Published in final edited form as:

Trends Cell Biol. 2011 September ; 21(9): 515–525. doi:10.1016/j.tcb.2011.05.005.

Membrane-trafficking sorting hubs: cooperation between PI4P and small GTPases at the *trans*-Golgi Network

Felipe H. Santiago-Tirado^{1,2} and Anthony Bretscher^{1,2,3}

¹ Department of Molecular Biology and Genetics, 434 Weill Hall, Cornell University, Ithaca, NY 14853-7202

² Weill Institute for Cell and Molecular Biology, 434 Weill Hall, Cornell University, Ithaca, NY 14853-7202

Abstract

Cell polarity in eukaryotes requires constant sorting, packaging, and transport of membrane-bound cargo within the cell. These processes occur in two sorting hubs: the recycling endosome for incoming material, and the *trans*-Golgi Network for outgoing. Phosphatidylinositol 3-phosphate and 4–5 phosphate are enriched at the endocytic and exocytic sorting hubs, respectively, where they act together with small GTPases to recruit factors to segregate cargo and regulate carrier formation and transport. In this review, we summarize the current understanding of how these lipids and GTPases directly regulate membrane trafficking, emphasizing the recent discoveries of phosphatidylinositol 4-phosphate functions at the *trans*-Golgi Network.

Phosphoinositides and small GTPases define membrane identity

Cell polarity is a characteristic of eukaryotic cells, requiring the synthesis and selective targeting of lipids and proteins to specific domains of the plasma membrane. Proteins of the secretory pathway are synthesized on the endoplasmic reticulum (ER) and subject to modifications as they traverse the Golgi cisternae before reaching the *trans*-Golgi Network (TGN). The TGN is the exocytic hub where cargoes must be sorted and packaged into distinct transport carriers that will fuse only with their target membrane, including endosomal destinations and the plasma membrane. The cell contains a second major sorting hub, the recycling endosome, where incoming endocytic cargo is sorted into specific subdomains and sent to different destinations. A major issue for both hubs is how sorting, packaging and transport are mediated. Although the amount of material processed by these hubs is immense, the cell handles it with exceptional accuracy, conferred, in part, by small GTPases of the Rab family. Rabs are master regulators of membrane trafficking, with specific members being associated with each organelle as well as with the transport vesicles they form^[1] (Figure 1). Over the last decade it has also become clear that the regulatory inositide phospholipids are also important signaling molecules in membrane traffic. By serving as binding platforms for proteins, they provide identity to membrane compartments^[2] (Figure 1). The concept is now emerging that specific phosphoinositides (PIs) and small GTPases cooperate at membrane trafficking sorting hubs, with the

© 2011 Elsevier Ltd. All rights reserved.

³Corresponding author: Bretscher, A. (apb5@cornell.edu).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

prototypical example being the involvement of phosphatidylinositol 3-phosphate (PI3P) and Rab5 at the endosome, and phosphatidylinositol 4-phosphate (PI4P) being recently appreciated as playing a central role with Arf1 and Rab proteins at the TGN^[3]. Here, we provide an overview of the endocytic hub and then focus on recent data revealing new contributions and roles for PI4P and small GTPases at the TGN sorting hub.

Coincidence detection and segregation at the endosomal system sorting hub

The original model for coincidence detection in membrane trafficking is that of PI3P and Rab5/7 in the endocytic pathway (Figure 2A). Early studies with the PI 3-kinase inhibitor wortmannin and with mutations in the catalytic domain of PI 3-kinase demonstrated that PI3P is a major determinant of early endosome identity and function^[4]. Moreover, the identification of yeast Vps34p as a PI 3-kinase necessary for vacuole protein sorting highlighted the importance of PI3P in endosomal transport^[5]. Mammalian Rab5 was also known to be the small GTPase involved in endosome formation as it localizes to endosomes, its overexpression leads to an increased rate of endocytosis, and its inhibition by expression of a GTPase-defective construct results in the formation of giant endosomes that are insensitive to wortmannin^[6, 7]. Initial activation of Rab5 occurs at the plasma membrane by guanine nucleotide exchange factors (GEFs) like RME-6 during the endocytotic event^[8, 9]. Concomitantly with vesicle fission, the mammalian PI 3-kinases Vps34 and PI3K β are recruited by Rab5-GTP initiating the generation of PI3P^[10]. Active Rab5 also recruits its effector Rabaptin5 in complex with another Rab5 GEF, Rabex5, generating a feedback loop resulting in more active Rab5, more PI 3-kinase, and thus more PI3P. The tethering and fusion factor early endosome antigen protein 1 (EEA1) is recruited by virtue of its Rab5-GTP and PI3P binding motifs^[11], each motif having a weak affinity for its ligand but together providing a strong specificity for early endosomal membranes. Since EEA1 forms homodimers^[12], it can tether internalized endosomes with other nascent and incoming endosomes, promoting fusion and allowing enlargement of the organelle.

Once established, the early endosome matures into a recycling endosome with different subdomains dictated in part by the cargos to be sorted. Ubiquitinated cargos are sorted into a Rab7 domain that will mature into the late endosome/multivesicular body (MVB), while other cargoes such as growth factor receptors can be recycled to the cell surface either rapidly via the Rab4 subdomain or more slowly via Rab11 (Figure 2A). Ubiquitinated cargo from the plasma membrane is recognized early in this pathway by the PI3P-binding protein Hrs, which then drives recruitment of the sequential ESCRT complexes that will ultimately internalize the cargo into an MVB for delivery to the lysosome^[13]. How this process is temporally coupled to Rab7 signaling is not known. However, an important factor for endosome maturation is the PI3P-binding protein SAND-1/Mon1^[14, 15] that, like EEA1, requires a bivalent interaction to localize to endosomal membranes. It binds to both PI3P and Rabex5, disrupting the Rabaptin5-Rabex5 complex and thereby breaking the active Rab5 positive feedback loop. This probably frees active Rab5 for binding, together with the localized SAND-1/Mon1, the homotypic fusion and vacuole protein sorting (HOPS) complex, which includes a GEF for Rab7. Active Rab7 can recruit more HOPS by binding another of its subunits, resulting in a self-amplification loop that drives conversion of Rab5-rich membranes into Rab7-labeled membranes. Recent studies have shown that the Ccz1 protein associates with Mon1 as a complex, suggesting that it may be the Ccz1-Mon1 complex rather than SAND-1/Mon1 alone that drives the transition^[14, 16]. Less is known mechanistically about how other endosomal domains occur. Specific activated receptors are recognized after internalization by the PI3P-binding protein SARA^[17–20] that regulates their signaling; G protein-coupled receptors are usually rapidly recycled through the Rab4 domain^[21, 22]; and the slower recycling pathway involves Rab11 and many proteins called

FIPs (Rab11 family of interacting proteins)[^{23, 24}]. Overall, endosomal Rabs and PI3P provide identity to the recycling endosome and by virtue of Rab effectors and PI3P-binding proteins can facilitate collection and sorting of cargo for distinct destinations. In general the affinity of proteins for PI3P is in the micromolar range, allowing sampling of the membranes until additional factors and ligands are found, restricting or segregating a specific cargo towards the appropriate carriers.

PI4P in Golgi function and organization

The synthesis of plasma membrane phosphatidylinositol 4,5-bisphosphate (PI4,5P₂) requires the sequential phosphorylation of phosphatidylinositol into PI4P and subsequently to PI4,5P₂. Thus, PI4P was initially regarded simply as a precursor of PI4,5P₂ (Box 1). Supporting this view were reports that synthesis of PI4,5P₂ by PI 4-kinase and PI4P 5-kinase were required for the regulated fusion of secretory granules and of synaptic vesicles with the plasma membrane[^{25–28}]. However, a dozen years ago a critical role for PI4P emerged in studies of the secretory pathway in budding yeast. Yeast with a temperature-sensitive mutation in the Golgi-localized PI 4-kinase, *Pik1p*, blocks exit from the Golgi when PI4P levels are reduced upon shifting to the restrictive temperature[^{29, 30}]. It was also shown that mutations in the phospholipid transfer protein (PITP) *Sec14p*, known to block transport out of the Golgi[³¹], reduced the cellular levels of PI4P. Moreover, over-expression of *PIK1*, or deletion of the PI4P phosphatase *SAC1*, conditions known to increase PI4P levels, rescued the conditional mutation in *SEC14*, again supporting a role for PI4P in exit from the Golgi. Further genetic studies revealed that two distinct and essential pools of PI4P exist, one synthesized at the Golgi by the PI 4-kinase *Pik1p* and the other at the plasma membrane generated by the PI 4-kinase *Stt4p*[³²]. In mammals, the expression of a kinase-dead version of PI4KIII β (the mammalian homolog of yeast *Pik1p*) disrupts Golgi architecture and secretion[³³] and knockdown of PI4KII α (a second Golgi-localized PI 4-kinase in mammals) disrupts the Golgi and inhibits transport of cargo out of it[^{34, 35}]. Because PI4KII α is membrane attached (via palmitoylation) and PI4KIII β has to be recruited, it has been suggested that the basal PI4P levels are generated by the former kinase while the latter generates specific pools of PI4P[³⁶]. These early studies imply that PI4P plays a critical role at the Golgi, suggesting it functions, at least in part, by its ability to recruit effector proteins to their sites of action (Table 1). However, many of these effectors only bind PI4P weakly and binding alone cannot account for their exquisite localization to specific compartments, especially since at least two distinct pools of PI4P exist in the cell. By using a dual recognition mechanism involving small GTPases and PI4P, the TGN sorting hub can recruit factors to segregate cargo for transport to specific locations.

BOX 1

The birth of phosphoinositide signaling and the rise of phosphatidylinositol 4-phosphate
The existence of inositol phospholipids was known since the 1940s, but it was not until the last two decades of the 20th century that a direct role for phosphoinositides (PIs) in cell signaling became widely accepted. In 1953 Mabel and Lowell Hokin showed that ³²P could be incorporated into phospholipids of the pancreas upon stimulation with acetylcholine, something they later showed in different cell types with different excitatory ligands⁸⁷. They also showed that most of the radioactivity was incorporated into PIs and not other neutral phospholipids⁸⁸. Before this work, it was believed that all intracellular signals were transmitted by specific protein-protein interactions. However, by the mid-seventies, it was shown that PI metabolism stimulated by extracellular signals raises intracellular calcium⁸⁹ and, subsequently, that phospholipase C hydrolyses PI4,5P₂ into IP₃ and DAG⁹⁰, with IP₃ then stimulating Ca⁺² release from intracellular stores⁹¹. The importance of PI4,5P₂ continued to grow with the discovery of PI 3-kinase and a

new signaling pathway involving PI3,4,5P₃^{92, 93}. By this time, receptor mediated hydrolysis of PI4,5P₂ was frequently found to accompany changes in the actin cytoskeleton, consistent with reports that PI4,5P₂ affected profilin, villin, cofilin, ezrin, and the function of many other actin-related proteins⁹⁴. With the discovery that PH domains can bind PI4,5P₂⁹⁵, many proteins, like dynamin⁹⁶, mSOS1⁹⁷, and N-WASP⁹⁸, were found to contain PH domains that linked PI4,5P₂ metabolism to their function. Up to this point, PI4P was simply regarded as the immediate precursor for PI4,5P₂ synthesis. However, in 1999 two articles implicated PI4P in yeast protein secretion^{29, 30}. It was then found that defects in animal cell Golgi PI4P metabolism affected Golgi morphology and function³³. The past decade has shown that Golgi PI4P is a master regulator of protein and lipid trafficking, doubling our understanding of PI4P functions (Figure I). With our current nascent understanding, this area will likely continue to be a central focus for many years to come.

In invertebrates and some fungi the Golgi stacks are scattered throughout the cell, whereas in most higher organisms it is present as a continuous perinuclear Golgi ribbon. The reason for this architecture is unclear, but presumably it makes the transport of cargo between cisternae more efficient. Each cisterna contains a set of resident enzymes involved in the modification and maturation of the cargo. The correct localization of these enzymes depends on their constant retrieval by COPI vesicles from more distal Golgi cisterna to earlier cisterna, or back from the cis-cisterna to the ER, whereupon they transit forward again to form an early Golgi *de novo* with newly synthesized secretory cargo. This type of structure is conserved in all eukaryotes, as is the presence of PI4P (Box 2).

BOX 2

Phosphoinositide signaling in plant membrane traffic

Although plants and animals diverged over a billion years ago, the basic mechanisms underlying polarized growth are shared between the kingdoms. In fact, because most of the main players discussed in this review are present in fungi, plants, and animals, it seems that phosphoinositides (PIs) and small GTPases already cooperated in the common ancestor from which these lineages evolved. Certain types of plant cells grow by rapid tip expansion (>1cm/h;⁹⁹) that depends on exo- and endocytosis, ionic gradients, cell wall synthesis, turgor pressure, and cytoplasmic streaming. These processes in turn depend on the actin cytoskeleton, small GTPases, and PI signaling. In fact, the first evidence for PI signaling in polarized growth also linked it to small GTPases¹⁰⁰. Rop GTPases localize to the growing tip where they regulate PI4P 5-kinase, resulting in a gradient of PI4,5P₂ which is strongest at the apex of the tip. The gradient is controlled in part by phospholipase C that localizes to the lateral sides of the cell, where it hydrolyses PI4,5P₂ to control Ca²⁺ gradients^{101, 102}. Sequestration of PI3P or inhibition of the only class of PI 3-kinases in plants inhibits endocytosis, affecting pollen tube growth rates¹⁰³. As in yeast, there is a spike in PI3,5P₂ synthesis upon osmotic stress¹⁰⁴ and it has been shown that AtFAB1, the PI3P 5-kinase, has a role in vacuole morphology and function^{105, 106}. However, PI4P is the most abundant PI in plants and been shown to coordinate membrane trafficking events with tip growth, cell plate formation, cytoplasmic streaming, and secretory organelle morphology^{107–109}. RabA4 (Rab11 class) labels the polarized TGN compartment in polarized cells where it recruits PI4Kβ1 to promote secretory vesicle production and tip growth^{65, 109}. Moreover, PI4P labels motile internal membranes as well as the plasma membrane, especially at the apical tip domain of growing cells. Interestingly, the *rhd4* mutation, with short and aberrant root hairs, was found to encode a PI4P phosphatase related to yeast Sac1p¹¹⁰. Finally, plants also have a set of P1TP¹¹¹ that when mutated exhibit similar phenotypes as cells with defects in PI4P

levels¹¹². In conclusion, tip growth in plants is very similar to polarized growth in yeast and animals, involving small GTPases, PI4P and PI4,5P₂, and cytoskeletal elements working together to achieve an exquisite level of regulation (Figure II).

The first mechanistic insight into how PI4P affects Golgi morphology was recently reported^[37]. The authors identified GOLPH3 as a new PI4P binding protein, and showed that this interaction is important for its localization to the TGN. Knockdown of GOLPH3 has two effects: it results in dilation of Golgi elements and reduction in the amount of VSVG-EGFP in transit through the secretory pathway that reaches the plasma membrane, and in condensation of the Golgi to one spot at the side of the nucleus, very similar to the effect of treating cells with the actin depolymerization agent Latrunculin B. Interestingly, GOLPH3 interacts with the unconventional myosin MYO18a, and knockdown of MYO18a has a phenotype similar to loss of GOLPH3. The authors suggest that the PI4P-GOLPH3-MYO18a linkages provide pulling forces to maintain the shape of the TGN and for the efficient budding of vesicles or extraction of tubules (Figure 2B). Both GOLPH3 and MYO18a are abundant proteins whose localization is not exclusively restricted to the TGN, although how they are localized elsewhere is unclear. It is likely that they recognize other proteins as determinants of their localization.

Yeast Vps74p, the homolog of mammalian GOLPH3, was identified nearly a decade ago in a screen for mutants that mis-sort the vacuolar protease carboxypeptidase Y^[38]. Recently, Vps74p has been implicated in retrieval of glycosyltransferases – enzymes that modify glycoproteins in a Golgi cisternae-specific manner. Vps74p has been found to bind both the cytoplasmic tails of glycosyltransferases and to coatomer – the coat of COPI vesicles^[39, 40]. In the absence of Vps74p, the glycosyltransferases are not retrieved but instead delivered to the vacuole where they are degraded. This retrieval function of Vps74p requires its ability to bind PI4P and localize to the Golgi as mutations that compromise lipid binding result in mis-localization away from the Golgi and under-glycosylation of secretory proteins^[41]. Interestingly, Vps74p is a tetramer, and requires oligomerization to perform its retrieval function. Since Vps74p binds PI4P weakly, the model emerges that the multiple sites present in the tetramer are required to bind both PI4P and the tails of glycosyltransferases for incorporation into COPI vesicles (Figure 2B). Since the formation of COPI vesicles requires the involvement of Arf1-GTP^[42], binding of Vps74p to PI4P and coatomer may coordinate its recruitment.

How do the results with yeast Vps74p relate to mammalian GOLPH3? GOLPH3 has a very similar structure to Vps74p, contains a modest PI4P-binding site, and can partially substitute for Vps74p in *vps74Δ* cells^[40, 41]. However, *vps74* mutants have no gross Golgi morphological or forward trafficking defects. At first glance this would suggest that they work differently, however this disparity might just be a reflection of the fact that in yeast, Golgi cisterna are not stacked, they are small and are scattered throughout the cell, not requiring a tensile force to keep morphology and efficient budding of carriers. Since MYO18a is not present in yeast, evolution may have added this additional function to make the Golgi complex more efficient in larger cells.

PI4P and Arfs in cargo sorting at the TGN

The first evidence for a relationship between the small GTPase Arf1 and PI4P was the finding that in mammalian cells activated Arf1 recruits PI4KIIIβ to the Golgi^[33]. It was then found that the clathrin adaptor AP-1, involved in collecting soluble lysosomal enzymes by the mannose-6-phosphate receptors for transport from the TGN to the early endosome, is an Arf1 effector that binds PI4P directly^[35, 43]. Likewise, the Golgi-localized, γ-ear containing, ADP-ribosylation factor binding proteins (GGAs) are also involved in transport

to the endosome and ultimately to the lysosome^[44]. The relationship between AP-1 and GGAs in this clathrin-dependent pathway is not yet clear, as they both localize to the TGN, both bind Arf1 and PI4P, yet they do not co-localize precisely^[45, 46]. This could be because there are two pathways, or a temporal involvement in the two classes of adaptor molecules. Knock-down of PI4KII α reduces the association of all three GGAs with the Golgi, especially that of GGA1^[44]. The GAT domain of GGAs mediates PI4P binding and mutations that abolish PI4P binding render the protein nonfunctional *in vivo*. A correlation was also found between PI4P binding and increased affinity for ubiquitin in GGA1, suggesting that PI4P-bound GGA1 is more accessible for binding ubiquitinated cargo. More recently, very rapid depletion of Golgi PI4P through the recruitment of a PIP phosphatase to the Golgi^[47] recapitulated most of these results, but also showed that the PI requirement for GGA3 might be distinct, or a second determinant drives its localization. Another important component of this system is EpsinR, a PI4P-, AP-1-, and clathrin-binding protein, again revealing how multiple interactions help coordinate cargo sorting^[34, 48]. This same type of mechanism may apply to other coat/adaptor complexes, like vertebrate endosomal AP-3 that is involved in direct transport from early endosomes to the lysosome or lysosome-related organelles^[49]. Although it is unclear if it binds any PI, it has been shown to bind Arf1- and Arf6-GTP *in vitro*^[43] and to associate with PI4KII α as a cargo⁵⁰. Moreover, both the kinase activity of PI4KII α and its AP-3 sorting motif are required for AP-3 function, indicating that not only recognition of the kinase is important, but its lipid product also regulates AP-3 complex function^[50, 51].

In yeast, both Arf1p and Pik1p localize to the Golgi coordinating the generation of the two signals, and their functions are closely related since *arf1* Δ cells also have a 40% reduction in PI4P levels and therefore exhibit most of the phenotypes of a *pik1* mutant³². Although no direct interaction between Arf1p and Pik1p has been reported in yeast, *arf1* Δ is synthetic lethal with *pik1* mutations and, recently, the TGN-localized Arf1-GEF Sec7p was shown to associate with Pik1p, so Arf1p and PI4P also provide Golgi-specific signals in yeast^[52, 53]. AP-1, GGAs and clathrin also function in yeast to divert lysosomal enzymes to the endosome. As in the mammalian case, TGN recruitment of yeast Gga2p (one of the two yeast homologues of GGA1) depends on Pik1p and Arf1p function. Moreover, GST-Gga2 only binds well to liposomes when both PI4P and membrane-bound active Arf1p are present^[54]. By analogy, it is expected that AP-1 function also requires PI4P, although this has not yet been reported. Nevertheless, the PIP phosphatase Inp53/Sjl3p has been implicated specifically in the AP-1-dependent pathway, suggesting that PI4P may also have a role^[55]. In yeast, the adaptin AP-3 binds Arf1-GTP at the TGN to collect and deliver lysosomal membrane proteins to the vacuole^[56], however it is not yet clear if this pathway requires PI4P. Interestingly, yet another coat has been found in yeast that is dependent on Arf1p, termed the exomer^[57]. This novel coat is involved in the cell-cycle-regulated delivery of a subset of enzymes and proteins to the plasma membrane. This pathway represents regulated secretion, involved in cell wall synthesis and the mating response, processes not conserved outside of yeast^[58, 59]. PI4P or PI4,5P₂ promote the assembly of exomer on liposomes, although an *in vivo* requirement for either lipid has yet to be demonstrated^[57]. Overall, the three pathways in yeast that divert selected cargo at the TGN require Arf1p, and those that have been studied require PI4P. This dual requirement for PI4P and Arf1 provide a code for TGN sorting in a similar way that PI3P and Rab5 provide a code for endosomal sorting. In both cases, coincidence detection appears to be critical for sorting, most probably by restricting cargo selection and vesicle assembly events to specific subdomains (Figure 2).

PI4P, Arf1 and Rabs in constitutive traffic

In contrast to the segregation of selected cargos for delivery to endosomes and vacuole/lysosomes described above, no coat proteins have been described for carriers mediating constitutive transport from the TGN to the plasma membrane. However, effectors for PI4P at the TGN play important roles in this pathway in both mammalian and yeast systems.

A role for TGN-localized PI4P-binding proteins was first reported with the characterization of FAPP1 and FAPP2, proteins containing a PH domain that bind PI4P and Arf1-GTP^[60]. Knockdown of both proteins blocks transport of cargo to the plasma membrane while displacement of the proteins by overexpression of the FAPP1 PH domain disrupts Golgi morphology and prevents membrane carrier fission. These results implicate them in the export of cargo from the TGN destined for the plasma membrane, a function shared between the two. However, FAPP2 has an additional glycolipid binding motif suggesting a unique function not shared with FAPP1. Consistently, RNAi-depletion of FAPP2 blocks apical, but not basolateral, transport, while depletion of FAPP1 alone has no effect^[60, 61]. FAPP2 and two other lipid-binding proteins, CERT (ceramide transfer) and OSBP (oxysterol-binding protein), have related PH domains that bind PI4P and another Golgi determinant. Just like the FAPPs, OSBP binds active Arf1, localizing to the TGN, while the other determinant for CERT localization is unclear. Since their PH domains are very similar, these proteins may participate in regulating lipid pathways and/or membrane tubulation by inserting a wedge into the cytosolic leaflet as shown for the FAPP1 PH domain^[62], but exactly how they contribute to TGN function is not clear (see ^[63] for review).

In yeast, there are no known factors that require both Arf1p and Golgi-localized PI4P for constitutive secretory traffic. The redundant genes *ARF1* and *ARF2* provide an essential function, with the retrograde traffic involving COPI probably being essential and the anterograde traffic from the TGN involving AP-1, GGAs and AP-3 described above each being non-essential. However, the GEF for TGN-localized Arf1p is Sec7p, an essential protein, suggesting that there is an essential requirement for Arf1-GTP at the TGN, presumably in forward constitutive transport. Despite this gap in understanding Arf1p, recent work has implicated a different class of GTPases in this step, namely Rab proteins. Indeed, mammalian Rab11 interacts directly with PI4KIII β , resulting in an association of Rab11 with subdomains of the Golgi^[64]. In tip-growing plant cells, the Rab11-related RabA4b binds a PI 4-kinase and helps localize it to the TGN^[65] (Box 2). The fly homologue of PI4KIII β , four wheel drive (Fwd), is required for the formation of PI4P- and Rab11-containing secretory organelles that localize to the midzone in dividing cells^[66]. More recently, it was reported that both PI4P and Rab1 are needed for the recruitment of an Arf GEF, GBF1, to Golgi membranes, although no direct interaction between the lipid or the Rab and GBF1 was shown^[67]. In yeast, the Rab11 homologues Ypt31/32p interact genetically with Pik1p and, although no direct interaction has been reported, they are part of a dual signal necessary to localize Sec2p, the GEF for the Rab Sec4p, an essential component of TGN to plasma membrane traffic. Sec2p associates with secretory vesicles as they emerge from the TGN and remains with them until they dock at sites of cell growth^[68]. Initial studies found that a C-terminal region of Sec2p is important for its correct localization^[69]. Since over-expression of Ypt31/32p can restore localization of the C-terminal truncation mutants, and Ypt31/32-GTP binds to a more N-terminal region, it appears that Sec2p must have another localization determinant that can be compensated for by *YPT31/32* over-expression. This other determinant turns out to be PI4P, with three positive-charged patches in the middle of Sec2p contributing a low affinity binding site for PI4P, and thereby establishing another example of a coincidence detection system at the Golgi^[70] (Figure 2B). In addition to providing a localization role, Golgi PI4P also regulates the properties of Sec2p. The region of Sec2p that interacts with Ypt31/32p, also interacts

with Sec15p, a component of the exocyst involved in tethering secretory vesicles at their site of exocytosis^[71]. Interestingly, it was found that GST-Sec2p does not bind Sec15p efficiently in the presence of liposomes containing PI4P, whereas PI4P had no effect on the binding to Ypt31/32p^[70]. The interpretation is that Ypt31/32p and PI4P recruit Sec2p to the TGN and, as PI4P levels drop, Sec2p switches from binding Ypt31/32p to binding Sec15p, which later helps tether the vesicles with the plasma membrane^[71].

Secretory vesicles emerging from the TGN are actively transported to the site of cell growth by the class V myosin Myo2p moving along polarized actin cables^[72–74]. Myo2p is also involved in the segregation of most organelles during the cell cycle, including the TGN marked by Ypt31/32p, although transport of secretory compartments are probably its only essential function^[75]. Recent work has revealed that Rab proteins and PI4P are necessary to link secretory compartments to Myo2p^[76] (Figure 2B). *Myo2* mutants conditionally defective in binding secretory vesicles^[73] can be suppressed by elevating the level of PI4P at the TGN. These same mutants can also be suppressed by over-expression of the post-Golgi Rab protein Sec4p, suggesting that both PI4P and Sec4p collaborate in the association of Myo2p with secretory cargo. In support of this model, Myo2p dissociates from secretory vesicles when the level of Golgi PI4P is rapidly reduced in a conditional *pik1* mutant, and Sec4p binds directly to the tail of Myo2p. Over-expression of *SEC2* does not suppress the *myo2* mutants, suggesting that the PI4P effects are not just a result of modulating Sec2p recruitment to vesicles. Moreover, a Myo2p construct in which the tail domain is replaced by the PI4P-binding PH domain from FAPP1 demonstrated a direct role for PI4P in the membrane association of Myo2p. Although this construct cannot complement *myo2Δ*, when introduced into *myo2* mutants sensitive to reduced PI4P levels, it dimerizes with the mutated Myo2p to suppress their temperature-sensitivity by enhancing association with PI4P. Moreover, this construct can suppress a lethal defect rendering Myo2p unable to bind Sec4p. Overall, these studies reveal that Myo2p binds to and transports compartments of the late secretory pathway in a Rab- and PI4P-dependent manner. So far the mechanism whereby Myo2p binds PI4P is not known; at least *in vitro*, the Myo2 cargo binding tail does not bind PI4P-containing liposomes. Could Myo2p link through Vps74p and perform a similar function to mammalian MYO18a, which binds GOLPH3/Vps74? Genetic approaches show no evidence for an interaction between Myo2p and Vps74p, making this scenario very unlikely. Moreover, the GOLPH3/Vps74 myosin-dependent function in mammals seems to be the generation or extraction of vesicles or tubular carriers from the TGN, while in yeast Myo2p is not necessary for secretory vesicle formation at the TGN, as secretion occurs normally, albeit in an unpolarized manner, in *myo2* mutants^[73].

Subversion of PI4P, Arfs, and Rabs by bacterial pathogens

Pathogenic microorganisms have evolved sophisticated strategies to gain access into cells and generate a permissive environment for their replication^[77]. Many of the virulence factors used by these pathogens are either PI effectors, PI metabolizing enzymes, or factors that interact with GTPases. By examining the association of these pathogens with specific PIs or GTPases we can begin to understand which branch of membrane trafficking they hijack for their benefit. Some, like *Mycobacterium* and *Salmonella*, manipulate host PI3P metabolism, and associate with Rab5/7 endocytic membranes. Others like *Legionella* and *Chlamydia*, prefer to use the exocytic pathway and hence encode effector proteins involved in PI4P metabolism and Rab1/6 recruitment and activation. The fact that when bacteria take advantage of a specific PI lipid it is always accompanied by the corresponding Rab GTPase underscores the close relationship the two factors have in regulating normal cellular functions.

Legionella species, the causative agent of Legionnaires' disease^[78], are natural parasites of free living amoebas. Within amoebas, or macrophages in humans, *Legionella* forms a new compartment known as the *Legionella*-containing vacuole (LCV) that mimics the Golgi apparatus by generating PI4P in a mechanism requiring host PI4KIII β . Three *Legionella* effectors bind PI4P: the Rab1 GEF SidM, which also acts as a Rab GDI dissociation factor, and the paralogues SidC and SdcA, which function as a tether for ER-derived vesicles^[79–81]. Another effector that binds PI3P and PI4P *in vitro* is LidA, which enhances SidM's GEF activity and also recruits Rab1, Rab6 and Rab8. Interestingly, SidC recruitment depends on multiple signals as depletion of Rab8, Arf1, or PI4KIII β prevents its association with the LCV^[82].

Chlamydia, the leading cause of bacterial sexually transmitted disease, replicates inside a membrane-bound compartment termed the inclusion. Of several GFP-Rab fusions tested, only Rab4, Rab11, and Rab1 were found to decorate the inclusion of all chlamydial species, while Rab6 and Rab10 were recruited to a subset of species^[83]. This indicates that *Chlamydia*, just like *Legionella*, resides in a niche formed by mimicking membranes of the exocytic pathway. Consistent with this, the inclusion membrane is rich in PI4P; Arf1, PI4KII α , and the PIP 5-phosphatase OCRL, proteins involved in the metabolism of this PI4P, are also present^[84]. OCRL is an effector of Rab1, Rab5, and Rab6 *in vitro*, but its localization in cells is at the TGN and endosomes where it plays a role in regulating the traffic between these compartments. Consistent with their collective importance, knock-down of any of these components individually and knock-down of multiple components simultaneously results in synergistic effects. Knock-down of OCRL, PI4KII α , and Arf1 simultaneously essentially eliminated inclusion formation and therefore infectivity of the progeny^[84].

Concluding remarks

Coincidence detection at membrane sorting hubs appears to be an ancient mechanism exploited by the common ancestor of fungi, plants, and animals. By combining two different identity codes, the cell can control the specificity of protein recruitment, cargo segregation, carrier generation and transport. This is especially true for the cell sorting hubs that constantly receive and transfer an immense flux of material, but also extends to other membrane-associated regulatory events such as plasma membrane kinases signaling (for a recent example see^[85]). Although this concept of coincidence detection was first appreciated in the endocytic pathway decades ago, many critical questions still remain. First, we still lack a detailed understanding of how the different domains and processes are segregated from each other. Second, we almost certainly do not know all the proteins involved – the PI4P-binding proteins GOLPH3/Vps74p and Sec2p have only just been discovered, so it seems likely that additional lipid binding proteins that function at the TGN are going to be found. Third, it is surprising that no coat has been found for constitutive secretion. Does this mean that the TGN generated by cisternal progression simply fragments into vesicles that constitute the constitutive pathway? This seems unlikely because, at least in yeast, post-Golgi secretory vesicles are of uniform size. Fourth, to what extent is the regulatory lipid PI4P included in compartments derived from the TGN? Most likely a gradient of this regulatory lipid occurs, as seems to be the case for PI4P in the constitutive secretory pathway in yeast and animal cells. If this gradient exists, which phosphatase generates the gradient, and how is it regulated? Additional questions relate to more distant roles. For example, Golgi-localized yeast PI 4-kinase, Pik1p, also has an essential role in the nucleus^[86], but what is this function and how does it interface with Pik1p's role at the TGN? Despite these questions, PI4P is now firmly established as a key regulator at the TGN and future studies on sorting, cargo selection and membrane transport will always have to consider a role for this important phosphoinositide.

Acknowledgments

We thank Chris Fromme and Bretscher lab members for reading and making comments on the manuscript, and the US National Institute of General Medical Sciences for funding research in the authors' laboratory.

References

1. Hutagalung AH, Novick PJ. Role of Rab GTPases in membrane traffic and cell physiology. *Physiol Rev.* 2011; 91:119–149. [PubMed: 21248164]
2. Behnia R, Munro S. Organelle identity and the signposts for membrane traffic. *Nature.* 2005; 438:597–604. [PubMed: 16319879]
3. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. *Nature.* 2006; 443:651–657. [PubMed: 17035995]
4. Clague MJ, et al. Regulation of early-endosome dynamics by phosphatidylinositol 3-phosphate binding proteins. *Biochem Soc Trans.* 1999; 27:662–666. [PubMed: 10917662]
5. Schu PV, et al. Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. *Science.* 1993; 260:88–91. [PubMed: 8385367]
6. Bucci C, et al. The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. *Cell.* 1992; 70:715–728. [PubMed: 1516130]
7. Li G, et al. Evidence for phosphatidylinositol 3-kinase as a regulator of endocytosis via activation of Rab5. *Proc Natl Acad Sci U S A.* 1995; 92:10207–10211. [PubMed: 7479754]
8. Sato M, et al. *Caenorhabditis elegans* RME-6 is a novel regulator of RAB-5 at the clathrin-coated pit. *Nat Cell Biol.* 2005; 7:559–569. [PubMed: 15895077]
9. Semerdjieva S, et al. Coordinated regulation of AP2 uncoating from clathrin-coated vesicles by rab5 and hRME-6. *J Cell Biol.* 2008; 183:499–511. [PubMed: 18981233]
10. Christoforidis S, et al. Phosphatidylinositol-3-OH kinases are Rab5 effectors. *Nat Cell Biol.* 1999; 1:249–252. [PubMed: 10559924]
11. Simonsen A, et al. EEA1 links PI(3)K function to Rab5 regulation of endosome fusion. *Nature.* 1998; 394:494–498. [PubMed: 9697774]
12. Dumas JJ, et al. Multivalent endosome targeting by homodimeric EEA1. *Mol Cell.* 2001; 8:947–958. [PubMed: 11741531]
13. Katzmann DJ, et al. Vps27 recruits ESCRT machinery to endosomes during MVB sorting. *J Cell Biol.* 2003; 162:413–423. [PubMed: 12900393]
14. Kinchen JM, Ravichandran KS. Identification of two evolutionarily conserved genes regulating processing of engulfed apoptotic cells. *Nature.* 2010; 464:778–782. [PubMed: 20305638]
15. Poteryaev D, et al. Identification of the switch in early-to-late endosome transition. *Cell.* 2010; 141:497–508. [PubMed: 20434987]
16. Nordmann M, et al. The Mon1-Ccz1 complex is the GEF of the late endosomal Rab7 homolog Ypt7. *Curr Biol.* 2010; 20:1654–1659. [PubMed: 20797862]
17. Bokel C, et al. Sara endosomes and the maintenance of Dpp signaling levels across mitosis. *Science.* 2006; 314:1135–1139. [PubMed: 17110576]
18. Shi W, et al. Endofin acts as a Smad anchor for receptor activation in BMP signaling. *J Cell Sci.* 2007; 120:1216–1224. [PubMed: 17356069]
19. Coumailleau F, et al. Directional Delta and Notch trafficking in Sara endosomes during asymmetric cell division. *Nature.* 2009; 458:1051–1055. [PubMed: 19295516]
20. Gillette JM, et al. Intercellular transfer to signalling endosomes regulates an ex vivo bone marrow niche. *Nat Cell Biol.* 2009; 11:303–311. [PubMed: 19198600]
21. van der Sluijs P, et al. The small GTP-binding protein rab4 controls an early sorting event on the endocytic pathway. *Cell.* 1992; 70:729–740. [PubMed: 1516131]
22. Yudowski GA, et al. Cargo-mediated regulation of a rapid Rab4-dependent recycling pathway. *Mol Biol Cell.* 2009; 20:2774–2784. [PubMed: 19369423]
23. Ullrich O, et al. Rab11 regulates recycling through the pericentriolar recycling endosome. *J Cell Biol.* 1996; 135:913–924. [PubMed: 8922376]

24. Hales CM, et al. Identification and characterization of a family of Rab11-interacting proteins. *J Biol Chem.* 2001; 276:39067–39075. [PubMed: 11495908]
25. Hay JC, Martin TF. Phosphatidylinositol transfer protein required for ATP-dependent priming of Ca(2+)-activated secretion. *Nature.* 1993; 366:572–575. [PubMed: 8255295]
26. Hay JC, et al. ATP-dependent inositide phosphorylation required for Ca(2+)-activated secretion. *Nature.* 1995; 374:173–177. [PubMed: 7877690]
27. Wiedemann C, et al. Chromaffin granule-associated phosphatidylinositol 4-kinase activity is required for stimulated secretion. *EMBO J.* 1996; 15:2094–2101. [PubMed: 8641275]
28. Wiedemann C, et al. An essential role for a small synaptic vesicle-associated phosphatidylinositol 4-kinase in neurotransmitter release. *J Neurosci.* 1998; 18:5594–5602. [PubMed: 9671651]
29. Hama H, et al. Direct involvement of phosphatidylinositol 4-phosphate in secretion in the yeast *Saccharomyces cerevisiae*. *J Biol Chem.* 1999; 274:34294–34300. [PubMed: 10567405]
30. Walch-Solimena C, Novick P. The yeast phosphatidylinositol-4-OH kinase pik1 regulates secretion at the Golgi. *Nat Cell Biol.* 1999; 1:523–525. [PubMed: 10587649]
31. Bankaitis VA, et al. An essential role for a phospholipid transfer protein in yeast Golgi function. *Nature.* 1990; 347:561–562. [PubMed: 2215682]
32. Audhya A, et al. Distinct roles for the yeast phosphatidylinositol 4-kinases, Stt4p and Pik1p, in secretion, cell growth, and organelle membrane dynamics. *Mol Biol Cell.* 2000; 11:2673–2689. [PubMed: 10930462]
33. Godi A, et al. ARF mediates recruitment of PtdIns-4-OH kinase-beta and stimulates synthesis of PtdIns(4,5)P2 on the Golgi complex. *Nat Cell Biol.* 1999; 1:280–287. [PubMed: 10559940]
34. Mills IG, et al. EpsinR: an AP1/clathrin interacting protein involved in vesicle trafficking. *J Cell Biol.* 2003; 160:213–222. [PubMed: 12538641]
35. Wang YJ, et al. Phosphatidylinositol 4 phosphate regulates targeting of clathrin adaptor AP-1 complexes to the Golgi. *Cell.* 2003; 114:299–310. [PubMed: 12914695]
36. Weixel KM, et al. Distinct Golgi populations of phosphatidylinositol 4-phosphate regulated by phosphatidylinositol 4-kinases. *J Biol Chem.* 2005; 280:10501–10508. [PubMed: 15634669]
37. Dippold HC, et al. GOLPH3 bridges phosphatidylinositol-4-phosphate and actomyosin to stretch and shape the Golgi to promote budding. *Cell.* 2009; 139:337–351. [PubMed: 19837035]
38. Bonangelino CJ, et al. Genomic screen for vacuolar protein sorting genes in *Saccharomyces cerevisiae*. *Mol Biol Cell.* 2002; 13:2486–2501. [PubMed: 12134085]
39. Schmitz KR, et al. Golgi localization of glycosyltransferases requires a Vps74p oligomer. *Dev Cell.* 2008; 14:523–534. [PubMed: 18410729]
40. Tu L, et al. Signal-mediated dynamic retention of glycosyltransferases in the Golgi. *Science.* 2008; 321:404–407. [PubMed: 18635803]
41. Wood CS, et al. PtdIns4P recognition by Vps74/GOLPH3 links PtdIns 4-kinase signaling to retrograde Golgi trafficking. *J Cell Biol.* 2009; 187:967–975. [PubMed: 20026658]
42. Spang A. ARF1 regulatory factors and COPI vesicle formation. *Curr Opin Cell Biol.* 2002; 14:423–427. [PubMed: 12383792]
43. Austin C, et al. Site-specific cross-linking reveals a differential direct interaction of class 1, 2, and 3 ADP-ribosylation factors with adaptor protein complexes 1 and 3. *Biochemistry.* 2002; 41:4669–4677. [PubMed: 11926829]
44. Wang J, et al. 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.* 2007; 18:2646–2655. [PubMed: 17494868]
45. Dell'Angelica EC, et al. GGAs: a family of ADP ribosylation factor-binding proteins related to adaptors and associated with the Golgi complex. *J Cell Biol.* 2000; 149:81–94. [PubMed: 10747089]
46. Bonifacino JS. The GGA proteins: adaptors on the move. *Nat Rev Mol Cell Biol.* 2004; 5:23–32. [PubMed: 14708007]
47. Szentpetery Z, et al. Acute manipulation of Golgi phosphoinositides to assess their importance in cellular trafficking and signaling. *Proc Natl Acad Sci U S A.* 2010; 107:8225–8230. [PubMed: 20404150]

48. Hirst J, et al. EpsinR: an ENTH domain-containing protein that interacts with AP-1. *Mol Biol Cell*. 2003; 14:625–641. [PubMed: 12589059]
49. Dell'Angelica EC. AP-3-dependent trafficking and disease: the first decade. *Curr Opin Cell Biol*. 2009; 21:552–559. [PubMed: 19497727]
50. Craig B, et al. 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*. 2008; 19:1415–1426. [PubMed: 18256276]
51. Salazar G, et al. Hermansky-Pudlak syndrome protein complexes associate with phosphatidylinositol 4-kinase type II alpha in neuronal and non-neuronal cells. *J Biol Chem*. 2009; 284:1790–1802. [PubMed: 19010779]
52. Sciorra VA, et al. Synthetic genetic array analysis of the PtdIns 4-kinase Pik1p identifies components in a Golgi-specific Ypt31/rab-GTPase signaling pathway. *Mol Biol Cell*. 2005; 16:776–793. [PubMed: 15574876]
53. Gloor Y, et al. Interaction between Sec7p and Pik1p: the first clue for the regulation of a coincidence detection signal. *Eur J Cell Biol*. 2010; 89:575–583. [PubMed: 20434792]
54. Demmel L, et al. The clathrin adaptor Gga2p is a phosphatidylinositol 4-phosphate effector at the Golgi exit. *Mol Biol Cell*. 2008; 19:1991–2002. [PubMed: 18287542]
55. Ha SA, et al. The synaptojanin-like protein Inp53/Sjl3 functions with clathrin in a yeast TGN-to-endosome pathway distinct from the GGA protein-dependent pathway. *Mol Biol Cell*. 2003; 14:1319–1333. [PubMed: 12686590]
56. Cowles CR, et al. The AP-3 adaptor complex is essential for cargo-selective transport to the yeast vacuole. *Cell*. 1997; 91:109–118. [PubMed: 9335339]
57. Wang CW, et al. Exomer: A coat complex for transport of select membrane proteins from the trans-Golgi network to the plasma membrane in yeast. *J Cell Biol*. 2006; 174:973–983. [PubMed: 17000877]
58. Barfield RM, et al. The exomer coat complex transports Fus1p to the plasma membrane via a novel plasma membrane sorting signal in yeast. *Mol Biol Cell*. 2009; 20:4985–4996. [PubMed: 19812245]
59. Trautwein M, et al. Arf1p, Chs5p and the ChAPs are required for export of specialized cargo from the Golgi. *EMBO J*. 2006; 25:943–954. [PubMed: 16498409]
60. Godi A, et al. FAPPs control Golgi-to-cell-surface membrane traffic by binding to ARF and PtdIns(4)P. *Nat Cell Biol*. 2004; 6:393–404. [PubMed: 15107860]
61. Vieira OV, et al. FAPP2 is involved in the transport of apical cargo in polarized MDCK cells. *J Cell Biol*. 2005; 170:521–526. [PubMed: 16103222]
62. Lenoir M, et al. Structural basis of wedging the Golgi membrane by FAPP pleckstrin homology domains. *EMBO Rep*. 2010; 11:279–284. [PubMed: 20300118]
63. Graham TR, Burd CG. Coordination of Golgi functions by phosphatidylinositol 4-kinases. *Trends Cell Biol*. 2011; 21:113–121. [PubMed: 21282087]
64. de Graaf P, et al. Phosphatidylinositol 4-kinasebeta is critical for functional association of rab11 with the Golgi complex. *Mol Biol Cell*. 2004; 15:2038–2047. [PubMed: 14767056]
65. Preuss ML, et al. A role for the RabA4b effector protein PI-4Kbeta1 in polarized expansion of root hair cells in *Arabidopsis thaliana*. *J Cell Biol*. 2006; 172:991–998. [PubMed: 16567499]
66. Poleyov G, et al. Dual roles for the *Drosophila* PI 4-kinase four wheel drive in localizing Rab11 during cytokinesis. *J Cell Biol*. 2009; 187:847–858. [PubMed: 19995935]
67. Dumaresq-Doiron K, et al. The phosphatidylinositol 4-kinase PI4KIIIalpha is required for the recruitment of GBF1 to Golgi membranes. *J Cell Sci*. 2010; 123:2273–2280. [PubMed: 20530568]
68. Walch-Solimena C, et al. Sec2p mediates nucleotide exchange on Sec4p and is involved in polarized delivery of post-Golgi vesicles. *J Cell Biol*. 1997; 137:1495–1509. [PubMed: 9199166]
69. Ortiz D, et al. Ypt32 recruits the Sec4p guanine nucleotide exchange factor, Sec2p, to secretory vesicles; evidence for a Rab cascade in yeast. *J Cell Biol*. 2002; 157:1005–1015. [PubMed: 12045183]
70. Mizuno-Yamasaki E, et al. Phosphatidylinositol 4-phosphate controls both membrane recruitment and a regulatory switch of the Rab GEF Sec2p. *Dev Cell*. 2010; 18:828–840. [PubMed: 20493815]

71. Medkova M, et al. The rab exchange factor Sec2p reversibly associates with the exocyst. *Mol Biol Cell*. 2006; 17:2757–2769. [PubMed: 16611746]
72. Pruyne DW, et al. Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. *J Cell Biol*. 1998; 143:1931–1945. [PubMed: 9864365]
73. Schott D, et al. The COOH-terminal domain of Myo2p, a yeast myosin V, has a direct role in secretory vesicle targeting. *J Cell Biol*. 1999; 147:791–808. [PubMed: 10562281]
74. Schott DH, et al. Secretory vesicle transport velocity in living cells depends on the myosin-V lever arm length. *J Cell Biol*. 2002; 156:35–39. [PubMed: 11781333]
75. Pruyne D, et al. Mechanisms of polarized growth and organelle segregation in yeast. *Annu Rev Cell Dev Biol*. 2004; 20:559–591. [PubMed: 15473852]
76. Santiago-Tirado FH, et al. PI4P and Rab inputs collaborate in myosin-V-dependent transport of secretory compartments in yeast. *Dev Cell*. 2011; 20:47–59. [PubMed: 21238924]
77. Cossart P, Roy CR. Manipulation of host membrane machinery by bacterial pathogens. *Curr Opin Cell Biol*. 2010; 22:547–554. [PubMed: 20542678]
78. McDade JE, et al. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. *N Engl J Med*. 1977; 297:1197–1203. [PubMed: 335245]
79. Weber SS, et al. Legionella pneumophila exploits PI(4)P to anchor secreted effector proteins to the replicative vacuole. *PLoS Pathog*. 2006; 2:e46. [PubMed: 16710455]
80. Brombacher E, et al. Rab1 guanine nucleotide exchange factor SidM is a major phosphatidylinositol 4-phosphate-binding effector protein of Legionella pneumophila. *J Biol Chem*. 2009; 284:4846–4856. [PubMed: 19095644]
81. Ragaz C, et al. The Legionella pneumophila phosphatidylinositol-4 phosphate-binding type IV substrate SidC recruits endoplasmic reticulum vesicles to a replication-permissive vacuole. *Cell Microbiol*. 2008; 10:2416–2433. [PubMed: 18673369]
82. Urwyler S, et al. Proteome analysis of Legionella vacuoles purified by magnetic immunoseparation reveals secretory and endosomal GTPases. *Traffic*. 2009; 10:76–87. [PubMed: 18980612]
83. Rzomp KA, et al. Rab GTPases are recruited to chlamydial inclusions in both a species-dependent and species-independent manner. *Infect Immun*. 2003; 71:5855–5870. [PubMed: 14500507]
84. Moorhead AM, et al. Multiple host proteins that function in phosphatidylinositol-4-phosphate metabolism are recruited to the chlamydial inclusion. *Infect Immun*. 2010; 78:1990–2007. [PubMed: 20231409]
85. Storchli TI, et al. Phosphoinositides are essential coactivators for p21-activated kinase 1. *Mol Cell*. 2010; 40:493–500. [PubMed: 21070974]
86. Strahl T, et al. Yeast phosphatidylinositol 4-kinase, Pik1, has essential roles at the Golgi and in the nucleus. *J Cell Biol*. 2005; 171:967–979. [PubMed: 16365163]
87. Hokin MR, Hokin LE. Enzyme secretion and the incorporation of P32 into phospholipides of pancreas slices. *J Biol Chem*. 1953; 203:967–977. [PubMed: 13084667]
88. Hokin LE, Hokin MR. Phosphoinositides and protein secretion in pancreas slices. *J Biol Chem*. 1958; 233:805–810. [PubMed: 13587496]
89. Michell RH. Inositol phospholipids and cell surface receptor function. *Biochim Biophys Acta*. 1975; 415:81–47. [PubMed: 164246]
90. Abdel-Latif AA, et al. Acetylcholine increases the breakdown of triphosphoinositide of rabbit iris muscle prelabelled with [32P] phosphate. *Biochem J*. 1977; 162:61–73. [PubMed: 192213]
91. Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*. 1984; 312:315–321. [PubMed: 6095092]
92. Kucera GL, Rittenhouse SE. Human platelets form 3-phosphorylated phosphoinositides in response to alpha-thrombin, U46619, or GTP gamma S. *J Biol Chem*. 1990; 265:5345–5348. [PubMed: 2156815]
93. Hawkins PT, et al. Platelet-derived growth factor stimulates synthesis of PtdIns(3,4,5)P3 by activating a PtdIns(4,5)P2 3-OH kinase. *Nature*. 1992; 358:157–159. [PubMed: 1319558]
94. Hilpela P, et al. Regulation of the actin cytoskeleton by PI(4,5)P2 and PI(3,4,5)P3. *Curr Top Microbiol Immunol*. 2004; 282:117–163. [PubMed: 14594216]

95. Harlan JE, et al. Pleckstrin homology domains bind to phosphatidylinositol-4,5-bisphosphate. *Nature*. 1994; 371:168–170. [PubMed: 8072546]
96. Artalejo CR, et al. Specific role for the PH domain of dynamin-1 in the regulation of rapid endocytosis in adrenal chromaffin cells. *EMBO J*. 1997; 16:1565–1574. [PubMed: 9130701]
97. Jefferson AB, et al. Inhibition of mSOS-activity by binding of phosphatidylinositol 4,5-P2 to the mSOS pleckstrin homology domain. *Oncogene*. 1998; 16:2303–2310. [PubMed: 9620547]
98. Rohatgi R, et al. Mechanism of N-WASP activation by CDC42 and phosphatidylinositol 4, 5-bisphosphate. *J Cell Biol*. 2000; 150:1299–1310. [PubMed: 10995436]
99. Bedinger PA, et al. Travelling in style: the cell biology of pollen. *Trends Cell Biol*. 1994; 4:132–138. [PubMed: 14731736]
100. Kost B, et al. Rac homologues and compartmentalized phosphatidylinositol 4, 5-bisphosphate act in a common pathway to regulate polar pollen tube growth. *J Cell Biol*. 1999; 145:317–330. [PubMed: 10209027]
101. Monteiro D, et al. Phosphoinositides and phosphatidic acid regulate pollen tube growth and reorientation through modulation of $[Ca^{2+}]_c$ and membrane secretion. *J Exp Bot*. 2005; 56:1665–1674. [PubMed: 15837704]
102. Helling D, et al. Pollen tube tip growth depends on plasma membrane polarization mediated by tobacco PLC3 activity and endocytic membrane recycling. *Plant Cell*. 2006; 18:3519–3534. [PubMed: 17172355]
103. Lee Y, et al. The Arabidopsis phosphatidylinositol 3-kinase is important for pollen development. *Plant Physiol*. 2008; 147:1886–1897. [PubMed: 18515640]
104. Dove SK, et al. Osmotic stress activates phosphatidylinositol-3,5-bisphosphate synthesis. *Nature*. 1997; 390:187–192. [PubMed: 9367158]
105. Whitley P, et al. Arabidopsis FAB1/PIKfyve proteins are essential for development of viable pollen. *Plant Physiol*. 2009; 151:1812–1822. [PubMed: 19846542]
106. Hirano T, et al. Loss-of-function and gain-of-function mutations in FAB1A/B impair endomembrane homeostasis, conferring pleiotropic developmental abnormalities in arabidopsis. *Plant Physiol*. 2011; 155:797–807. [PubMed: 21173023]
107. Thole JM, Nielsen E. Phosphoinositides in plants: novel functions in membrane trafficking. *Curr Opin Plant Biol*. 2008; 11:620–631. [PubMed: 19028349]
108. Vermeer JE, et al. Imaging phosphatidylinositol 4-phosphate dynamics in living plant cells. *Plant J*. 2009; 57:356–372. [PubMed: 18785997]
109. Kang BH, et al. Electron Tomography of RabA4b- and PI-4Kbeta1-Labeled Trans Golgi Network Compartments in Arabidopsis. *Traffic*. 2011; 12:313–329. [PubMed: 21134079]
110. Thole JM, et al. Root hair defective4 encodes a phosphatidylinositol-4-phosphate phosphatase required for proper root hair development in Arabidopsis thaliana. *Plant Cell*. 2008; 20:381–395. [PubMed: 18281508]
111. Vincent P, et al. A Sec14p-nodulin domain phosphatidylinositol transfer protein polarizes membrane growth of Arabidopsis thaliana root hairs. *J Cell Biol*. 2005; 168:801–812. [PubMed: 15728190]
112. Bohme K, et al. The Arabidopsis COW1 gene encodes a phosphatidylinositol transfer protein essential for root hair tip growth. *Plant J*. 2004; 40:686–698. [PubMed: 15546352]
113. Levine TP, Munro S. The pleckstrin homology domain of oxysterol-binding protein recognizes a determinant specific to Golgi membranes. *Curr Biol*. 1998; 8:729–739. [PubMed: 9651677]
114. Levine TP, Munro S. Dual targeting of Osh1p, a yeast homologue of oxysterol-binding protein, to both the Golgi and the nucleus-vacuole junction. *Mol Biol Cell*. 2001; 12:1633–1644. [PubMed: 11408574]
115. Li X, et al. Analysis of oxysterol binding protein homologue Kes1p function in regulation of Sec14p-dependent protein transport from the yeast Golgi complex. *J Cell Biol*. 2002; 157:63–77. [PubMed: 11916983]
116. Wild AC, et al. The p21-activated protein kinase-related kinase Cla4 is a coincidence detector of signaling by Cdc42 and phosphatidylinositol 4-phosphate. *J Biol Chem*. 2004; 279:17101–17110. [PubMed: 14766750]

117. Yamashita S, et al. PI4P-signaling pathway for the synthesis of a nascent membrane structure in selective autophagy. *J Cell Biol.* 2006; 173:709–717. [PubMed: 16754956]
118. Stahelin RV, et al. Structural and membrane binding analysis of the Phox homology domain of Bem1p: basis of phosphatidylinositol 4-phosphate specificity. *J Biol Chem.* 2007; 282:25737–25747. [PubMed: 17581820]
119. Natarajan P, et al. Regulation of a Golgi flippase by phosphoinositides and an ArfGEF. *Nat Cell Biol.* 2009; 11:1421–1426. [PubMed: 19898464]
120. Dowler S, et al. Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. *Biochem J.* 2000; 351:19–31. [PubMed: 11001876]
121. Levine TP, Munro S. Targeting of Golgi-specific pleckstrin homology domains involves both PtdIns 4-kinase-dependent and -independent components. *Curr Biol.* 2002; 12:695–704. [PubMed: 12007412]

Figure 1.

Intracellular distribution of phosphoinositides and small GTPases involved in membrane traffic. The localization of the different phosphoinositides and the transport steps that small GTPases regulate are shown in yeast (a) and mammalian (b) cells. EE, early endosome; LE/MVB, late endosome/multivesicular body; ER, endoplasmic reticulum.

Figure 2.

The cell's central sorting hubs and their effector proteins. (a) The generation of a recycling endosome and the different subdomains present. (1) PI 3-kinase, as well as the Rab5 GEF complex, is recruited by active Rab5 to a nascent endosome. Generation of both Rab5-GTP and PI3P recruits the tethering and fusion factor EEA1, promoting the fusion of incoming endocytic cargo. (2) Fusion, tubulation, and fission, as well as maturation of the membranes results in the sorting of cargo at the recycling endosome. (3) The SAND-1/Mon1-Ccz1 protein complex is required for maturation of the Rab5 domain into Rab7 by disrupting the Rab5 self-amplification loop and promoting the generation of a Rab7 feedback loop. Cargo recognition by specific adaptor proteins, such as Hrs, collects and sorts cargo destined for the late endosome/MVB. (4) Other factors, such as SARA, sort cargo into domains for

recycling through the Rab4 or Rab11 pathways. (b) Involvement of PI4P at the TGN sorting hub in yeast (left) and mammals (right). (1) Arf1 and PI 4-kinase contribute to Golgi PI4P and recruitment of Rab11 to PI4P-rich domains. (2) Normal Golgi morphology and tubulation by GOLPH3 and the unconventional myosin MYO18a. (3) Recruitment of GGAs/AP1/clathrin for sorting of proteins destined for endosomes. (4) Involvement of the PI4P- and Arf1-binding proteins FAPP1 and FAPP2 in traffic to the plasma membranes. (5) Retention and retrieval of a subset of Golgi resident enzymes by Vps74p and COPI vesicles. (6) Recruitment of Gga2/AP1/clathrin for sorting of proteins destined for endosomes. (7) Recruitment and assembly of the exomer coat for sorting of specialized cargo into vesicles. (8) Recruitment and conformational regulation of the Rab-GEF protein Sec2p for constitutive secretion. (9) Transport of secretory membranes by the myosin V, Myo2p.

Figure I.

Graph showing the recent increase in reports about PI4P. The number of publications was obtained from a Pubmed search of the phrase “phosphatidylinositol 4-phosphate functions” and subsequent manual validation. Although the numbers are clearly an underestimate, it shows an increasing trend in our knowledge of PI4P functions.

Figure II.

Schematic representing the localization of various proteins involved in tip growth. EE, early endosome; ER, endoplasmic reticulum; PI-PLC, PI specific phospholipase C.

TABLE 1

Proteins shown to bind PI4P directly in yeast, mammals, and bacteria (intracellular pathogens)*

Yeast		
Osh1/2/3/4p	Sterol traffic, control of PI4P levels, and of membrane contact sites	113–115
Cla4p	p21-activated kinase, involved in cell polarity regulation	116
PcAtg26	Ergosterol glucoside synthesizing enzyme required for autophagy	117
Gga2p	Clathrin adaptor, TGN to endosomal traffic	54
Bem1p	Multidomain scaffold protein critical for polarity establishment	118
Vps74p	Glycosyltransferase retention factor/retrieval receptor	37, 41
Drs2p	ATPase involved in phospholipid translocation (flippase)	119
Sec2p	GEF for exocytic Rab Sec4p	70
Mammalian		
OSBP/CERT/FAPP	Nonvesicular traffic of lipids, lipid balance in membranes, membrane deformation	113, 120, 121
epsinR	Clathrin adaptor, TGN to endosomal traffic	48
AP-1	Clathrin adaptor, TGN to endosomal traffic	35
GGAs	Clathrin adaptor, TGN to endosomal traffic	44
GOLPH3	Golgi function, coupling of Golgi function to cell metabolism	37
Bacteria (intracellular pathogens)		
SdcA/SidC	Paralogues involved in tethering ER-derived vesicles to the <i>Legionella</i> -containing vacuole	79, 81
SidM	<i>Legionella</i> GEF for Rab1	80

* Not a comprehensive list.