase or with nutrient deprivation, Pik1 shifts from the TGN to the nucleolus or to cytoplasmic puncta, where it forms a complex with 14-3-3 proteins (Demmel et al., 2008).

Sac1 retention in the ER depends on its interaction with the ER transmembrane protein dolichol phosphate mannosyltransferase (Dpm1). Sac1 translocation to the Golgi upon starvation requires COPII-mediated exit, and in mammalian cells, also requires prior oligomerization of Sac1 in the ER (Blagoveshchenskaya et al., 2008). Retrieval of Sac1 back to the ER upon addition of glucose requires COPI, and in mammalian cells, requires prior dissociation of Sac1 oligomers in the Golgi. Curiously, in yeast, the Rer1 adaptor for COPI retrograde transport is also required for ER exit of Sac1, although its role in this context is in disruption of the Sac1-Dpm1 interaction (Faulhammer et al., 2007).

This effect of nutrient status on PI4P changes in the ER and Golgi appears to be mediated by MAPK signaling. Treating NIH3T3 mouse embryonic fibroblasts with FGF and PDGF simulates nutrient addition and promotes relocation of Sac1 from the Golgi to the ER, an effect eliminated by addition of a p38 MAPK inhibitor (Blagoveshchenskaya et al., 2008). In yeast, retrieval of Sac1 from the Golgi to the ER upon glucose stimulation of starved cells requires the AMP-activated kinase Snf1 and the MAPK Hog1 (Piao et al., 2012).

PKC-mediated MAPK pathway activation in yeast occurs in response to nutrient

deprivation and other stresses. Recently, inositol starvation was shown to induce MAPK pathway activation and an increase in PM PI4P, revealing an interesting link between inositol-containing sphingolipid biosynthesis and PI4P signaling (Jesch et al., 2010). This was dependent on Stt4. Intriguingly, perturbation of inositol-containing sphingolipid production by other means, even in the presence of inositol, also led to Stt4 and PKC pathway stimulation. This suggests that Stt4 responds to cellular levels of inositol-containing sphingolipids, perhaps PM sphingolipid composition. In this context, changes in sphingolipid composition may facilitate formation of active Stt4 signaling complexes (PIK patches) (Baird et al., 2008), restrict access of PI4P effectors to PM PI4P, or directly alter the activity of Stt4. Interestingly, PI4KIIα is also regulated by lipid composition, with membrane cholesterol increasing the mobile fraction of laterally-diffusing PI4KIIα in the TGN, thereby leading to increased PI4P production (Minogue et al., 2010). The interdependency of sphingolipid homeostasis and Stt4-regulated PKC pathway activation, and of cholesterol metabolism and PI4KIIα activity, remain to be examined.

Type II PI4Ks and communication with other signaling pathways

PI4P participates in signal transduction not only by providing precursors for PLC and PI3K pathways, but also by regulating endosomal trafficking in response to receptor-stimulated endocytosis. Type II PI4K activity has long been detected in microsomal membrane fractions (Harwood and Hawthorne, 1969; Collins and Wells, 1983; Balla et al., 2002). The finding that PI4KIIα is present on vesicles that are secreted in response to external cues, such as synaptic vesicles (Guo et al., 2003) and chromaffin granules (Barylko et al., 2001), led to the idea that this enzyme plays a role in regulated secretion downstream of receptor activation. However, the finding that PI4KIIα also localizes specifically to a pool of insulin-nonresponsive vesicles

carrying the glucose transporter GLUT4 suggested a more general role in trafficking that supports signaling (Xu et al., 2006).

Such a role for PI4KIIα can affect the trafficking of many cell surface receptors as well as signaling outcomes, as some ligand-receptor complexes continue to signal until they are sent to multivesicular bodies (Futter et al., 1996). In mammalian cells, the EGF, transferrin, and Angiotensin II receptors were all found to pass through PI4KIIα-positive vesicles. In addition, PI4KIIα activity is upregulated upon EGFR activation (Kauffmann-Zeh et al., 1994). Knockdown of PI4KIIα in human cell lines leads to accumulation of ligand-bound EGFR in small cytoplasmic vesicles, as well as to decreased degradation of EGFR over a two hour period (Minogue et al., 2006). The concomitant loss of late endosomes, which are large LAMP1-positive perinuclear structures, suggested that EGFR signaling complexes fail to traffic along the endocytic pathway in the absence of PI4KIIα. Thus, PI4KIIα regulates trafficking downstream of early endosomes. The yeast homologue Lsb6 has similarly been implicated in actin-based endosome motility. Unlike in mammals, however, its PI4K activity is not required and it is still unclear whether Lsb6 facilitates early endosome movement away from sites of endocytosis or later stages of endosome motility (Chang et al., 2005; Kim et al., 2006).

Much less is known about PI4KIIβ, whose properties differ significantly from PI4KIIα in that approximately 75% of the enzyme is unpalmitoylated and shows either cytoplasmic or peripheral membrane-association (Jung et al., 2008). Only palmitoylated, membrane-bound PI4KIIβ is catalytically active and only 25-30% of type II PI4K activity is contributed by this enzyme in resting cells (Balla et al., 2002; Jung et al., 2008). The cytosolic pool of PI4KIIβ is sensitive to proteasome degradation and is stabilized by binding Hsp90 (Jung et al., 2011). This sequestration is released upon stimulation by EGF or PDGF, which results in palmitoylation of a

subset of cytosolic PI4KIIβ. This membrane-bound PI4KIIβ subsequently translocates to the PM, where it becomes active (Wei et al., 2002; Jung et al., 2011). Relocation of cytosolic PI4KIIβ to plasma membrane ruffles was also seen with overexpression of constitutively active Rac. The pool of PI4P stimulated by growth factor may regulate early steps of endocytic trafficking since PI4KIIβ can be detected on clathrin- and AP-2-containing vesicles (Li et al., 2012b). Thus, it is tempting to speculate that PI4P synthesis in early trafficking of some receptors is subject to dynamic regulation by PI4KIIβ and that, as endosomes mature, their cargo is sorted in a manner that depends on PI4KIIα.

PI4KIIα has also been implicated in Wnt signaling in HEK293 cells and in *Xenopus laevis* embryos (Pan et al., 2008; Qin et al., 2009). Activation of the canonical Wnt signaling pathway by Wnt3a leads to Dishevelled (Dvl) binding to PI4KIIα and PIP5KI, and increased cellular PI4P and PIP_2. The rise in PIP_2 is required for phosphorylation of the Wnt co-receptor, low density lipoprotein receptor-related protein (Lrp) 5/6; co-aggregation of Wnt3a and Lrp5/6 into signalsomes; and recruitment of clathrin and AP-2 for receptor endocytosis (Kim et al., 2013). Importantly, knockdown of type IIα PI4K prevented PI4P and PIP_2 elevation, Lrp5/6 phosphorylation, and β-catenin stabilization (Pan et al., 2008). Dvl itself can stimulate PI4KIIα activity *in vitro*, suggesting that PI4KIIα may produce the PI4P precursor to PIP_2 in this context. Indeed, kinase-dead PI4KIIα greatly reduced endocytosis of the Wnt receptor Frizzled 4 (Fz4) (Mossinger et al., 2012). However, PI4KIIα undergoes ubiquitination at multiple sites, and expression of PI4KIIα that is unable to bind the E3 ubiquitin ligase Itch only moderately restored Fz4 colocalization with early endosome markers. This suggests that PI4KIIα has a ubiquitin-mediated, non-catalytic role in Fz4 internalization or sorting into early endosomes. Furthermore,

siRNA of either *PI4KIIα* or *itch* delays lysosomal degradation of Fz4, suggesting that these proteins act together in late endosome trafficking. Itch also inhibits PI4KIIα kinase activity independently of ubiquitination, which may help to limit Wnt signaling at the cell surface; indeed, *itch* knockdown increases Lrp6 phosphorylation. It would be interesting to know whether PI4KIIα kinase activity is required for PIP_2 elevation and/or Lrp5/6 phosphorylation.

The small size of type II PI4Ks and their membrane association by palmitoylation allow for dynamic regulation of their localization. Indeed, PI4KIIα may act as a spatial landmark connecting Wnt signaling, PIP5KI activity, and PI4P-dependent trafficking, rather than directly providing PI4P for PIP_2 synthesis. Alternatively, complex formation between PI4KIIα and a PIP5K facilitated by a core signaling pathway component such as Dvl may allow for production of a pool of PM PIP_2 reserved for Wnt signaling, thereby mediating crosstalk between the Wnt and PIP signaling pathways. A similar mechanism occurs at the Golgi, where PKD forms a complex with PI4KIIIβ and PIP5KI (Nishikawa et al., 1998) that may be important for producing a Golgi-specific pool of PIP_2, which is known to be present at low levels at this organelle (Watt et al., 2002). Monitoring where PIP increases occur in response to signaling *in vivo* would prove useful in deciphering the precise involvement of PI4KIIα in this pathway, and will add to our understanding of spatiotemporal control of PI4P.

PI4P regulation by enzyme localization

In addition to PI4KIIβ, other phosphoinositide enzymes have also been found to shuttle between sites. For example, Sac1 is ER-localized during periods of cell growth, but relocalizes to the Golgi during periods of starvation where it halts PI4P-dependent secretion (Faulhammer et al., 2005; see above). The C-terminus of Sac1 is responsible for its ER localization, whereas its N-

terminus localizes Sac1 to the Golgi. Both type II and type III PI4K activities have been detected in the nucleus (Fiume et al., 2012), although little is known about the nuclear type II enzymes. Budding yeast Pik1 contains a nuclear localization signal (NLS) and a nuclear export signal (NES). Pik1 cycles through three different locales. Phosphorylation at S396 during times of nutrient limitation decreases Golgi localization and increases its association with 14-3-3 proteins in the cytoplasm, as well as its accumulation in the nucleolus (Demmel et al., 2008). A S396D phosphomimetic mutant accumulates to a greater extent in the nucleus, suggesting that dephosphorylation may be required for nuclear exit. The Golgi and nuclear functions of Pik1 are both essential, since restriction of the enzyme to either the Golgi (by addition of a CAAX box) or the nucleus (by deletion of the NES) results in lethality that can be rescued by expression of the reciprocal mutant (Strahl et al., 2005). Mammalian PI4KIIIβ and PI4KIIIα have also been found in the nucleus. Nucleolar localization of endogenous PI4KIIIα was abolished with DNase or RNase treatment of permeabilized rat B50 cells, suggesting that PI4KIIIα complexes with nucleic acids (Kakuk et al., 2006). The targets of nuclear PI4P are unknown, although undoubtedly a proportion is used to produce nuclear PIP_2. The budding yeast PIP5K Mss4 (Audhya and Emr, 2003) and its mammalian and *Drosophila* homologues undergo nucleocytoplasmic shuttling and have nuclear functions (Cheng and Shearn, 2004; Schill and Anderson, 2009). PIP_2 has been implicated in a number of nuclear processes, including RNA processing, nuclear export, regulation of nuclear actin and chromatin remodeling (Barlow et al., 2010). In mammals, PIP_2 and PIP5Ks are detected in so-called nuclear speckles, interchromatin granule clusters that are enriched for pre-mRNA splicing machinery (Boronenkov et al., 1998). Notably, the PI4Ks involved in these processes have yet to be identified.

A requirement for some phosphoinositide kinases in multiple organelles can be explained

by shuttling. However, in the case of PI4KIIIα, until recently, there has been a disconnect between its apparent location and its site of action. As described above, experiments in yeast and mammals point to PI4KIIIα controlling PM PI4P (Audhya and Emr, 2002; Balla et al., 2008). In yeast, localization of the PM PI4P marker GFP-Osh2-PH is dependent on Stt4 (Roy and Levine, 2004). In mammalian cells, PI4KIIIα is required for replenishment of PM PI4P and PIP_2 in response to PLC activation. In yeast, consistent with its PM role, Stt4 localizes to PM PIK patches, which are stable complexes of Stt4 molecules with the accessory proteins Ypp1 and Efr3 (Baird et al., 2008). Nonetheless, epitope tagging and immunoelectron microscopy indicated that mammalian PI4KIIIα localizes to the Golgi, nucleolus, vacuoles, and pericentriolar regions, but not the PM (Balla and Balla, 2006). Immunocytochemistry and cell fractionation experiments consistently identified endogenous PI4KIIIα at the ER (Wong et al., 1997; Balla et al., 2000), but it was still unclear how an enzyme at this location could mediate acute responses to signaling at the PM. These discrepancies were recently resolved when a conserved ~50 amino acid sequence upstream of the reported translational start site was shown to confer targeting of mammalian PI4KIIIα to the PM (Nakatsu et al., 2012). Total internal reflection fluorescence (TIRF) microscopy revealed that full length GFP-PI4KIIIα exhibits dynamic localization at the cell surface, with abundant puncta transiently appearing at the PM. Consistent with a role for PI4KIIIα at the PM, ER-PM contact sites known to be dependent on PI4P and the ER protein stromal interacting molecule 1 (STIM1) were greatly reduced in *PI4KIIIα*-knockout MEFs. In addition, pre-association of mouse Ypp1 (TTC7B) and Efr3 (EFR3B) at the PM was necessary for targeting of PI4KIIIα to this site.

In mammals, regulation of PI4P signaling at the PM is shaping up to be more dynamic than expected, requiring continuous recruitment of PI4KIIIα, perhaps from the ER (see below).

One potential moderator of PI4KIIIα dynamics is With no lysine 1 (WNK1), a serine/threonine protein kinase that regulates many ion transporters. WNK1 was shown to stimulate DAG-activated TRPC6 channels in a kinase-independent but PLC- and PI4KIIIα-dependent manner (An et al., 2011). Interestingly, WNK1 promoted PI4P production as well as association of PI4KIIIα with membrane, suggesting that WNK1 regulates PI4KIIIα localization.

Mammalian PI4KIIIα localization is reminiscent of findings for PI synthase (PIS), the enzyme that catalyzes addition of *myo*-inositol to CDP-DAG to generate PI as part of the phosphoinositide cycle. Although its synthesis is associated with the ER, PI must be made available for PI4Ks in different cellular compartments. Recently, the Balla laboratory discovered that the vast majority of enzymatically active PIS is found on a novel system of highly mobile vesicles, the formation of which is dependent on the ER membrane remodeling GTPase and COPII nucleator Sar1 (Kim et al., 2011). This population of vesicles can be separated from heavier ER membranes using a shallow fractionation gradient. In addition, by cell imaging, the vesicles did not colocalize with typical ER, Golgi or endosomal markers. Photoactivation of PIS fused to photoactivatable GFP in the perinuclear ER generates a similar highly mobile pool of vesicles emanating from this compartment. In addition, PIS-positive vesicles were seen to make contact with STIM1-positive ER-PM sites, although no fusion events were detected. Interestingly, this dynamic PIS pool is also associated with CEPT1, an enzyme that converts DAG to phosphatidylethanolamine or phosphatidylcholine (English and Voeltz, 2013). PIS and CEPT1 colocalize with Rab10 at the tip of ER tubules, which are reduced upon treatment with Rab10 siRNA or expression of GDP-locked Rab10. Thus, phospholipid synthesis may be coordinated on a common, dynamic membrane platform. Colocalization of PI and PI4KIIIα on ER-derived vesicles has yet to be demonstrated, but it is possible that synthesis and delivery of

PI may be coupled to the provision of PI4KIIIα in a common mobile compartment. It is currently unknown how PI, or PI4P, would be transferred from these vesicles into the PM, or whether as yet unidentified PITPs are required for this process.

ER-PM contact sites are emerging as important locales for PI4P metabolism. Research from fission yeast suggests that the iconic, reticular nature of ER results from the necessity of ER-PM contacts (Zhang et al., 2012a). At these sites in mammalian cells, PI4P regulates calcium entry through the STIM1/Orai1 complex when intracellular stores are depleted (Korzeniowski et al., 2009; Walsh et al., 2010). Recent data in budding yeast suggest Sac1 is involved in forming ER-PM contact sites. Sac1 is associated with the ER and Golgi and, similar to the conundrum for PI4KIIIα, it was not clear how it controls PM PI4P (Foti et al., 2001). Osh proteins are thought to mediate this process by detecting PM PI4P, either via PH domains (for Osh1-3) or ORD domains, and tethering peripheral ER membranes to these PI4P patches through interaction of their FFAT motifs with ER VAP proteins Scs2/22 (Stefan et al., 2011). Yeast cells mutant for Osh proteins or Scs2/22 accumulate excess PM PI4P, suggesting these proteins may promote Sac1 activity at ER-PM contact sites, where it could potentially act *in trans* on PM PI4P. A possible role for Sac1 in dephosphorylating PI4P *in trans* has also been postulated for ER-early Golgi contact sites (Wood et al., 2012). In support of this idea, the ORD domains of Osh3 and Osh4 stimulate Sac1 turnover of PI4P *in trans* when incubated with PI4P-containing liposomes (Stefan et al., 2011). However, since Osh4 transfers PI4P between liposomes (de Saint-Jean et al., 2011), an alternative possibility is that *in vivo*, Osh3/4 could deliver PI4P to the ER, where it could be consumed by Sac1 *in cis*. In any case, PI4P-binding by Osh3/4 is a prerequisite for ORD stimulation of Sac1, suggesting that a key role for Osh proteins is to mediate substrate presentation to Sac1 (Stefan et al., 2011). Importantly, the Sac1-containing ER-PM sites form in

response to high PM PI4P. Hence, these interactions constitute feedback regulation. A functional relationship between *Drosophila* Sac1 and DVAP in modulating PI4P levels was also identified in the control of neuromuscular morphology and neurotransmission (Forrest et al., 2013). It will be interesting to see if this interaction requires *Drosophila* OSBP.

As it turns out, mechanisms regulating PM PI4P may have more in common than originally anticipated. Although Stt4 is stably localized to PIK patches on the yeast PM, it contains an FFAT motif (Nakatsu et al., 2012) that could potentially bind to ER VAP proteins, thereby regulating ER-PM junctions. Indeed, Stt4 co-precipitates with Scs2 (Gavin et al., 2002). Mammalian PI4KIIIα contains a partially conserved FFAT motif, leaving open the question of whether its access to the PM is similar to ER-PM contact sites in yeast, involving tethering of tubular ER, or more similar to that of mammalian PIS, via delivery by a highly mobile ER-derived vesicular population.

These developments highlight the regulation of PM PI4P at specific organelle contact sites and suggest that organelle-specific enrichment of phosphoinositides is not simply achieved by restricted localization of phosphoinositide-generating enzymes. A dynamic method of PM PI4P accumulation holds important implications for PI4P signaling. Whether the sites of PI/PI4P deposition are regulated and how this regulation is coordinated with PI4P effectors remain to be seen.

Regulation by Calcium

Support for the idea of dynamic regulation of PI4P production comes from the ancient and conserved physical interaction between homologues of PI4KIIIβ and members of the neuronal calcium sensor (NCS) family of proteins, Frequenin (Frq)/NCS-1. Intracellular calcium levels

frequently rise in response to signaling downstream of receptor stimulation. Thus, coupling PI4K activity with calcium suggests a mechanism to control PI4P production in response to physiological changes. In yeast and mammalian cells, this interaction is required for many types of PI4P-dependent secretion.

Frq was first identified in *Drosophila* due to its ability to increase neurotransmitter release when overexpressed (Pongs et al., 1993). This effect was also demonstrated in frogs (Olafsson et al., 1995) and mammals (McFerran et al., 1998; Pan et al., 2002). The high level of conservation between Frq/NCS-1 homologues was shown in rescue experiments, where yeast *frq1* mutants could be suppressed through expression of frog or human NCS-1 (Hendricks et al., 1999; Strahl et al., 2003). NCS family proteins are less than 30 kDa in size and contain four calcium-binding EF-hand motifs. Direct binding and stimulation of PI4KIIIβ activity by Frq1/NCS-1 has been demonstrated in yeast and mammalian cells (Hendricks et al., 1999; Weisz et al., 2000; Zhao et al., 2001; Haynes et al., 2005). Overexpression of Pik1 rescues *frq1^{ts}* mutants at restrictive temperature and vice versa, whereas *pik1^{ts}* and *frq1^{ts}* show synthetic lethality (Hendricks et al., 1999; Huttner et al., 2003). Removing elements required for optimal binding to each other (myristoylation of Frq1 or the N-terminal LKU domain of Pik1) reduced the ability of one protein to rescue a temperature-sensitive mutant of the other.

In mammalian cells, the NCS-1- PI4KIIIβ interaction has been studied in models of regulated and constitutive exocytosis. One model is the ATP-dependent activation of purinergic receptor signaling that results in release of dense core granules in PC12 neuroendocrine cells. The purinergic receptor is coupled to PLC, which, through hydrolysis of PIP_2 and formation of second messengers following stimulation, leads to a rise in intracellular calcium. In these cells, NCS-1 normally binds and stimulates PI4KIIIβ activity to promote secretion when intracellular

calcium levels rise. Overexpression of NCS-1 that cannot be myristoylated prevented the stimulatory effect of overexpressed PI4KIIIβ on secretion, and siRNA knockdown of *PI4KIIIβ* prevented NCS-1-stimulated exocytosis in evoked cells (de Barry et al., 2006). In addition, the PI4K inhibitor PAO prevented ATP-evoked exocytosis, but overexpression of NCS-1 overcame this effect (Rajebhosale et al., 2003). Indeed, PI4KIIIβ has been suggested to act downstream of NCS-1 in both regulated and constitutive exocytosis in a variety of cell types (Weisz et al., 2000; Koizumi et al., 2002; Kapp-Barnea et al., 2003; Gromada et al., 2005; de Barry et al., 2006).

Calcium regulation of PI4KIIIβ-dependent secretion by Frq1/NCS-1 is thought to occur via a calcium-to-myristoyl switch, as initially proposed for the related NCS family protein recoverin (Ames et al., 1997; Ames and Lim, 2012). Ca^{2+} binding to NCS-1 induces a large conformational shift in the protein (Cox et al., 1994; McFerran et al., 1999), allowing for increased membrane association via a more extruded N-terminal myristoyl group (Ames et al., 2000). The conformational shift also exposes two large hydrophobic crevices that interact with PI4KIIIβ (Lim et al., 2011), thus making membrane-bound NCS-1 more efficient in anchoring PI4KIIIβ. Indeed, PI4KIIIβ activity was stimulated by Frq1/NCS-1 three- to ten-fold in yeast (Hendricks et al., 1999), and in a dose-dependent manner in COS-7 cells (Zhao et al., 2001). Oddly, in contrast to what is predicted by the calcium-to-myristoyl switch, localization of myristoylated NCS-1 and its interaction with PI4KIIIβ does not depend on Ca^{2+} binding *in vivo* (Hendricks et al., 1999; Zhao et al., 2001). However, Ca^{2+} binding does enhance PI4K activity (Zhao et al., 2001; Haynes et al., 2005), possibly by forcing a conformational change in PI4KIIIβ (Strahl et al., 2007).

Additional roles for calcium in fine-tuning PI4P accumulation and localization come from identification of Arf1, a PI4KIIIβ interactor, as an NCS-1 binding partner in bovine brain

cells (Haynes et al., 2005). Although both proteins partially colocalize with PI4KIIIβ at the TGN, a complex of all three could not be detected under conditions that allow Arf1-NCS-1 binding, which occurs with or without Ca^{2+}. Curiously, although Arf1 increases PI4KIIIβ activity by 125% above basal levels in *in vitro* kinase assays, and NCS-1 increases PI4KIIIβ activity by 70%, when both regulators are incubated in the presence of Ca^{2+}, kinase activity was reduced below the level elicited with either protein alone. This suggests a regulatory network in which PI4KIIIβ is efficient at producing PI4P only in the presence of either Arf1 or Ca^{2+}-bound NCS-1, but not both. Therefore, membrane sites occupied by both regulators serve as PI4KIIIβ-inactive zones so that Arf1- and Ca^{2+}-dependent pathways do not interfere with each other, and these zones act as boundaries demarcating pools of PI4P dedicated to either Arf1-mediated secretion or Ca^{2+}-dependent NCS-1 signaling. In support of this, overexpressed NCS-1 interferes with formation of the activated $Arf1^{Q71L}$ tubular Golgi phenotype, and overexpression of Arf1 abolishes Ca^{2+}-dependent NCS-1 stimulation of secretion in PC12 cells (Haynes et al., 2005). Thus, precise and dynamic regulation of separate pools of PI4P is crucial even within the same organelle and when produced by the same PI4K. Importantly, effective mechanisms exist to link spatial regulation of PI4P to different signaling pathways.

Adding another layer of regulation to Ca^{2+}-induced PI4P-mediated secretion is the inhibitory effect of calcium sensor proteins calneuron-1 and calneuron-2 on PI4KIIIβ activity (Mikhaylova et al., 2009). Under low Ca^{2+} conditions, the calneurons outcompete NCS-1 for binding to PI4KIIIβ and strongly inhibit PI4P production. At high Ca^{2+} concentrations, calneurons relinquish PI4KIIIβ to NCS-1, possibly due to unfolding of calneurons upon Ca^{2+} binding. Calneuron-1 affects secretory traffic in a manner consistent with its effects on PI4P levels because in primary cortical neurons, RNA interference (RNAi) mediated knockdown of

calneuron-1 increases Golgi to axonal PM trafficking of the synaptic vesicle marker mCherry-Synaptophysin in fluorescence recovery after photobleaching experiments. Overexpression of Calneuron-1 has the opposite effect. Interestingly, the related protein Caldendron has no effect on PI4KIIIβ activity, indicating that the enzyme is regulated by a specific set of calcium sensors.

In *Arabidopsis*, interaction between the Pik1 homologue and calcium sensors plays a role in root hair tip development. Members of the calcineurin B-like (AtCBL) family of proteins detect changes in intracellular calcium and their expression is either induced or repressed in response to various environmental stresses to regulate downstream gene expression (Albrecht et al., 2003; Cheong et al., 2003). In addition, a tip-focused calcium gradient is essential for growth of root hair cells and pollen tubes (Li et al., 1999; Akimana et al., 2009; Cardenas, 2009), processes that depend on polarized membrane trafficking of Golgi-derived vesicles (Samaj et al., 2006). These two requirements for root hair tip growth were integrated with the discovery that AtCBL1 binds to an amino-terminal domain of AtPI4KIIIβ1 (Preuss et al., 2006). Abolishing the calcium gradient with an ionophore led to inhibition of tip growth and dispersal of tip-localized RabA4b/Rab11-positive post-Golgi compartments. PI4KIIIβ1 colocalizes with and physically binds EYFP-RabA4b, similar to their mammalian counterparts. *PI4KIIIβ1/β2* double mutant plants have short and aberrant root hairs and fewer distinct TGN budding profiles visualized by transmission electron microscopy. Thus, a model emerges whereby the tip-focused calcium gradient, via AtCBL1, directs PI4KIIIβ1 to sites of membrane growth, where it produces PI4P required for directed post-Golgi trafficking.

Extracellular PI4P

Curiously, in plants, treatment with fungal xylanase, a potent activator of plant defenses, induces

a rise in extracellular PI4P in tomato cell suspensions, which is then responsible for an intracellular burst of reactive oxygen species (Gonorazky et al., 2008). Oxidative burst is an early response to pathogen recognition and is thwarted with a PLC inhibitor (Gonorazky et al., 2010). Uptake of this extracellular PI4P was observed, although instead of conversion to PIP_2, metabolism to PI was detected, leading the authors to suggest that PLC may hydrolyze PI4P in this system. Extracellular PI4P, along with several other phospholipids, was also found in the intercellular space of tomato plants under basal conditions (Gonorazky et al., 2012). Microarray analysis showed that application of extracellular PI4P to *Arabidopsis* induced expression of genes required for environmental defense responses (Alvarez-Venegas et al., 2006), together suggesting that PI4P may function in cell-to-cell communication in plants under both normal and stress conditions.

PI4P in Health and Disease

Phosphoinositide regulation is involved in numerous aspects of human health and disease (McCrea and De Camilli, 2009; Skwarek and Boulianne, 2009). *PI4KIIIα* is situated at chromosomal 22q11.2, deletion of which has been associated with higher susceptibility to psychiatric conditions such as bipolar disorder, autism, and schizophrenia (Clayton et al., 2013). Reduced PI4KIIα activity has been correlated with Alzheimer's disease (Zubenko et al., 1999; Wu et al., 2004). Various PI4Ks are upregulated in polycystic kidney disease (Cuozzo et al., 2002), malignant melanoma, breast ductal carcinoma, pancreatic cancer and others (Waugh, 2012). Also, the PI4P effector GOLPH3 is an oncogene (Scott et al., 2009; Kunigou et al., 2011; Li et al., 2011; Zeng et al., 2012) associated with poor clinical outcome (Hua et al., 2012; Li et al., 2012a; Wang et al., 2012; Hu et al., 2013). Thus, an optimal balance of PI4K activity is of

great physiological importance.

PI4P regulation and membrane trafficking are key targets in pathogen invasion. PI4P metabolism is subverted during bacterial infections. Type II PI4Ks and AP-1 are required for *Listeria monocytogenes* phagocytosis, and PI4P is found at the entry site in HeLa cells (Pizarro-Cerda et al., 2007). Formation of intracellular vacuolar replication complexes by various *Chlamydia* species requires PI4KIIα and Arf1, which are detected on the vacuole along with GFP-OSBP-PH (Moorhead et al., 2010). Upon infection, *Legionella pneumophila* establishes a replication vacuole, the *Legionella*-containing vacuole (LCV), and avoids fusion with lysosomes by intercepting and fusing with ER-derived vesicles, disguising the LCV with host markers. The *Legionella* proteins SidC and DrrA/SidM are released into the host cytoplasm via the type IV Icm/Dot secretion system. Once cytoplasmic, these proteins bind PI4KIIIβ-dependent PI4P on the LCV (Brombacher et al., 2009). The Rab1 GEF domain of DrrA and the N-terminal region of SidC are then able to misdirect ER vesicles en route to the Golgi by binding to Rab1 and calnexin, respectively (Ragaz et al., 2008; Brombacher et al., 2009).

Cellular PI4Ks and PI4P are also co-opted by positive-sense RNA viruses, including hepatitis C virus (HCV), coxsackievirus, and poliovirus (Alvisi et al., 2011; Altan-Bonnet and Balla, 2012; Bishe et al., 2012a; Delang et al., 2012). These viruses induce formation of PI4P-enriched 'membranous webs' derived of ER and other cellular membranes, which serve as platforms for viral replication. In genome-wide and targeted siRNA screens, PI4KIIIα was identified as a host factor required for HCV replication and membranous web formation (Berger et al., 2009; Borawski et al., 2009; Vaillancourt et al., 2009; Reiss et al., 2011). The HCV nonstructural protein 5a (NS5A) interacts with and stimulates PI4KIIIα activity at replication sites (Berger et al., 2011; Lim and Hwang, 2011; Reiss et al., 2011). Knockdown of *PI4KIIIα*

abrogates replication and leads to clusters of NS5A instead of the reticular distribution seen during competent infections.

Involvement of PI4KIIIβ in HCV replication is debated, with some groups reporting that it is required (Borawski et al., 2009; Zhang et al., 2012b) and others that it is not (Arita et al., 2011; Berger et al., 2011). This inconsistency may be due to the study of distinct HCV genotypes and the use of different assays in each case (Bishe et al., 2012a). PI4KIIIβ may be hijacked at other time-points in the virus life cycle (Tai and Salloum, 2011). Assembled and infectious viral particles are thought to be released via the very low density lipoprotein (VLDL) secretion pathway, and HCV transit through the Golgi, tightly coupled with Apolipoprotein E, has been visualized in live cells (Coller et al., 2012). Knockdown of *PI4KIIIβ*, *Rab11a*, *GOLPH3*, or *MYO18A* leads to retention of HCV particles in the cell, as does expression of Golgi-targeted Sac1, suggesting that Golgi PI4P and its effectors are required for virus secretion (Bishe et al., 2012b; Coller et al., 2012). It has also been suggested that PI4KIIIβ is required for clathrin-mediated endocytosis of some HCV strains, although PI4KIIIα was similarly implicated in this process (Trotard et al., 2009). Compounds that inhibit PI4KIIIα and HCV web formation show promise as potential therapeutics (Bianco et al., 2012; Vaillancourt et al., 2012), as do those targeting PI4KIIIβ to halt poliovirus and coxsackievirus replication (Hsu et al., 2010; van der Schaar et al., 2012). However, viruses were still able to evolve resistance to compounds targeting these host factors (Arita et al., 2011; Vaillancourt et al., 2012; van der Schaar et al., 2012), which remains a current challenge in deploying PI4K inhibitors as antiviral therapeutic agents.

Concluding Remarks and Future Perspectives

The wealth of new information regarding the versatile roles of PI4P has brought us a long way

from the notion that this lipid functions mainly as a precursor to higher phosphorylated inositides. Certainly, PI4P conversion to PIP_2 is essential for PLC signaling, and likely also for PIP_3 signaling; however, the bona fide independent roles for PI4P are more numerous than previously suspected, involving many aspects of membrane trafficking, including budding, tubulation, scission, linkage to motors, docking, fusing, sorting, and establishing membrane identity. These discoveries bring new questions, and the next wave of research will focus on regulators that govern specific PI4P-dependent processes. Furthermore, whereas many of the cellular roles for PI4P have been identified at the ER and Golgi, independent roles for PI4P on other membranes, such as endosomes, lysosomes and the PM, are emerging.

A broad but important question is what determines functional specificity of PI4P pools derived from different PI4Ks at the same organelle. Aside from the catalytic commonality of their kinase domains, the N-termini of PI4K isoforms are unique, and a non-overlapping set of PI4K binding partners may preclude compensation by other isoforms. Unique partnerships with Rabs present ways to turn PI4P signaling on or off, as do interactions with activators that are mutually exclusive and jointly inhibiting, as in the case of Arf1, NCS-1, and PI4KIIIβ. In addition, because recent developments illustrate the interdependencies of phosphoinositide and sphingolipid metabolism, PI4K isoform-specific control of cholesterol and sphingolipid composition may be relevant in feedback mechanisms defining compartments for PI4K-specific signaling events and regulation of PI4K activity. Uncovering the full web of proteomic and lipid interactions for each PI4K will help elucidate isoform-specific mechanisms and provide insight for the development and treatment of diseases resulting from their dysfunction.

Future studies on PI4P regulation in animal systems will be valuable to this end, as roles for PIP enzymes revealed by RNAi approaches in tissue culture do not always predict the

phenotypes revealed by knockout in cells or organisms. The phenotypes can be either milder or stronger than predicted. For example, whereas essential trafficking roles at the TGN were reported for PI4KIIα in cultured cells, *PI4KIIα* mutant mice are born healthy and survive without obvious phenotypes prior to developing late-onset neurodegeneration. In contrast, a crucial role for *PI4KIIIα* in PM integrity and steady state PIP_2 (similar to what was observed in *PI4KIIIα* mutant flies) was discovered using a knockout approach in the MEF cell line, after treatment of other cell lines with *PI4KIIIα* siRNA had little effect. The observed differences are likely due to off-target effects or incomplete knockdown associated with siRNA. Studies in whole organisms will be of critical importance as new roles for PI4P and PI4Ks in human diseases continue to be revealed, and as drug candidates targeting these pathways need to be validated in physiologically relevant contexts.

Although PI4P regulation is known to affect many signaling pathways, including Wnt, FGF, EGF, Hh, and Hippo signaling, we are only beginning to uncover mechanisms governing crosstalk and regulation. Recent discoveries of dynamic regulation of PI4P levels, including frequent transient contacts of PI4KIIIα with the PM, hint that this system may be more complicated than expected. Future research focusing on intersection of pathways regulating PI4P will lead to greater mechanistic understanding of a multitude of specific cellular processes, as well as a greater understanding of the molecular role for PI4P in cell homeostasis and organismal health.

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References

Akhtar RA, Abdel-Latif AA. (1984). Carbachol causes rapid phosphodiesteratic cleavage of phosphatidylinositol 4,5-bisphosphate and accumulation of inositol phosphates in rabbit iris smooth muscle; prazosin inhibits noradrenaline- and ionophore A23187-stimulated accumulation of inositol phosphates. Biochem J, 224, 291-300.

Akimana C, Al-Khodor S, Kwaik YA. (2009). Host factors required for modulation of phagosome biogenesis and proliferation of Francisella tularensis within the cytosol. PloS One, 11, e11025.

Albrecht V, Weinl S, Blazevic D, D'Angelo C, Batistic O, Kolukisaoglu U, Bock R, Schulz B, Harter K, Kudla J. (2003). The calcium sensor CBL1 integrates plant responses to abiotic stresses. The Plant Journal, 36, 457-70.

Alfaro G, Johansen J, Dighe SA, Duamel G, Kozminski KG, Beh CT. (2011). The sterol-binding protein Kes1/Osh4p is a regulator of polarized exocytosis. Traffic, 12, 1521-36.

Altan-Bonnet N, Balla T. (2012). Phosphatidylinositol 4-kinases: hostages harnessed to build panviral replication platforms. Trends Biochem Sci, 37, 293-302.

Alvarez-Venegas R, Xia Y, Lu G, Avramova Z. (2006). Phosphoinositide 5-phosphate and phosphoinositide 4-phosphate trigger distinct specific responses of Arabidopsis genes: genome-wide expression analyses. Plant Signal Behav, 1, 140-51.

Alvisi G, Madan V, Bartenschlager R. (2011). Hepatitis C virus and host cell lipids: an intimate connection. RNA Biol, 8, 258-69.

Ames JB, Hendricks KB, Strahl T, Huttner IG, Hamasaki N, Thorner J. (2000). Structure and calcium-binding properties of Frq1, a novel calcium sensor in the yeast Saccharomyces cerevisiae. Biochemistry, 39, 12149-61.

Ames JB, Ishima R, Tanaka T, Gordon JI, Stryer L, Ikura M. (1997). Molecular mechanics of calcium-myristoyl switches. Nature, 389, 198-202.

Ames JB, Lim S. (2012). Molecular structure and target recognition of neuronal calcium sensor proteins. Biochim Biophys Acta, 1820, 1205-13.

An SW, Cha SK, Yoon J, Chang S, Ross EM, Huang CL. (2011). WNK1 promotes PIP(2) synthesis to coordinate growth factor and GPCR-Gq signaling. Curr Biol, 21, 1979-87.

Arita M, Kojima H, Nagano T, Okabe T, Wakita T, Shimizu H. (2011). Phosphatidylinositol 4-kinase III beta is a target of enviroxime-like compounds for antipoliovirus activity. J Virol, 85, 2364-72.

Audhya A, Emr SD. (2002). Stt4 PI 4-kinase localizes to the plasma membrane and functions in the Pkc1-mediated MAP kinase cascade. Dev Cell, 2, 593-605.

Audhya A, Emr SD. (2003). Regulation of PI4,5P2 synthesis by nuclear-cytoplasmic shuttling of the Mss4 lipid kinase. EMBO J, 22, 4223-36.

Audhya A, Foti M, Emr SD. (2000). Distinct roles for the yeast phosphatidylinositol 4-kinases, Stt4p and Pik1p, in secretion, cell growth, and organelle membrane dynamics. Mol Biol Cell, 11, 2673-89.

Baird D, Stefan C, Audhya A, Weys S, Emr SD. (2008). Assembly of the PtdIns 4-kinase Stt4 complex at the plasma membrane requires Ypp1 and Efr3. J Cell Biol, 183, 1061-74.

Balla A, Balla T. (2006). Phosphatidylinositol 4-kinases: old enzymes with emerging functions. Trends Cell Biol, 16, 351-61.

Balla A, Kim YJ, Varnai P, Szentpetery Z, Knight Z, Shokat KM, Balla T. (2008). Maintenance of hormone-sensitive phosphoinositide pools in the plasma membrane requires phosphatidylinositol 4-kinase IIIalpha. Mol Biol Cell, 19, 711-21.

Balla A, Tuymetova G, Barshishat M, Geiszt M, Balla T. (2002). Characterization of type II phosphatidylinositol 4-kinase isoforms reveals association of the enzymes with endosomal vesicular compartments. J Biol Chem, 277, 20041-50.

Balla A, Tuymetova G, Tsiomenko A, Varnai P, Balla T. (2005). A plasma membrane pool of phosphatidylinositol 4-phosphate is generated by phosphatidylinositol 4-kinase type-III alpha: studies with the PH domains of the oxysterol binding protein and FAPP1. Mol Biol Cell, 16, 1282-95.

Balla A, Vereb G, Gulkan H, Gehrmann T, Gergely P, Heilmeyer LM, Jr., Antal M. (2000). Immunohistochemical localisation of two phosphatidylinositol 4-kinase isoforms, PI4K230 and PI4K92, in the central nervous system of rats. Exp Brain Res, 134, 279-88.

Banerji S, Ngo M, Lane CF, Robinson CA, Minogue S, Ridgway ND. (2010). Oxysterol binding protein-dependent activation of sphingomyelin synthesis in the golgi apparatus requires phosphatidylinositol 4-kinase IIalpha. Mol Biol Cell, 21, 4141-50.

Barlow CA, Laishram RS, Anderson RA. (2010). Nuclear phosphoinositides: a signaling enigma wrapped in a compartmental conundrum. Trends Cell Biol, 20, 25-35.

Barylko B, Gerber SH, Binns DD, Grichine N, Khvotchev M, Sudhof TC, Albanesi JP. (2001). A novel family of phosphatidylinositol 4-kinases conserved from yeast to humans. J Biol Chem, 276, 7705-8.

Behar-Bannelier M, Murray RK. (1980). An electrophoretic study of endogenous phosphorylation in vitro of the polypeptides of microsomal membrane fractions of mouse liver. Biochem J, 187, 147-56.

Berger KL, Cooper JD, Heaton NS, Yoon R, Oakland TE, Jordan TX, Mateu G, Grakoui A, Randall G. (2009). Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. Proc Natl Acad Sci U S A, 106, 7577-82.

Berger KL, Kelly SM, Jordan TX, Tartell MA, Randall G. (2011). Hepatitis C virus stimulates the phosphatidylinositol 4-kinase III alpha-dependent phosphatidylinositol 4-phosphate production that is essential for its replication. J Virol, 85, 8870-83.

Berridge MJ. (1983). Rapid accumulation of inositol trisphosphate reveals that agonists hydrolyse polyphosphoinositides instead of phosphatidylinositol. Biochem J, 212, 849-58.

Bhattacharya N, J OD, Stagg SM. (2012). The structure of the Sec13/31 COPII cage bound to Sec23. J Mol Biol, 420, 324-34.

Bianco A, Reghellin V, Donnici L, Fenu S, Alvarez R, Baruffa C, Peri F, Pagani M, Abrignani S, Neddermann P, De Francesco R. (2012). Metabolism of phosphatidylinositol 4-kinase IIIalpha-dependent PI4P Is subverted by HCV and is targeted by a 4-anilino quinazoline with antiviral activity. PLoS Pathog, 8, e1002576.

Bishe B, Syed G, Siddiqui A. (2012a). Phosphoinositides in the hepatitis C virus life cycle. Viruses, 4, 2340-58.

Bishe B, Syed GH, Field SJ, Siddiqui A. (2012b). Role of phosphatidylinositol 4-phosphate (PI4P) and its binding protein GOLPH3 in hepatitis C virus secretion. J Biol Chem, 287, 27637-47.

Blagoveshchenskaya A, Cheong FY, Rohde HM, Glover G, Knodler A, Nicolson T, Boehmelt G, Mayinger P. (2008). Integration of Golgi trafficking and growth factor signaling by the lipid phosphatase SAC1. J Cell Biol, 180, 803-12.

Blumental-Perry A, Haney CJ, Weixel KM, Watkins SC, Weisz OA, Aridor M. (2006). Phosphatidylinositol 4-phosphate formation at ER exit sites regulates ER export. Dev Cell, 11, 671-82.

Boman AL, Salo PD, Hauglund MJ, Strand NL, Rensink SJ, Zhdankina O. (2002). ADP-ribosylation factor (ARF) interaction is not sufficient for yeast GGA protein function or localization. Mol Biol Cell, 13, 3078-95.

Bonazzi M, Spano S, Turacchio G, Cericola C, Valente C, Colanzi A, Kweon HS, Hsu VW, Polishchuck EV, Polishchuck RS, Sallese M, Pulvirenti T, Corda D, Luini A. (2005). CtBP3/BARS drives membrane fission in dynamin-independent transport pathways. Nat Cell Biol, 7, 570-80.

Bonekamp NA, Vormund K, Jacob R, Schrader M. (2010). Dynamin-like protein 1 at the Golgi complex: a novel component of the sorting/targeting machinery en route to the plasma membrane. Exp Cell Res, 316, 3454-67.

Borawski J, Troke P, Puyang X, Gibaja V, Zhao S, Mickanin C, Leighton-Davies J, Wilson CJ, Myer V, Cornellataracido I, Baryza J, Tallarico J, Joberty G, Bantscheff M, Schirle M, Bouwmeester T, Mathy JE, Lin K, Compton T, Labow M, Wiedmann B, Gaither LA. (2009). Class III phosphatidylinositol 4-kinase alpha and beta are novel host factor regulators of hepatitis C virus replication. J Virol, 83, 10058-74.

Boronenkov IV, Loijens JC, Umeda M, Anderson RA. (1998). Phosphoinositide signaling pathways in nuclei are associated with nuclear speckles containing pre-mRNA processing factors. Mol Biol Cell, 9, 3547-60.

Brill JA, Hime GR, Scharer-Schuksz M, Fuller MT. (2000). A phospholipid kinase regulates actin organization and intercellular bridge formation during germline cytokinesis. Development, 127, 3855-64.

Brombacher E, Urwyler S, Ragaz C, Weber SS, Kami K, Overduin M, Hilbi H. (2009). Rab1 guanine nucleotide exchange factor SidM is a major phosphatidylinositol 4-phosphate-binding effector protein of Legionella pneumophila. J Biol Chem, 284, 4846-56.

Burgess J, Del Bel LM, Ma C-I, Barylko B, Polevoy G, Rollins J, Albanesi JP, Kramer H, Brill JA. (2012). Type II phosphatidylinositol 4-kinase regulates trafficking of secretory granule proteins in Drosophila. Development, 139, 3040-50.

Campellone KG, Webb NJ, Znameroski EA, Welch MD. (2008). WHAMM is an Arp2/3 complex activator that binds microtubules and functions in ER to Golgi transport. Cell, 134, 148-61.

Campelo F, Malhotra V. (2012). Membrane fission: the biogenesis of transport carriers. Annu Rev Biochem, 81, 407-27.

Cao X, Coskun U, Rossle M, Buschhorn SB, Grzybek M, Dafforn TR, Lenoir M, Overduin M, Simons K. (2009). Golgi protein FAPP2 tubulates membranes. Proc Natl Acad Sci U S A, 106, 21121-5.

Cardenas L. (2009). New findings in the mechanisms regulating polar growth in root hair cells. Plant Signal Behav, 4, 4-8.

Carvou N, Holic R, Li M, Futter C, Skippen A, Cockcroft S. (2010). Phosphatidylinositol- and phosphatidylcholine-transfer activity of PITPbeta is essential for COPI-mediated retrograde transport from the Golgi to the endoplasmic reticulum. J Cell Sci, 123, 1262-73.

Chang FS, Han GS, Carman GM, Blumer KJ. (2005). A WASp-binding type II phosphatidylinositol 4-kinase required for actin polymerization-driven endosome motility. J Cell Biol, 171, 133-42.

Chang-Ileto B, Frere SG, Chan RB, Voronov SV, Roux A, Di Paolo G. (2011). Synaptojanin 1-mediated PI(4,5)P2 hydrolysis is modulated by membrane curvature and facilitates membrane fission. Dev Cell, 20, 206-18.

Cheng MK, Shearn A. (2004). The direct interaction between ASH2, a Drosophila trithorax group protein, and SKTL, a nuclear phosphatidylinositol 4-phosphate 5-kinase, implies a role for phosphatidylinositol 4,5-bisphosphate in maintaining transcriptionally active chromatin. Genetics, 167, 1213-23.

Cheong FY, Sharma V, Blagoveshchenskaya A, Oorschot VM, Brankatschk B, Klumperman J, Freeze HH, Mayinger P. (2010). Spatial regulation of Golgi phosphatidylinositol-4-phosphate is required for enzyme localization and glycosylation fidelity. Traffic, 11, 1180-90.

Cheong YH, Kim K-N, Pandey GK, Gupta R, Grant JJ, Luan S. (2003). CBL1, a calcium sensor that differentially regulates salt, drought, and cold responses in Arabidopsis. The Plant Cell, 15, 1833-45.

Chu KM, Minogue S, Hsuan JJ, Waugh MG. (2010). Differential effects of the phosphatidylinositol 4-kinases, PI4KIIalpha and PI4KIIIbeta, on Akt activation and apoptosis. Cell Death Dis, 1, e106.

Clark J, Anderson KE, Juvin V, Smith TS, Karpe F, Wakelam MJ, Stephens LR, Hawkins PT. (2011). Quantification of PtdInsP3 molecular species in cells and tissues by mass spectrometry. Nat Methods, 8, 267-72.

Clayton EL, Minogue S, Waugh MG. (2013). Phosphatidylinositol 4-kinases and PI4P metabolism in the nervous system: roles in psychiatric and neurological diseases. Mol Neurobiol, 47, 361-72.

Coller KE, Heaton NS, Berger KL, Cooper JD, Saunders JL, Randall G. (2012). Molecular determinants and dynamics of hepatitis C virus secretion. PLoS Pathog, 8, e1002466.

Collins CA, Wells WW. (1983). Identification of phosphatidylinositol kinase in rat liver lysosomal membranes. J Biol Chem, 258, 2130-4.

Cooper PH, Hawthorne JN. (1976). Phosphatidylinositol kinase and diphosphoinositide kinase of rat kidney cortex: properties and subcellular localization. Biochem J, 160, 97-105.

Corda D, Colanzi A, Luini A. (2006). The multiple activities of CtBP/BARS proteins: the Golgi view. Trends Cell Biol, 16, 167-73.

Cox JA, Durussel I, Comte M, Nef S, Nef P, Lenz SE, Gundelfinger ED. (1994). Cation binding and conformational changes in VILIP and NCS-1, two neuron-specific calcium-binding proteins. J Biol Chem, 269, 32807-13.

Craige B, Salazar G, Faundez V. (2008). Phosphatidylinositol-4-kinase type II alpha contains an AP-3-sorting motif and a kinase domain that are both required for endosome traffic. Mol Biol Cell, 19, 1415-26.

Creba JA, Downes CP, Hawkins PT, Brewster G, Michell RH, Kirk CJ. (1983). Rapid breakdown of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate in rat hepatocytes stimulated by vasopressin and other Ca2+-mobilizing hormones. Biochem J, 212, 733-47.

Cuozzo FP, Mishra S, Jiang J, Aukema HM. (2002). Overexpression of kidney phosphatidylinositol 4-kinasebeta and phospholipase C(gamma1) proteins in two rodent models of polycystic kidney disease. Biochim Biophys Acta, 1587, 99-106.

D'Angelo G, Vicinanza M, Di Campli A, De Matteis MA. (2008). The multiple roles of PtdIns(4)P -- not just the precursor of PtdIns(4,5)P2. J Cell Sci, 121, 1955-63.

Daboussi L, Costaguta G, Payne GS. (2012). Phosphoinositide-mediated clathrin adaptor progression at the trans-Golgi network. Nat Cell Biol, 14, 239-48.

de Barry J, Janoshazi A, Dupont JL, Procksch O, Chasserot-Golaz S, Jeromin A, Vitale N. (2006). Functional implication of neuronal calcium sensor-1 and phosphoinositol 4-kinase-beta interaction in regulated exocytosis of PC12 cells. J Biol Chem, 281, 18098-111.

de Graaf P, Klapisz EE, Schulz TK, Cremers AF, Verkleij AJ, van Bergen en Henegouwen PM. (2002). Nuclear localization of phosphatidylinositol 4-kinase beta. J Cell Sci, 115, 1769-75.

de Graaf P, Zwart WT, van Dijken RA, Deneka M, Schulz TK, Geijsen N, Coffer PJ, Gadella BM, Verkleij AJ, van der Sluijs P, van Bergen en Henegouwen PM. (2004). Phosphatidylinositol 4-kinasebeta is critical for functional association of rab11 with the Golgi complex. Mol Biol Cell, 15, 2038-47.

De Matteis MA, Di Campli A, D'Angelo G. (2007). Lipid-transfer proteins in membrane trafficking at the Golgi complex. Biochim Biophys Acta, 1771, 761-8.

De Matteis MA, Luini A. (2008). Exiting the Golgi complex. Nat Rev Mol Cell Biol, 9, 273-84.

de Saint-Jean M, Delfosse V, Douguet D, Chicanne G, Payrastre B, Bourguet W, Antonny B, Drin G. (2011). Osh4p exchanges sterols for phosphatidylinositol 4-phosphate between lipid bilayers. J Cell Biol, 195, 965-78.

Delage E, Puyaubert J, Zachowski A, Ruelland E. (2012a). Signal transduction pathways involving phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate: Convergences and divergences among eukaryotic kingdoms. Prog Lipid Res, 52, 1-14.

Delage E, Ruelland E, Guillas I, Zachowski A, Puyaubert J. (2012b). Arabidopsis type-III phosphatidylinositol 4-kinases beta1 and beta2 are upstream of the phospholipase C pathway triggered by cold exposure. Plant Cell Physiol, 53, 565-76.

Delang L, Paeshuyse J, Neyts J. (2012). The role of phosphatidylinositol 4-kinases and phosphatidylinositol 4-phosphate during viral replication. Biochem Pharmacol, 84, 1400-8.

Demmel L, Beck M, Klose C, Schlaitz AL, Gloor Y, Hsu PP, Havlis J, Shevchenko A, Krause E, Kalaidzidis Y, Walch-Solimena C. (2008). Nucleocytoplasmic shuttling of the Golgi phosphatidylinositol 4-kinase Pik1 is regulated by 14-3-3 proteins and coordinates Golgi function with cell growth. Mol Biol Cell, 19, 1046-61.

Deng Y, Golinelli-Cohen MP, Smirnova E, Jackson CL. (2009). A COPI coat subunit interacts directly with an early-Golgi localized Arf exchange factor. EMBO Rep, 10, 58-64.

Dippold HC, Ng MM, Farber-Katz SE, Lee SK, Kerr ML, Peterman MC, Sim R, Wiharto PA, Galbraith KA, Madhavarapu S, Fuchs GJ, Meerloo T, Farquhar MG, Zhou H, Field SJ. (2009).

GOLPH3 bridges phosphatidylinositol-4- phosphate and actomyosin to stretch and shape the Golgi to promote budding. Cell, 139, 337-51.

Du X, Kumar J, Ferguson C, Schulz TA, Ong YS, Hong W, Prinz WA, Parton RG, Brown AJ, Yang H. (2011). A role for oxysterol-binding protein-related protein 5 in endosomal cholesterol trafficking. J Cell Biol, 192, 121-35.

Dumaresq-Doiron K, Savard MF, Akam S, Costantino S, Lefrancois S. (2010). The phosphatidylinositol 4-kinase PI4KIIIalpha is required for the recruitment of GBF1 to Golgi membranes. J Cell Sci, 123, 2273-80.

Dunlop ME, Larkins RG. (1986). Muscarinic-agonist and guanine nucleotide activation of polyphosphoinositide phosphodiesterase in isolated islet-cell membranes. Biochem J, 240, 731-7.

Emr S, Glick BS, Linstedt AD, Lippincott-Schwartz J, Luini A, Malhotra V, Marsh BJ, Nakano A, Pfeffer SR, Rabouille C, Rothman JE, Warren G, Wieland FT. (2009). Journeys through the Golgi--taking stock in a new era. J Cell Biol, 187, 449-53.

Endemann GC, Graziani A, Cantley LC. (1991). A monoclonal antibody distinguishes two types of phosphatidylinositol 4-kinase. Biochem J, 273(Pt 1), 63-6.

English AR, Voeltz GK. (2013). Rab10 GTPase regulates ER dynamics and morphology. Nat Cell Biol, 15, 169-78.

Fairn GD, Curwin AJ, Stefan CJ, McMaster CR. (2007). The oxysterol binding protein Kes1p regulates Golgi apparatus phosphatidylinositol-4-phosphate function. Proc Natl Acad Sci U S A, 104, 15352-7.

Falkenburger BH, Dickson EJ, Hille B. (2013). Quantitative properties and receptor reserve of the DAG and PKC branch of G(q)-coupled receptor signaling. J Gen Physiol, 141, 537-55.

Falkenburger BH, Jensen JB, Hille B. (2010). Kinetics of PIP2 metabolism and KCNQ2/3 channel regulation studied with a voltage-sensitive phosphatase in living cells. J Gen Physiol, 135, 99-114.

Farhan H, Weiss M, Tani K, Kaufman RJ, Hauri HP. (2008). Adaptation of endoplasmic reticulum exit sites to acute and chronic increases in cargo load. EMBO J, 27, 2043-54.

Faulhammer F, Kanjilal-Kolar S, Knodler A, Lo J, Lee Y, Konrad G, Mayinger P. (2007). Growth control of Golgi phosphoinositides by reciprocal localization of sac1 lipid phosphatase and pik1 4-kinase. Traffic, 8, 1554-67.

Faulhammer F, Konrad G, Brankatschk B, Tahirovic S, Knodler A, Mayinger P. (2005). Cell growth-dependent coordination of lipid signaling and glycosylation is mediated by interactions between Sac1p and Dpm1p. J Cell Biol, 168, 185-91.

Fili N, Calleja V, Woscholski R, Parker PJ, Larijani B. (2006). Compartmental signal modulation: Endosomal phosphatidylinositol 3-phosphate controls endosome morphology and selective cargo sorting. Proc Natl Acad Sci U S A, 103, 15473-8.

Fiume R, Keune WJ, Faenza I, Bultsma Y, Ramazzotti G, Jones DR, Martelli AM, Somner L, Follo MY, Divecha N, Cocco L. (2012). Nuclear phosphoinositides: location, regulation and function. Subcell Biochem, 59, 335-61.

Flanagan CA, Schnieders EA, Emerick AW, Kunisawa R, Admon A, Thorner J. (1993). Phosphatidylinositol 4-kinase: gene structure and requirement for yeast cell viability. Science, 262, 1444-8.

Forrest S, Chai A, Sanhueza M, Marescotti M, Parry K, Georgiev A, Sahota V, Mendez-Castro R, Pennetta G. (2013). Increased levels of phosphoinositides cause neurodegeneration in a Drosophila model of amyotrophic lateral sclerosis. Hum Mol Genet.

Foti M, Audhya A, Emr SD. (2001). Sac1 lipid phosphatase and Stt4 phosphatidylinositol 4-kinase regulate a pool of phosphatidylinositol 4-phosphate that functions in the control of the actin cytoskeleton and vacuole morphology. Mol Biol Cell, 12, 2396-411.

Futter CE, Pearse A, Hewlett LJ, Hopkins CR. (1996). Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes. J Cell Biol, 132, 1011-23.

Garcia-Bustos JF, Marini F, Stevenson I, Frei C, Hall MN. (1994). PIK1, an essential phosphatidylinositol 4-kinase associated with the yeast nucleus. EMBO J, 13, 2352-61.

Garrenton LS, Stefan CJ, McMurray MA, Emr SD, Thorner J. (2010). Pheromone-induced anisotropy in yeast plasma membrane phosphatidylinositol-4,5-bisphosphate distribution is required for MAPK signaling. Proceedings of the National Academy of Sciences of the United States of America, 107, 11805-10.

Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G. (2002). Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature, 415, 141-7.

Gaynor EC, Graham TR, Emr SD. (1998). COPI in ER/Golgi and intra-Golgi transport: do yeast COPI mutants point the way? Biochim Biophys Acta, 1404, 33-51.

Godi A, Di Campli A, Konstantakopoulos A, Di Tullio G, Alessi DR, Kular GS, Daniele T, Marra P, Lucocq JM, De Matteis MA. (2004). FAPPs control Golgi-to-cell-surface membrane traffic by binding to ARF and PtdIns(4)P. Nat Cell Biol, 6, 393-404.

Godi A, Pertile P, Meyers R, Marra P, Di Tullio G, Iurisci C, Luini A, Corda D, De Matteis MA. (1999). ARF mediates recruitment of PtdIns-4-OH kinase-beta and stimulates synthesis of PtdIns(4,5)P2 on the Golgi complex. Nat Cell Biol, 1, 280-7.

Gonorazky G, Laxalt AM, de la Canal L. (2010). Involvement of phospholipase C in the responses triggered by extracellular phosphatidylinositol 4-phosphate. J Plant Physiol, 167, 411-5.

Gonorazky G, Laxalt AM, Dekker HL, Rep M, Munnik T, Testerink C, de la Canal L. (2012). Phosphatidylinositol 4-phosphate is associated to extracellular lipoproteic fractions and is detected in tomato apoplastic fluids. Plant Biol (Stuttg), 14, 41-9.

Gonorazky G, Laxalt AM, Testerink C, Munnik T, de la Canal L. (2008). Phosphatidylinositol 4-phosphate accumulates extracellularly upon xylanase treatment in tomato cell suspensions. Plant Cell Environ, 31, 1051-62.

Graham TR, Burd CG. (2011). Coordination of Golgi functions by phosphatidylinositol 4-kinases. Trends Cell Biol, 21, 113-21.

Gromada J, Bark C, Smidt K, Efanov AM, Janson J, Mandic SA, Webb DL, Zhang W, Meister B, Jeromin A, Berggren PO. (2005). Neuronal calcium sensor-1 potentiates glucose-dependent exocytosis in pancreatic beta cells through activation of phosphatidylinositol 4-kinase beta. Proc Natl Acad Sci U S A, 102, 10303-8.

Guo J, Wenk MR, Pellegrini L, Onofri F, Benfenati F, De Camilli P. (2003). Phosphatidylinositol 4-kinase type IIalpha is responsible for the phosphatidylinositol 4-kinase activity associated with synaptic vesicles. Proc Natl Acad Sci U S A, 100, 3995-4000.

Haga Y, Miwa N, Jahangeer S, Okada T, Nakamura S. (2009). CtBP1/BARS is an activator of phospholipase D1 necessary for agonist-induced macropinocytosis. EMBO J, 28, 1197-207.

Hammond GRV, Fischer MJ, Anderson KE, Holdich J, Koteci A, Balla T, Irvine RF. (2012). PI4P and PI(4,5)P2 are essential but independent lipid determinants of membrane identity. Science, 337, 727-30.

Hammond GRV, Schiavo G, Irvine RF. (2009). Immunocytochemical techniques reveal multiple, distinct cellular pools of PtdIns4P and PtdIns(4,5)P2. Biochemical Journal, 422, 23-35.

Han GS, Audhya A, Markley DJ, Emr SD, Carman GM. (2002). The Saccharomyces cerevisiae LSB6 gene encodes phosphatidylinositol 4-kinase activity. J Biol Chem, 277, 47709-18.

Harwood JL, Hawthorne JN. (1969). The properties and subcellular distribution of phosphatidylinositol kinase in mammalian tissues. Biochim Biophys Acta, 171, 75-88.

Hausser A, Link G, Hoene M, Russo C, Selchow O, Pfizenmaier K. (2006). Phospho-specific binding of 14-3-3 proteins to phosphatidylinositol 4-kinase III beta protects from dephosphorylation and stabilizes lipid kinase activity. J Cell Sci, 119, 3613-21.

Haynes LP, Thomas GM, Burgoyne RD. (2005). Interaction of neuronal calcium sensor-1 and ADP-ribosylation factor 1 allows bidirectional control of phosphatidylinositol 4-kinase beta and trans-Golgi network-plasma membrane traffic. J Biol Chem, 280, 6047-54.

He B, Guo W. (2009). The exocyst complex in polarized exocytosis. Curr Opin Cell Biol, 21, 537-42.

Heldwein EE, Macia E, Wang J, Yin HL, Kirchhausen T, Harrison SC. (2004). Crystal structure of the clathrin adaptor protein 1 core. Proc Natl Acad Sci U S A, 101, 14108-13.

Hendricks KB, Wang BQ, Schnieders EA, Thorner J. (1999). Yeast homologue of neuronal frequenin is a regulator of phosphatidylinositol-4-OH kinase. Nat Cell Biol, 1, 234-41.

Heo WD, Inoue T, Park WS, Kim ML, Park BO, Wandless TJ, Meyer T. (2006). PI(3,4,5)P3 and PI(4,5)P2 lipids target proteins with polybasic clusters to the plasma membrane. Science, 314, 1458-61.

Hirst J, Miller SE, Taylor MJ, von Mollard GF, Robinson MS. (2004). EpsinR is an adaptor for the SNARE protein Vti1b. Mol Biol Cell, 15, 5593-602.

Hirst J, Motley A, Harasaki K, Peak Chew SY, Robinson MS. (2003). EpsinR: an ENTH domain-containing protein that interacts with AP-1. Mol Biol Cell, 14, 625-41.

Hokin LE, Hokin MR. (1955). Effects of acetylcholine on phosphate turnover in phospholipides of brain cortex in vitro. Biochim Biophys Acta, 16, 229-37.

Hokin LE, Hokin MR. (1958). Acetylcholine and the exchange of inositol and phosphate in brain phosphoinositide. J Biol Chem, 233, 818-21.

Hsu NY, Ilnytska O, Belov G, Santiana M, Chen YH, Takvorian PM, Pau C, van der Schaar H, Kaushik-Basu N, Balla T, Cameron CE, Ehrenfeld E, van Kuppeveld FJ, Altan-Bonnet N. (2010). Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. Cell, 141, 799-811.

Hu BS, Hu H, Zhu CY, Gu YL, Li JP. (2013). Overexpression of GOLPH3 is associated with poor clinical outcome in gastric cancer. Tumour Biol, 34, 515-20.

Hua X, Yu L, Pan W, Huang X, Liao Z, Xian Q, Fang L, Shen H. (2012). Increased expression of Golgi phosphoprotein-3 is associated with tumor aggressiveness and poor prognosis of prostate cancer. Diagn Pathol, 7, 127.

Huttner IG, Strahl T, Osawa M, King DS, Ames JB, Thorner J. (2003). Molecular interactions of yeast frequenin (Frq1) with the phosphatidylinositol 4-kinase isoform, Pik1. J Biol Chem, 278, 4862-74.

Jacquot A, Montigny C, Hennrich H, Barry R, le Maire M, Jaxel C, Holthuis J, Champeil P, Lenoir G. (2012). Phosphatidylserine stimulation of Drs2p.Cdc50p lipid translocase

dephosphorylation is controlled by phosphatidylinositol-4-phosphate. J Biol Chem, 287, 13249-61.

Jean S, Kiger AA. (2012). Coordination between RAB GTPase and phosphoinositide regulation and functions. Nat Rev Mol Cell Biol, 13, 463-70.

Jesch SA, Gaspar ML, Stefan CJ, Aregullin MA, Henry SA. (2010). Interruption of inositol sphingolipid synthesis triggers Stt4p-dependent protein kinase C signaling. J Biol Chem, 285, 41947-60.

Jia D, Gomez TS, Metlagel Z, Umetani J, Otwinowski Z, Rosen MK, Billadeau DD. (2010). WASH and WAVE actin regulators of the Wiskott-Aldrich syndrome protein (WASP) family are controlled by analogous structurally related complexes. Proc Natl Acad Sci U S A, 107, 10442-7.

Jovic M, Kean MJ, Szentpetery Z, Polevoy G, Gingras AC, Brill JA, Balla T. (2012). Two phosphatidylinositol 4-kinases control lysosomal delivery of the Gaucher disease enzyme, beta-glucocerebrosidase. Mol Biol Cell, 23, 1533-45.

Jovic M, Kieken F, Naslavsky N, Sorgen PL, Caplan S. (2009). Eps15 homology domain 1-associated tubules contain phosphatidylinositol-4-phosphate and phosphatidylinositol-(4,5)-bisphosphate and are required for efficient recycling. Mol Biol Cell, 20, 2731-43.

Jung G, Barylko B, Lu D, Shu H, Yin H, Albanesi JP. (2011). Stabilization of phosphatidylinositol 4-kinase type IIbeta by interaction with Hsp90. J Biol Chem, 286, 12775-84.

Jung G, Wang J, Wlodarski P, Barylko B, Binns DD, Shu H, Yin HL, Albanesi JP. (2008). Molecular determinants of activation and membrane targeting of phosphoinositol 4-kinase IIbeta. Biochem J, 409, 501-9.

Kakuk A, Friedlander E, Vereb G, Jr., Kasa A, Balla A, Balla T, Heilmeyer LM, Jr., Gergely P, Vereb G. (2006). Nucleolar localization of phosphatidylinositol 4-kinase PI4K230 in various mammalian cells. Cytometry A, 69, 1174-83.

Kapp-Barnea Y, Melnikov S, Shefler I, Jeromin A, Sagi-Eisenberg R. (2003). Neuronal calcium sensor-1 and phosphatidylinositol 4-kinase beta regulate IgE receptor-triggered exocytosis in cultured mast cells. J Immunol, 171, 5320-7.

Kauffmann-Zeh A, Klinger R, Endemann G, Waterfield MD, Wetzker R, Hsuan JJ. (1994). Regulation of human type II phosphatidylinositol kinase activity by epidermal growth factor-dependent phosphorylation and receptor association. J Biol Chem, 269, 31243-51.

Kim I, Pan W, Jones SA, Zhang Y, Zhuang X, Wu D. (2013). Clathrin and AP2 are required for PtdIns(4,5)P2-mediated formation of LRP6 signalosomes. J Cell Biol, 200, 419-28.

Kim K, Galletta BJ, Schmidt KO, Chang FS, Blumer KJ, Cooper JA. (2006). Actin-based motility during endocytosis in budding yeast. Mol Biol Cell, 17, 1354-63.

Kim YJ, Guzman-Hernandez ML, Balla T. (2011). A highly dynamic ER-derived phosphatidylinositol-synthesizing organelle supplies phosphoinositides to cellular membranes. Dev Cell, 21, 813-24.

Klemm RW, Ejsing CS, Surma MA, Kaiser HJ, Gerl MJ, Sampaio JL, de Robillard Q, Ferguson C, Proszynski TJ, Shevchenko A, Simons K. (2009). Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. J Cell Biol, 185, 601-12.

Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, Balla T, Weiss WA, Williams RL, Shokat KM. (2006). A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. Cell, 125, 733-47.

Kobuna H, Inoue T, Shibata M, Gengyo-Ando K, Yamamoto A, Mitani S, Arai H. (2010). Multivesicular body formation requires OSBP-related proteins and cholesterol. PLoS Genet, 6.

Koizumi S, Rosa P, Willars GB, Challiss RA, Taverna E, Francolini M, Bootman MD, Lipp P, Inoue K, Roder J, Jeromin A. (2002). Mechanisms underlying the neuronal calcium sensor-1-evoked enhancement of exocytosis in PC12 cells. J Biol Chem, 277, 30315-24.

Korzeniowski MK, Popovic MA, Szentpetery Z, Varnai P, Stojilkovic SS, Balla T. (2009). Dependence of STIM1/Orai1-mediated calcium entry on plasma membrane phosphoinositides. J Biol Chem, 284, 21027-35.

Kunigou O, Nagao H, Kawabata N, Ishidou Y, Nagano S, Maeda S, Komiya S, Setoguchi T. (2011). Role of GOLPH3 and GOLPH3L in the proliferation of human rhabdomyosarcoma. Oncol Rep, 26, 1337-42.

Lamb CA, McCann RK, Stockli J, James DE, Bryant NJ. (2010). Insulin-regulated trafficking of GLUT4 requires ubiquitination. Traffic, 11, 1445-54.

Lanzetti L. (2007). Actin in membrane trafficking. Curr Opin Cell Biol, 19, 453-8.

Larimore J, Tornieri K, Ryder PV, Gokhale A, Zlatic SA, Craige B, Lee JD, Talbot K, Pare JF, Smith Y, Faundez V. (2011). The schizophrenia susceptibility factor dysbindin and its associated complex sort cargoes from cell bodies to the synapse. Mol Biol Cell, 22, 4854-67.

LeBlanc MA, McMaster CR. (2010). Lipid binding requirements for oxysterol-binding protein Kes1 inhibition of autophagy and endosome-trans-Golgi trafficking pathways. J Biol Chem, 285, 33875-84.

Lee MC, Orci L, Hamamoto S, Futai E, Ravazzola M, Schekman R. (2005). Sar1p N-terminal helix initiates membrane curvature and completes the fission of a COPII vesicle. Cell, 122, 605-17.

Lefebvre YA, White DA, Hawthorne JN. (1976). Diphosphoinositide metabolism in bovine adrenal medulla. Can J Biochem, 54, 746-53.

Lefrancois S, McCormick PJ. (2007). The Arf GEF GBF1 is required for GGA recruitment to Golgi membranes. Traffic, 8, 1440-51.

Lemmon MA. (2008). Membrane recognition by phospholipid-binding domains. Nat Rev Mol Cell Biol, 9, 99-111.

Lenoir M, Coskun U, Grzybek M, Cao X, Buschhorn SB, James J, Simons K, Overduin M. (2010). Structural basis of wedging the Golgi membrane by FAPP pleckstrin homology domains. EMBO Rep, 11, 279-84.

Li H, Guo L, Chen SW, Zhao XH, Zhuang SM, Wang LP, Song LB, Song M. (2012a). GOLPH3 overexpression correlates with tumor progression and poor prognosis in patients with clinically N0 oral tongue cancer. J Transl Med, 10, 168.

Li H, Lin Y, Heath RM, Zhu MX, Yang Z. (1999). Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. The Plant Cell, 11, 1731-42.

Li J, Barylko B, Johnson J, Mueller JD, Albanesi JP, Chen Y. (2012b). Molecular brightness analysis reveals phosphatidylinositol 4-Kinase IIbeta association with clathrin-coated vesicles in living cells. Biophys J, 103, 1657-65.

Li X, Rivas MP, Fang M, Marchena J, Mehrotra B, Chaudhary A, Feng L, Prestwich GD, Bankaitis VA. (2002). Analysis of oxysterol binding protein homologue Kes1p function in regulation of Sec14p-dependent protein transport from the yeast Golgi complex. J Cell Biol, 157, 63-77.

Li XY, Liu W, Chen SF, Zhang LQ, Li XG, Wang LX. (2011). Expression of the Golgi phosphoprotein-3 gene in human gliomas: a pilot study. J Neurooncol, 105, 159-63.

Lim S, Strahl T, Thorner J, Ames JB. (2011). Structure of a Ca2+-myristoyl switch protein that controls activation of a phosphatidylinositol 4-kinase in fission yeast. J Biol Chem, 286, 12565-77.

Lim YS, Hwang SB. (2011). Hepatitis C virus NS5A protein interacts with phosphatidylinositol 4-kinase type IIIalpha and regulates viral propagation. J Biol Chem, 286, 11290-8.

Lorente-Rodriguez A, Barlowe C. (2011). Requirement for Golgi-localized PI(4)P in fusion of COPII vesicles with Golgi compartments. Mol Biol Cell, 22, 216-29.

Low PC, Misaki R, Schroder K, Stanley AC, Sweet MJ, Teasdale RD, Vanhaesebroeck B, Meunier FA, Taguchi T, Stow JL. (2010). Phosphoinositide 3-kinase delta regulates membrane fission of Golgi carriers for selective cytokine secretion. J Cell Biol, 190, 1053-65.

Ma H, Blake T, Chitnis A, Liu P, Balla T. (2009). Crucial role of phosphatidylinositol 4-kinase IIIalpha in development of zebrafish pectoral fin is linked to phosphoinositide 3-kinase and FGF signaling. J Cell Sci, 122, 4303-10.

McCrea HJ, De Camilli P. (2009). Mutations in phosphoinositide metabolizing enzymes and human disease. Physiology (Bethesda), 24, 8-16.

McFerran BW, Graham ME, Burgoyne RD. (1998). Neuronal Ca2+ sensor 1, the mammalian homologue of frequenin, is expressed in chromaffin and PC12 cells and regulates neurosecretion from dense-core granules. J Biol Chem, 273, 22768-72.

McFerran BW, Weiss JL, Burgoyne RD. (1999). Neuronal Ca(2+) sensor 1. Characterization of the myristoylated protein, its cellular effects in permeabilized adrenal chromaffin cells, Ca(2+)-independent membrane association, and interaction with binding proteins, suggesting a role in rapid Ca(2+) signal transduction. J Biol Chem, 274, 30258-65.

Mikhaylova M, Reddy PP, Munsch T, Landgraf P, Suman SK, Smalla KH, Gundelfinger ED, Sharma Y, Kreutz MR. (2009). Calneurons provide a calcium threshold for trans-Golgi network to plasma membrane trafficking. Proc Natl Acad Sci U S A, 106, 9093-8.

Miller SE, Collins BM, McCoy AJ, Robinson MS, Owen DJ. (2007). A SNARE-adaptor interaction is a new mode of cargo recognition in clathrin-coated vesicles. Nature, 450, 570-4.

Mills IG, Praefcke GJ, Vallis Y, Peter BJ, Olesen LE, Gallop JL, Butler PJ, Evans PR, McMahon HT. (2003). EpsinR: an AP1/clathrin interacting protein involved in vesicle trafficking. J Cell Biol, 160, 213-22.

Minogue S, Anderson JS, Waugh MG, dos Santos M, Corless S, Cramer R, Hsuan JJ. (2001). Cloning of a human type II phosphatidylinositol 4-kinase reveals a novel lipid kinase family. J Biol Chem, 276, 16635-40.

Minogue S, Chu KM, Westover EJ, Covey DF, Hsuan JJ, Waugh MG. (2010). Relationship between phosphatidylinositol 4-phosphate synthesis, membrane organization, and lateral diffusion of PI4KIIalpha at the trans-Golgi network. J Lipid Res, 51, 2314-24.

Minogue S, Waugh MG, De Matteis MA, Stephens DJ, Berditchevski F, Hsuan JJ. (2006). Phosphatidylinositol 4-kinase is required for endosomal trafficking and degradation of the EGF receptor. J Cell Sci, 119, 571-81.

Mizuno-Yamasaki E, Medkova M, Coleman J, Novick P. (2010). Phosphatidylinositol 4-phosphate controls both membrane recruitment and a regulatory switch of the Rab GEF Sec2p. Dev Cell, 18, 828-40.

Monetta P, Slavin I, Romero N, Alvarez C. (2007). Rab1b interacts with GBF1 and modulates both ARF1 dynamics and COPI association. Mol Biol Cell, 18, 2400-10.

Montell C. (2012). Drosophila visual transduction. Trends Neurosci, 35, 356-63.

Moorhead AM, Jung JY, Smirnov A, Kaufer S, Scidmore MA. (2010). Multiple host proteins that function in phosphatidylinositol-4-phosphate metabolism are recruited to the chlamydial inclusion. Infect Immun, 78, 1990-2007.

Mossinger J, Wieffer M, Krause E, Freund C, Gerth F, Krauss M, Haucke V. (2012). Phosphatidylinositol 4-kinase IIalpha function at endosomes is regulated by the ubiquitin ligase Itch. EMBO Rep, 13, 1087-94.

Mousley CJ, Yuan P, Gaur NA, Trettin KD, Nile AH, Deminoff SJ, Dewar BJ, Wolpert M, Macdonald JM, Herman PK, Hinnebusch AG, Bankaitis VA. (2012). A sterol-binding protein integrates endosomal lipid metabolism with TOR signaling and nitrogen sensing. Cell, 148, 702-15.

Murray MJ, Ng MM, Fraval H, Tan J, Liu W, Smallhorn M, Brill JA, Field SJ, Saint R. (2012). Regulation of Drosophila mesoderm migration by phosphoinositides and the PH domain of the Rho GTP exchange factor Pebble. Dev Biol, 372, 17-27.

Nakagawa T, Goto K, Kondo H. (1996a). Cloning and characterization of a 92 kDa soluble phosphatidylinositol 4-kinase. Biochem J, 320 (Pt 2), 643-9.

Nakagawa T, Goto K, Kondo H. (1996b). Cloning, expression, and localization of 230-kDa phosphatidylinositol 4-kinase. J Biol Chem, 271, 12088-94.

Nakatsu F, Baskin JM, Chung J, Tanner LB, Shui G, Lee SY, Pirruccello M, Hao M, Ingolia NT, Wenk MR, De Camilli P. (2012). PtdIns4P synthesis by PI4KIIIalpha at the plasma membrane and its impact on plasma membrane identity. J Cell Biol, 199, 1003-16.

Natarajan P, Liu K, Patil DV, Sciorra VA, Jackson CL, Graham TR. (2009). Regulation of a Golgi flippase by phosphoinositides and an ArfGEF. Nat Cell Biol, 11, 1421-6.

Ng MM, Dippold HC, Buschman MD, Noakes CJ, Field SJ. (2013). GOLPH3L antagonizes GOLPH3 to determine Golgi morphology. Mol Biol Cell, 24, 796-808.

Ngo M, Ridgway ND. (2009). Oxysterol binding protein-related Protein 9 (ORP9) is a cholesterol transfer protein that regulates Golgi structure and function. Mol Biol Cell, 20, 1388-99.

Nishikawa K, Toker A, Wong K, Marignani PA, Johannes FJ, Cantley LC. (1998). Association of protein kinase Cmu with type II phosphatidylinositol 4-kinase and type I phosphatidylinositol-4-phosphate 5-kinase. J Biol Chem, 273, 23126-33.

Olafsson P, Wang T, Lu B. (1995). Molecular cloning and functional characterization of the Xenopus Ca(2+)-binding protein frequenin. Proc Natl Acad Sci U S A, 92, 8001-5.

Olayioye MA, Hausser A. (2012). Integration of non-vesicular and vesicular transport processes at the Golgi complex by the PKD-CERT network. Biochim Biophys Acta, 1821, 1096-103.

Olsen HL, Hoy M, Zhang W, Bertorello AM, Bokvist K, Capito K, Efanov AM, Meister B, Thams P, Yang SN, Rorsman P, Berggren PO, Gromada J. (2003). Phosphatidylinositol 4-kinase serves as a metabolic sensor and regulates priming of secretory granules in pancreatic beta cells. Proc Natl Acad Sci U S A, 100, 5187-92.

Pan CY, Jeromin A, Lundstrom K, Yoo SH, Roder J, Fox AP. (2002). Alterations in exocytosis induced by neuronal Ca2+ sensor-1 in bovine chromaffin cells. J Neurosci, 22, 2427-33.

Pan W, Choi SC, Wang H, Qin Y, Volpicelli-Daley L, Swan L, Lucast L, Khoo C, Zhang X, Li L, Abrams CS, Sokol SY, Wu D. (2008). Wnt3a-mediated formation of phosphatidylinositol 4,5-bisphosphate regulates LRP6 phosphorylation. Science, 321, 1350-3.

Peretti D, Dahan N, Shimoni E, Hirschberg K, Lev S. (2008). Coordinated lipid transfer between the endoplasmic reticulum and the Golgi complex requires the VAP proteins and is essential for Golgi-mediated transport. Mol Biol Cell, 19, 3871-84.

Perry RJ, Ridgway ND. (2006). Oxysterol-binding protein and vesicle-associated membrane protein-associated protein are required for sterol-dependent activation of the ceramide transport protein. Mol Biol Cell, 17, 2604-16.

Piao H, MacLean Freed J, Mayinger P. (2012). Metabolic activation of the HOG MAP kinase pathway by Snf1/AMPK regulates lipid signaling at the Golgi. Traffic, 13, 1522-31.

Pizarro-Cerda J, Payrastre B, Wang YJ, Veiga E, Yin HL, Cossart P. (2007). Type II phosphatidylinositol 4-kinases promote Listeria monocytogenes entry into target cells. Cell Microbiol, 9, 2381-90.

Polevoy G, Wei HC, Wong R, Szentpetery Z, Kim YJ, Goldbach P, Steinbach SK, Balla T, Brill JA. (2009). Dual roles for the Drosophila PI 4-kinase four wheel drive in localizing Rab11 during cytokinesis. J Cell Biol, 187, 847-58.

Pongs O, Lindemeier J, Zhu XR, Theil T, Engelkamp D, Krah-Jentgens I, Lambrecht HG, Koch KW, Schwemer J, Rivosecchi R, et al. (1993). Frequenin--a novel calcium-binding protein that modulates synaptic efficacy in the Drosophila nervous system. Neuron, 11, 15-28.

Popoff V, Adolf F, Brugger B, Wieland F. (2011). COPI budding within the Golgi stack. Cold Spring Harb Perspect Biol, 3, a005231.

Preuss ML, Schmitz AJ, Thole JM, Bonner HK, Otegui MS, Nielsen E. (2006). A role for the RabA4b effector protein PI-4Kbeta1 in polarized expansion of root hair cells in Arabidopsis thaliana. J Cell Biol, 172, 991-8.

Qin Y, Li L, Pan W, Wu D. (2009). Regulation of phosphatidylinositol kinases and metabolism by Wnt3a and Dvl. J Biol Chem, 284, 22544-8.

Ragaz C, Pietsch H, Urwyler S, Tiaden A, Weber SS, Hilbi H. (2008). The Legionella pneumophila phosphatidylinositol-4 phosphate-binding type IV substrate SidC recruits endoplasmic reticulum vesicles to a replication-permissive vacuole. Cell Microbiol, 10, 2416-33.

Rajebhosale M, Greenwood S, Vidugiriene J, Jeromin A, Hilfiker S. (2003). Phosphatidylinositol 4-OH kinase is a downstream target of neuronal calcium sensor-1 in enhancing exocytosis in neuroendocrine cells. J Biol Chem, 278, 6075-84.

Ramachandran R. (2011). Vesicle scission: dynamin. Semin Cell Dev Biol, 22, 10-7.

Raychaudhuri S, Im YJ, Hurley JH, Prinz WA. (2006). Nonvesicular sterol movement from plasma membrane to ER requires oxysterol-binding protein-related proteins and phosphoinositides. J Cell Biol, 173, 107-19.

Reiss S, Rebhan I, Backes P, Romero-Brey I, Erfle H, Matula P, Kaderali L, Poenisch M, Blankenburg H, Hiet MS, Longerich T, Diehl S, Ramirez F, Balla T, Rohr K, Kaul A, Buhler S, Pepperkok R, Lengauer T, Albrecht M, Eils R, Schirmacher P, Lohmann V, Bartenschlager R. (2011). Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment. Cell Host Microbe, 9, 32-45.

Ren X, Farias GG, Canagarajah BJ, Bonifacino JS, Hurley JH. (2013). Structural Basis for Recruitment and Activation of the AP-1 Clathrin Adaptor Complex by Arf1. Cell, 152, 755-67.

Rohde G, Wenzel D, Haucke V. (2002). A phosphatidylinositol (4,5)-bisphosphate binding site within mu2-adaptin regulates clathrin-mediated endocytosis. J Cell Biol, 158, 209-14.

Rong Y, Liu M, Ma L, Du W, Zhang H, Tian Y, Cao Z, Li Y, Ren H, Zhang C, Li L, Chen S, Xi J, Yu L. (2012). Clathrin and phosphatidylinositol-4,5-bisphosphate regulate autophagic lysosome reformation. Nat Cell Biol, 14, 924-34.

Roy A, Levine TP. (2004). Multiple pools of phosphatidylinositol 4-phosphate detected using the pleckstrin homology domain of Osh2p. J Biol Chem, 279, 44683-9.

Ryder PV, Vistein R, Gokhale A, Seaman MN, Puthenveedu MA, Faundez V. (2013). The WASH complex, an endosomal Arp2/3 activator, interacts with the Hermansky-Pudlak syndrome complex BLOC-1 and its cargo phosphatidylinositol-4-kinase type IIalpha. Mol Biol Cell, 24, 2269-84.

Saint-Pol A, Yelamos B, Amessou M, Mills IG, Dugast M, Tenza D, Schu P, Antony C, McMahon HT, Lamaze C, Johannes L. (2004). Clathrin adaptor epsinR is required for retrograde sorting on early endosomal membranes. Dev Cell, 6, 525-38.

Salazar G, Zlatic S, Craige B, Peden AA, Pohl J, Faundez V. (2009). Hermansky-Pudlak syndrome protein complexes associate with phosphatidylinositol 4-kinase type II alpha in neuronal and non-neuronal cells. J Biol Chem, 284, 1790-802.

Salcedo-Sicilia L, Granell S, Jovic M, Sicart A, Mato E, Johannes L, Balla T, Egea G. (2013). betaIII Spectrin Regulates the Structural Integrity and the Secretory Protein Transport of the Golgi Complex. J Biol Chem, 288, 2157-66.

Samaj J, Muller J, Beck M, Bohm N, Menzel D. (2006). Vesicular trafficking, cytoskeleton and signalling in root hairs and pollen tubes. Trends Plant Sci, 11, 594-600.

Santiago-Tirado FH, Legesse-Miller A, Schott D, Bretscher A. (2011). PI4P and Rab inputs collaborate in myosin-V-dependent transport of secretory compartments in yeast. Dev Cell, 20, 47-59.

Schill NJ, Anderson RA. (2009). Two novel phosphatidylinositol-4-phosphate 5-kinase type Igamma splice variants expressed in human cells display distinctive cellular targeting. Biochem J, 422, 473-82.

Schmitz KR, Liu J, Li S, Setty TG, Wood CS, Burd CG, Ferguson KM. (2008). Golgi localization of glycosyltransferases requires a Vps74p oligomer. Dev Cell, 14, 523-34.

Schulz TA, Choi MG, Raychaudhuri S, Mears JA, Ghirlando R, Hinshaw JE, Prinz WA. (2009). Lipid-regulated sterol transfer between closely apposed membranes by oxysterol-binding protein homologues. J Cell Biol, 187, 889-903.

Scott KL, Kabbarah O, Liang MC, Ivanova E, Anagnostou V, Wu J, Dhakal S, Wu M, Chen S, Feinberg T, Huang J, Saci A, Widlund HR, Fisher DE, Xiao Y, Rimm DL, Protopopov A, Wong KK, Chin L. (2009). GOLPH3 modulates mTOR signalling and rapamycin sensitivity in cancer. Nature, 459, 1085-90.

Scott PM, Bilodeau PS, Zhdankina O, Winistorfer SC, Hauglund MJ, Allaman MM, Kearney WR, Robertson AD, Boman AL, Piper RC. (2004). GGA proteins bind ubiquitin to facilitate sorting at the trans-Golgi network. Nat Cell Biol, 6, 252-9.

Seifert JP, Wing MR, Snyder JT, Gershburg S, Sondek J, Harden TK. (2004). RhoA activates purified phospholipase C-epsilon by a guanine nucleotide-dependent mechanism. J Biol Chem, 279, 47992-7.

Shen QT, Hsiue PP, Sindelar CV, Welch MD, Campellone KG, Wang HW. (2012). Structural insights into WHAMM-mediated cytoskeletal coordination during membrane remodeling. J Cell Biol, 199, 111-24.

Shiba T, Takatsu H, Nogi T, Matsugaki N, Kawasaki M, Igarashi N, Suzuki M, Kato R, Earnest T, Nakayama K, Wakatsuki S. (2002). Structural basis for recognition of acidic-cluster dileucine sequence by GGA1. Nature, 415, 937-41.

Shiba Y, Katoh Y, Shiba T, Yoshino K, Takatsu H, Kobayashi H, Shin HW, Wakatsuki S, Nakayama K. (2004). GAT (GGA and Tom1) domain responsible for ubiquitin binding and ubiquitination. J Biol Chem, 279, 7105-11.

Simons JP, Al-Shawi R, Minogue S, Waugh MG, Wiedemann C, Evangelou S, Loesch A, Sihra TS, King R, Warner TT, Hsuan JJ. (2009). Loss of phosphatidylinositol 4-kinase 2alpha activity causes late onset degeneration of spinal cord axons. Proc Natl Acad Sci U S A, 106, 11535-9.

Skwarek LC, Boulianne GL. (2009). Great expectations for PIP: phosphoinositides as regulators of signaling during development and disease. Dev Cell, 16, 12-20.

Smrcka AV, Hepler JR, Brown KO, Sternweis PC. (1991). Regulation of polyphosphoinositide-specific phospholipase C activity by purified Gq. Science, 251, 804-7.

Sridhar S, Patel B, Aphkhazava D, Macian F, Santambrogio L, Shields D, Cuervo AM. (2013). The lipid kinase PI4KIIIbeta preserves lysosomal identity. EMBO J, 32, 324-39.

Stefan CJ, Manford AG, Baird D, Yamada-Hanff J, Mao Y, Emr SD. (2011). Osh proteins regulate phosphoinositide metabolism at ER-plasma membrane contact sites. Cell, 144, 389-401.

Strahl T, Grafelmann B, Dannenberg J, Thorner J, Pongs O. (2003). Conservation of regulatory function in calcium-binding proteins: human frequenin (neuronal calcium sensor-1) associates productively with yeast phosphatidylinositol 4-kinase isoform, Pik1. J Biol Chem, 278, 49589-99.

Strahl T, Hama H, DeWald DB, Thorner J. (2005). Yeast phosphatidylinositol 4-kinase, Pik1, has essential roles at the Golgi and in the nucleus. J Cell Biol, 171, 967-79.

Strahl T, Huttner IG, Lusin JD, Osawa M, King D, Thorner J, Ames JB. (2007). Structural insights into activation of phosphatidylinositol 4-kinase (Pik1) by yeast frequenin (Frq1). J Biol Chem, 282, 30949-59.

Surma MA, Klose C, Simons K. (2012). Lipid-dependent protein sorting at the trans-Golgi network. Biochim Biophys Acta, 1821, 1059-67.

Szentpetery Z, Varnai P, Balla T. (2010). Acute manipulation of Golgi phosphoinositides to assess their importance in cellular trafficking and signaling. Proc Natl Acad Sci U S A, 107, 8225-30.

Sztul E, Lupashin V. (2006). Role of tethering factors in secretory membrane traffic. Am J Physiol Cell Physiol, 290, C11-26.

Tai AW, Salloum S. (2011). The role of the phosphatidylinositol 4-kinase PI4KA in hepatitis C virus-induced host membrane rearrangement. PLoS One, 6, e26300.

Tan J, Oh K, Burgess J, Hipfner, DR, Brill JA. (2014). PI4KIIIα is required for cortical integrity and cell polarity during *Drosophila* oogenesis. J Cell Sci, in press.

Toth B, Balla A, Ma H, Knight ZA, Shokat KM, Balla T. (2006). Phosphatidylinositol 4-kinase IIIbeta regulates the transport of ceramide between the endoplasmic reticulum and Golgi. J Biol Chem, 281, 36369-77.

Trotard M, Lepere-Douard C, Regeard M, Piquet-Pellorce C, Lavillette D, Cosset FL, Gripon P, Le Seyec J. (2009). Kinases required in hepatitis C virus entry and replication highlighted by small interference RNA screening. FASEB J, 23, 3780-9.

Tu L, Chen L, Banfield DK. (2012). A conserved N-terminal arginine-motif in GOLPH3-family proteins mediates binding to coatomer. Traffic, 13, 1496-507.

Tu L, Tai WC, Chen L, Banfield DK. (2008). Signal-mediated dynamic retention of glycosyltransferases in the Golgi. Science, 321, 404-7.

Vaillancourt FH, Brault M, Pilote L, Uyttersprot N, Gaillard ET, Stoltz JH, Knight BL, Pantages L, McFarland M, Breitfelder S, Chiu TT, Mahrouche L, Faucher AM, Cartier M, Cordingley MG, Bethell RC, Jiang H, White PW, Kukolj G. (2012). Evaluation of phosphatidylinositol-4-kinase IIIalpha as a hepatitis C virus drug target. J Virol, 86, 11595-607.

Vaillancourt FH, Pilote L, Cartier M, Lippens J, Liuzzi M, Bethell RC, Cordingley MG, Kukolj G. (2009). Identification of a lipid kinase as a host factor involved in hepatitis C virus RNA replication. Virology, 387, 5-10.

Valente C, Turacchio G, Mariggio S, Pagliuso A, Gaibisso R, Di Tullio G, Santoro M, Formiggini F, Spano S, Piccini D, Polishchuk RS, Colanzi A, Luini A, Corda D. (2012). A 14-3-3gamma dimer-based scaffold bridges CtBP1-S/BARS to PI(4)KIIIbeta to regulate post-Golgi carrier formation. Nat Cell Biol, 14, 343-54.

van den Bogaart G, Meyenberg K, Risselada HJ, Amin H, Willig KI, Hubrich BE, Dier M, Hell SW, Grubmuller H, Diederichsen U, Jahn R. (2011). Membrane protein sequestering by ionic protein-lipid interactions. Nature, 479, 552-5.

van der Schaar HM, van der Linden L, Lanke KH, Strating JR, Purstinger G, de Vries E, de Haan CA, Neyts J, van Kuppeveld FJ. (2012). Coxsackievirus mutants that can bypass host factor PI4KIIIbeta and the need for high levels of PI4P lipids for replication. Cell Res, 22, 1576-92.

Varnai P, Balla T. (2008). Live cell imaging of phosphoinositides with expressed inositide binding protein domains. Methods, 46, 167-76.

Varnai P, Thyagarajan B, Rohacs T, Balla T. (2006). Rapidly inducible changes in phosphatidylinositol 4,5-bisphosphate levels influence multiple regulatory functions of the lipid in intact living cells. J Cell Biol, 175, 377-82.

Vicinanza M, D'Angelo G, Di Campli A, De Matteis MA. (2008). Function and dysfunction of the PI system in membrane trafficking. EMBO J, 27, 2457-70.

Vieira OV, Verkade P, Manninen A, Simons K. (2005). FAPP2 is involved in the transport of apical cargo in polarized MDCK cells. J Cell Biol, 170, 521-6.

Walsh CM, Chvanov M, Haynes LP, Petersen OH, Tepikin AV, Burgoyne RD. (2010). Role of phosphoinositides in STIM1 dynamics and store-operated calcium entry. Biochem J, 425, 159-68.

Wang J, Sun HQ, Macia E, Kirchhausen T, Watson H, Bonifacino JS, Yin HL. (2007). PI4P promotes the recruitment of the GGA adaptor proteins to the trans-Golgi network and regulates their recognition of the ubiquitin sorting signal. Mol Biol Cell, 18, 2646-55.

Wang JH, Chen XT, Wen ZS, Zheng M, Deng JM, Wang MZ, Lin HX, Chen K, Li J, Yun JP, Luo RZ, Song LB. (2012). High expression of GOLPH3 in esophageal squamous cell carcinoma correlates with poor prognosis. PLoS One, 7, e45622.

Wang YJ, Wang J, Sun HQ, Martinez M, Sun YX, Macia E, Kirchhausen T, Albanesi JP, Roth MG, Yin HL. (2003). Phosphatidylinositol 4 phosphate regulates targeting of clathrin adaptor AP-1 complexes to the Golgi. Cell, 114, 299-310.

Watt SA, Kular G, Fleming IN, Downes CP, Lucocq JM. (2002). Subcellular localization of phosphatidylinositol 4,5-bisphosphate using the pleckstrin homology domain of phospholipase C delta1. Biochem J, 363, 657-66.

Waugh MG. (2012). Phosphatidylinositol 4-kinases, phosphatidylinositol 4-phosphate and cancer. Cancer Lett, 325, 125-31.

Wei YJ, Sun HQ, Yamamoto M, Wlodarski P, Kunii K, Martinez M, Barylko B, Albanesi JP, Yin HL. (2002). Type II phosphatidylinositol 4-kinase beta is a cytosolic and peripheral membrane protein that is recruited to the plasma membrane and activated by Rac-GTP. J Biol Chem, 277, 46586-93.

Weisz OA, Gibson GA, Leung SM, Roder J, Jeromin A. (2000). Overexpression of frequenin, a modulator of phosphatidylinositol 4-kinase, inhibits biosynthetic delivery of an apical protein in polarized madin-darby canine kidney cells. J Biol Chem, 275, 24341-7.

Weller SG, Capitani M, Cao H, Micaroni M, Luini A, Sallese M, McNiven MA. (2010). Src kinase regulates the integrity and function of the Golgi apparatus via activation of dynamin 2. Proc Natl Acad Sci U S A, 107, 5863-8.

Whitters EA, Cleves AE, McGee TP, Skinner HB, Bankaitis VA. (1993). SAC1p is an integral membrane protein that influences the cellular requirement for phospholipid transfer protein function and inositol in yeast. J Cell Biol, 122, 79-94.

Wilson DB, Bross TE, Hofmann SL, Majerus PW. (1984). Hydrolysis of polyphosphoinositides by purified sheep seminal vesicle phospholipase C enzymes. J Biol Chem, 259, 11718-24.

Wong K, Meyers dd R, Cantley LC. (1997). Subcellular locations of phosphatidylinositol 4-kinase isoforms. J Biol Chem, 272, 13236-41.

Wood CS, Hung CS, Huoh YS, Mousley CJ, Stefan CJ, Bankaitis V, Ferguson KM, Burd CG. (2012). Local control of phosphatidylinositol 4-phosphate signaling in the Golgi apparatus by Vps74 and Sac1 phosphoinositide phosphatase. Mol Biol Cell, 23, 2527-36.

Wood CS, Schmitz KR, Bessman NJ, Setty TG, Ferguson KM, Burd CG. (2009). PtdIns4P recognition by Vps74/GOLPH3 links PtdIns 4-kinase signaling to retrograde Golgi trafficking. J Cell Biol, 187, 967-75.

Wu B, Kitagawa K, Zhang NY, Liu B, Inagaki C. (2004). Pathophysiological concentrations of amyloid beta proteins directly inhibit rat brain and recombinant human type II phosphatidylinositol 4-kinase activity. J Neurochem, 91, 1164-70.

Wuttke A, Sagetorp J, Tengholm A. (2010). Distinct plasma-membrane PtdIns(4)P and PtdIns(4,5)P2 dynamics in secretagogue-stimulated beta-cells. J Cell Sci, 123, 1492-502.

Xu Z, Huang G, Kandror KV. (2006). Phosphatidylinositol 4-kinase type IIalpha is targeted specifically to cellugyrin-positive glucose transporter 4 vesicles. Mol Endocrinol, 20, 2890-7.

Yamaji T, Kumagai K, Tomishige N, Hanada K. (2008). Two sphingolipid transfer proteins, CERT and FAPP2: their roles in sphingolipid metabolism. IUBMB Life, 60, 511-8.

Yan Y, Denef N, Tang C, Schupbach T. (2011). Drosophila PI4KIIIalpha is required in the follicle cells for oocyte polarization and Hippo signaling. Development, 138, 1697-703.

Yang JS, Lee SY, Spano S, Gad H, Zhang L, Nie Z, Bonazzi M, Corda D, Luini A, Hsu VW. (2005). A role for BARS at the fission step of COPI vesicle formation from Golgi membrane. EMBO J, 24, 4133-43.

Yang JS, Valente C, Polishchuk RS, Turacchio G, Layre E, Moody DB, Leslie CC, Gelb MH, Brown WJ, Corda D, Luini A, Hsu VW. (2011). COPI acts in both vesicular and tubular transport. Nat Cell Biol, 13, 996-1003.

Yarar D, Waterman-Storer CM, Schmid SL. (2007). SNX9 couples actin assembly to phosphoinositide signals and is required for membrane remodeling during endocytosis. Dev Cell, 13, 43-56.

Yavari A, Nagaraj R, Owusu-Ansah E, Folick A, Ngo K, Hillman T, Call G, Rohatgi R, Scott MP, Banerjee U. (2010). Role of lipid metabolism in smoothened derepression in hedgehog signaling. Dev Cell, 19, 54-65.

Yorimitsu T, Sato K. (2012). Insights into structural and regulatory roles of Sec16 in COPII vesicle formation at ER exit sites. Mol Biol Cell, 23, 2930-42.

Yoshida S, Ohya Y, Goebl M, Nakano A, Anraku Y. (1994). A novel gene, STT4, encodes a phosphatidylinositol 4-kinase in the PKC1 protein kinase pathway of Saccharomyces cerevisiae. J Biol Chem, 269, 1166-72.

Yu X, Breitman M, Goldberg J. (2012). A structure-based mechanism for Arf1-dependent recruitment of coatomer to membranes. Cell, 148, 530-42.

Zanetti G, Pahuja KB, Studer S, Shim S, Schekman R. (2012). COPII and the regulation of protein sorting in mammals. Nat Cell Biol, 14, 20-8.

Zeng Z, Lin H, Zhao X, Liu G, Wang X, Xu R, Chen K, Li J, Song L. (2012). Overexpression of GOLPH3 promotes proliferation and tumorigenicity in breast cancer via suppression of the FOXO1 transcription factor. Clin Cancer Res, 18, 4059-69.

Zhang D, Vjestica A, Oliferenko S. (2012a). Plasma membrane tethering of the cortical ER necessitates its finely reticulated architecture. Curr Biol, 22, 2048-52.

Zhang L, Hong Z, Lin W, Shao RX, Goto K, Hsu VW, Chung RT. (2012b). ARF1 and GBF1 generate a PI4P-enriched environment supportive of hepatitis C virus replication. PLoS One, 7, e32135.

Zhang L, Malik S, Pang J, Wang H, Park KM, Yule DI, Blaxall BC, Smrcka AV. (2013). Phospholipase Cepsilon hydrolyzes perinuclear phosphatidylinositol 4-phosphate to regulate cardiac hypertrophy. Cell, 153, 216-27.

Zhao X, Varnai P, Tuymetova G, Balla A, Toth ZE, Oker-Blom C, Roder J, Jeromin A, Balla T. (2001). Interaction of neuronal calcium sensor-1 (NCS-1) with phosphatidylinositol 4-kinase beta stimulates lipid kinase activity and affects membrane trafficking in COS-7 cells. J Biol Chem, 276, 40183-9.

Zubenko GS, Stiffler JS, Hughes HB, Martinez AJ. (1999). Reductions in brain phosphatidylinositol kinase activities in Alzheimer's disease. Biol Psychiatry, 45, 731-6.

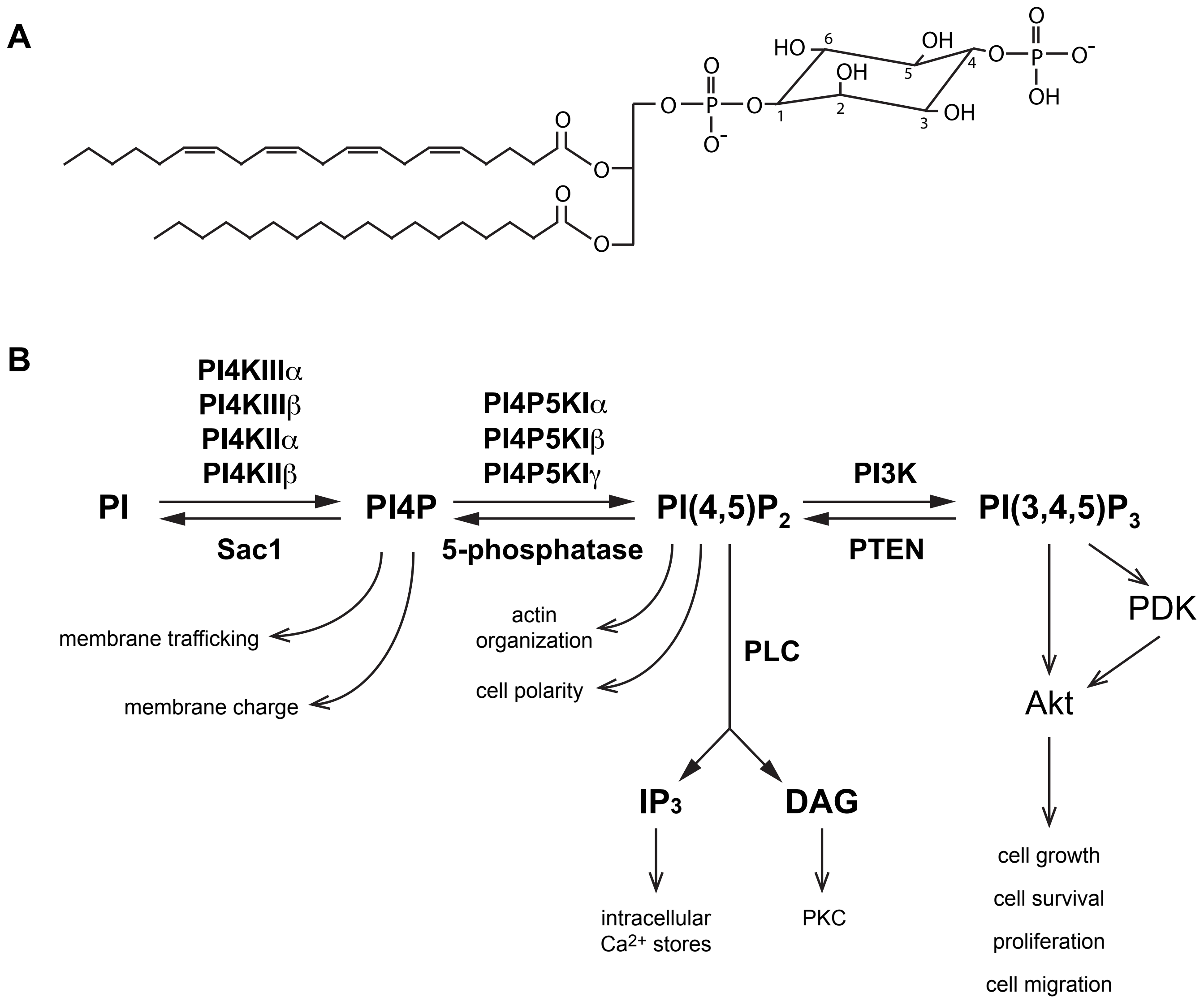

Figure 1. Major pathway for phosphoinositide biosynthesis and degradation. (**A**) Structure of phosphatidylinositol 4-phosphate (PI4P). A variety of fatty acids can be found to populate the lipid tails of the molecule, varying in the length of their hydrocarbon chains and degree of saturation. Shown here are stearic acid (18:0) and arachadonic acid (20:4). (**B**) Major PIP pathway. PI is phosphorylated by PI4Ks on the D-4 position of the inositol ring to produce PI4P. PI4P can be dephosphorylated by the PI4P phosphatase Sac1, or act as the substrate for PI4P5Ks to produce PI(4,5)P_2 [PIP_2]. PIP_2 may be hydrolyzed by PLC to generate the second messengers IP_3 and DAG, dephosphosphorylated by 5-phosphatases, or phosphorylated by class I PI3Ks to produce PI(3,4,5)P_3 [PIP_3]. PIP_3 may be dephosphorylated on the D-3 position by the PTEN phosphatase.

Tan and Brill, Fig 2

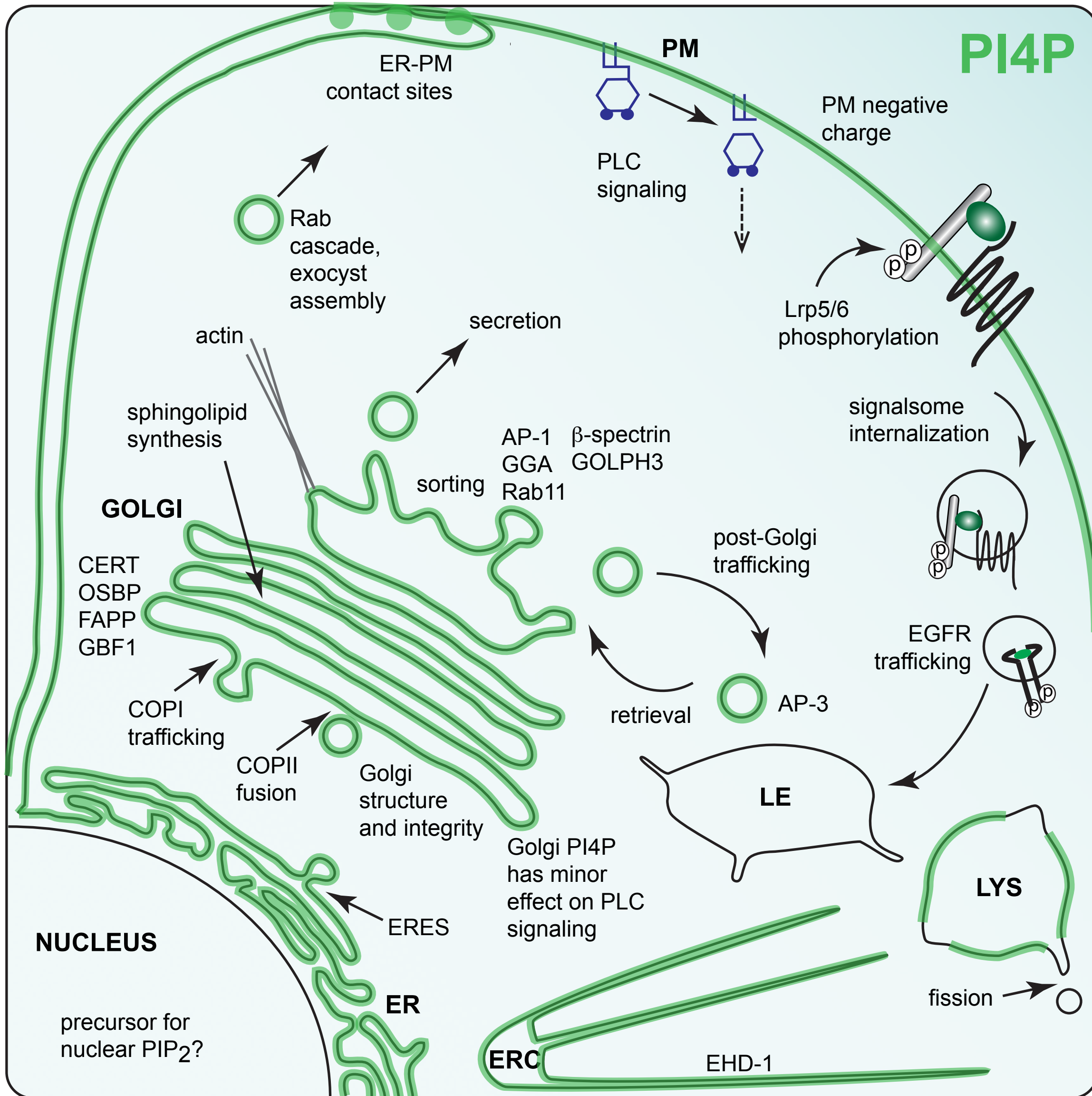

Figure 2. PI4P localization and PI4P-regulated cellular processes. Green highlighting represents locations at which PI4P has been detected, using collective data from mammalian cells, yeast and animal model systems. Methods of detection include antibody recognition, visualization of PI4P-specific binding domains fused to fluorescent proteins, and detection of radiolabeled mono-phosphorylated PIPs isolated from membrane fractions, the presence of which is abolished by inhibition or siRNA of PI4Ks. Abbreviations: ERES, ER exit sites; ERC, endocytic recycling compartment; LE, late endosome; LYS, lysosome.

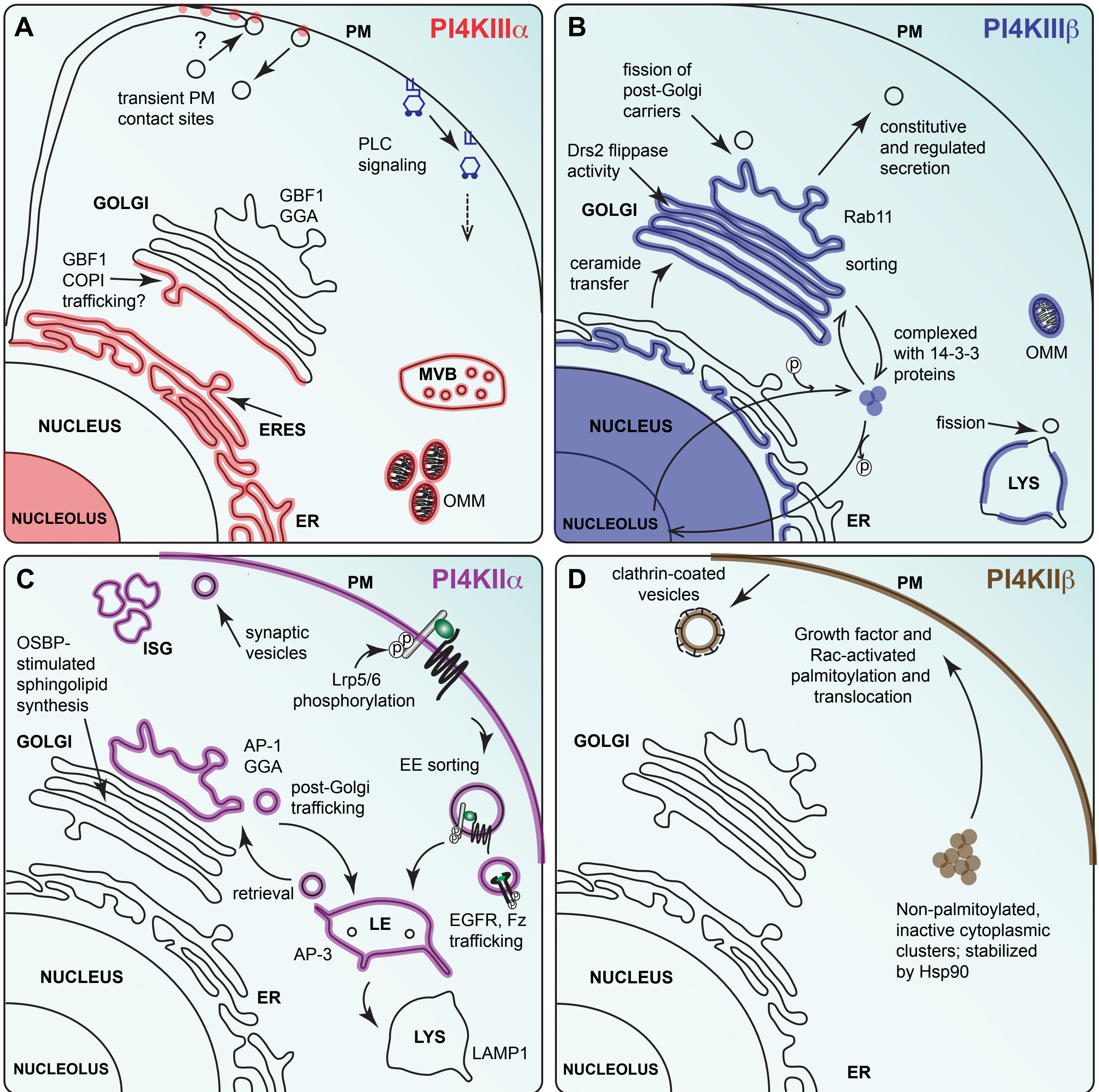

Figure 3. Cellular localizations reported for PI4Ks and the processes they regulate. Each panel represents collective data from mammalian cells, yeast and animal model systems. Localization of endogenous and expressed kinases as determined using immunofluorescence, immunoelectron microscopy, immunoblotting and kinase assays using fractionated membranes, and live imaging of fluorescent fusions to PI4Ks. Known protein effectors are listed next to the organelle at which they function. Abbreviations: EE, early endosome; ISG, immature secretory granules; LE, late endosome; LYS, lysosome; MVB, multivesicular bodies; OMM, outer mitochondrial membrane.

Table 1. Methods to detect cellular PI4P.

Method	Advantages	Disadvantages	Recent References
Metabolic labeling of ^{32}P or ^{3}H incorporation into PIPs	Quantitative; direct lipid analysis; high level labeling can detect low abundance PIPs and distinguish between regioisomers; allows examination of membrane fractions	Difficult to examine subpopulations of cells within a tissue	Wood et al., 2012; Stefan et al., 2011; Cheong et al., 2010; Balla et al., 2008
Expression of PI4P-binding modules fused to fluorescent proteins	Amenable to live imaging; information on organelle and spatial distribution	Modules often harbor second binding site to other proteins; high-level expression may titrate lipid away from normal function	Santiago-Tirado et al., 2011; Stefan et al., 2011; Mizuno-Yamasaki et al., 2010; Balla et al., 2008;
Antibody detection	Detection of endogenous PIPs	Results are sensitive to experimental conditions	Forrest et al., 2013; Hammond et al., 2009
High performance lipid chromatography followed by mass spectrometry	High sensitivity; can be applied to total tissue; information on fatty acid composition	Does not distinguish subcellular distribution	Hammond et al., 2012; Clark et al., 2011

Table 2. Methods used to manipulate PI4P levels.

Method	Advantages	Disadvantages	Recent References
Pharmacological inhibitors Wortmannin, LY294002 PAO PIK93 Adenosine AL-9 T-00127 HEV1 GW5074 Compound A, Compound B	Inhibit enzyme without affecting structural roles; identify contribution by PI4K class; use in combination with other methods	Lack of complete and specific inhibition of specific PI4K isoforms (Wortmannin, LY294002, PAO); some with high selectivity for PI4Ks isoforms also inhibit PI3Ks (PIK93, GW5074)	Bianco et al., 2012; Hammond et al., 2012; Jovic et al., 2012; Vaillancourt et al., 2012; van der Schaar et al., 2012; Arita et al., 2011; Zakharian et al., 2011; Wuttke et al., 2010; Hsu et al., 2010; Balla et al., 2008; Toth et al., 2006
Inhibition of type II PI4K with 4C5G monoclonal antibody	>90% inhibition of type II PI4K activity with little effect on type III	Effect on type IIβ activity unknown; requires protein transfection	Mingoue et al., 2006; Endemann et al., 1991
Inhibition of PI4Ks by ATP depletion	Reversible	Requires cell permeabilization, may deplete small molecules of interest; may inhibit other kinases that affect PI4K activity	Wuttke et al., 2010
Titration by PI4P-binding modules	Second site binding means sequestration of specific pools	Toxic effects of overexpression	Salcedo-Sicilia et al., 2013; Lorente-Rodriguez et al., 2011; Dippold et al., 2009; Bluemental-Perry et al., 2006
Direct delivery with lipid carrier	Relatively acute action in cultured cells	Lack of membrane-specific targeting	Pan et al., 2008
Water-soluble PIP analogs	Technical convenience for in vitro/patch assays	Do not mimic native conformation of cellular PIPs	Zakharian et al., 2011; Caromile et al., 2010
Knockdown of PI4Ks or Sac1	Incomplete silencing may reduce activity without causing lethality	Residual activity from incomplete silencing may mask phenotype; RNAi off-targets	Sridhar et al., 2013; Jovic et al., 2012; Yavari et al., 2010
Overexpression of PI4Ks or Sac1	Manipulate PIP levels with varying strength	May produce artifactual phenotype	Daboussi et al., 2012; Zhang et al., 2012; Santiago-Tirado et al., 2011;
Genetic ablation of PI4Ks or Sac1	Complete and specific removal of enzyme	Essential nature of gene may necessitate genetic tools to examine effects in specific cells; cell/organism may compensate	Nakatsu et al., 2012; Yan et al., 2011; Burgess et al., 2012; Simons et al., 2009; Wei et al., 2003; Foti et al., 2001; Brill et al., 2000; Audhya et al., 2000
FKBP12-FRB rapamycin-inducible lipid phosphatase recruitment	Acute disruption and reversible; depletes PIPs without generating second messengers; membrane-specific	Does not reveal enzymes responsible for depleted lipid pool; dependent on specificity of phosphatase recruited	Hammond et al., 2012; Szentpetery et al., 2010; Varnai et al., 2006; Fili et al., 2006; Heo et al., 2006
Fusion of Sac1 phosphatase domain to PLCδ-PH membrane-targeting element	Rapamycin treatment not required (eg., for expression in animals)	Does not reveal enzymes responsible; cell/organism may compensate for loss of PI4P	Jesch et al., 2010