Published in final edited form as:
Trends Cell Biol. 2011 February ; 21(2): 113–121. doi:10.1016/j.tcb.2010.10.002.

Coordination of Golgi functions by phosphatidylinositol 4-kinases

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Abstract

Phosphatidylinositol 4-kinases (PI4Ks) regulate vesicle-mediated export from the Golgi apparatus via phosphatidylinositol 4-phosphate (PtdIns4*P*) binding effector proteins that control vesicle budding reactions and regulate membrane dynamics. From the characterization of Golgi PI4K effectors emerges evidence that vesicle budding and lipid dynamics are tightly coupled via a regulatory network that ensures that the appropriate membrane composition is established before a transport vesicle buds from the Golgi. An important hub of this network is protein kinase D, which regulates the activity of PI4K and several PtdIns4*P* effectors that control sphingolipid and sterol content of Golgi membranes. Other newly identified PtdIns4*P* effectors include Vps74/GOLPH3, a phospholipid flippase and a Rab GEF that orchestrate membrane transformation events facilitating vesicle formation and targeting. Here, we discuss how PtdIns4*P* signaling is integrated with membrane biosynthetic and vesicle budding machineries to potentially coordinate these critical functions of the Golgi apparatus.

Introduction

The Golgi apparatus occupies a central position in the secretory pathway playing well-known roles in glycoprotein maturation and the sorting of proteins into transport vesicles for exocytosis or delivery to the endosomal system. The Golgi also has an underappreciated function in lipid biosynthesis and many enzymes engaged in producing different sphingolipid species are localized to this organelle (Box 1). How the various activities of the Golgi apparatus are integrated to ensure appropriate membrane assembly and distribution of cargo is poorly understood. However, a number of studies over the past decade have implicated PtdIns4*P* in membrane biogenesis and vesicular transport at the *trans*-Golgi network suggesting a central regulatory role for this signaling lipid.

Box 1

Topology of sphingolipid biosynthetic events in the yeast and mammalian Golgi complex

In the mammalian Golgi complex (Figure Ia), ceramide (Cer) can spontaneously flip-flop between the two leaflets and is converted to either sphingomyelin (SM) in the luminal leaflet, or glucosylceramide (GlcCer) in the cytosolic leaflet. The luminal sphingomyelin synthase transfers the phosphorylcholine headgroup from phosphatidylcholine (PtdCho)

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to ceramide generating sphingomyelin and diacylglycerol (DAG) [86]. DAG also flip-flops spontaneously between leaflets and can be consumed in the cytosolic leaflet by conversion to PtdCho or phosphatidylethanolamine (not shown) through the Kennedy pathway. GlcCer synthase adds a glucose headgroup to ceramide in the cytosolic leaflet of the *cis*-Golgi. GlcCer must then translocate to the luminal leaflet for further glycosylation to produce a diverse group of complex glycosphingolipids (GSL) [87]. Although GlcCer can flip-flop spontaneously in artificial membranes [40], precisely how GlcCer gains access to the luminal glycosyltransferases is unclear (see text for Discussion).

A similar set of reactions is catalyzed in the yeast Golgi (Figure Ib) although a unique set of sphingolipids is produced. A luminal inositol phosphorylceramide (IPC) synthase transfers the inositol phosphate headgroup from phosphatidylinositol (PtdIns) to ceramide generating IPC and DAG. The IPC can be further glycosylated in the lumen with a mannose and an additional inositol phosphate to generate the mature glycosphingolipid (GSL).

Phosphorylated derivatives of phosphatidylinositol (phosphoinositides) regulate myriad aspects of eukaryotic cell physiology by serving as signaling molecules that convey spatial and temporal information to downstream effectors. Up to seven different phosphoinositides are produced in the cytosolic leaflets of cellular membranes and their site of localization and abundance is determined by the combined activities of phosphatidylinositol kinases and phosphatases that generate distinct phosphoinositide species. Phosphoinositide signaling operates in two general ways. First, acute stimulation of signaling leads to rapid, highly localized production of a phosphoinositide that serves as a relatively short-lived second messenger that conveys information to downstream pathway components. For example, within minutes of activation of the epidermal growth factor receptor, PtdIns(3,4,5)P_3 is produced in the plasma membrane via phosphorylation of PtdIns(4,5)P_2 by phosphatidylinositol 3-kinase, ultimately leading to activation of the Akt protein kinase which regulates cell growth and survival [1]. In the second general mode of signaling, specific phosphoinositides display a steady-state enrichment in particular organelle membranes where they constitute a defining feature of that organelle. For example, PtdIns(4,5)P_2 is a hallmark feature of the plasma membrane (PM), phosphatidylinositol 3-phosphate (PtdIns3*P*) marks endosomes, and phosphatidylinositol 4-phosphate (PtdIns4*P*) marks the Golgi apparatus. In this context phosphoinositides typically function as organelle specific ‘platforms’ upon which cytosolic proteins are recruited via specific phosphoinositide recognition, in conjunction with other organelle-specific features such as Ras-related GTPases [2]. This review summarizes recent progress in understanding the functions of PtdIns4*P*, the most abundant monophosphorylated phosphatidylinositol (PtdIns), with particular emphasis on the roles and regulation of PtdIns4*P* effectors at the Golgi apparatus, including lipid transfer proteins and vesicle budding factors.

Determinants of PtdIns4*P* levels in Golgi membranes

Two classes of phosphatidylinositol 4-kinases (PI4Ks), distinguished by their sensitivity to wortmannin and adenine, phosphorylate the D-4 position of the phosphatidylinositol headgroup to produce PtdIns4*P*. The human genome encodes two Type II PI4Ks (PI4KIIα and PI4KIIβ) and two Type III (PI4KIIIα and PI4KIIIβ) kinases, while the genome of the yeast *Saccharomyces cerevisiae*, an organism that has been enormously useful for investigating phosphoinositide signaling, encodes one Type II PI4K (Lsb6), and two Type III PI4Ks (Stt4 and Pik1). In all species and cell types examined the membranes of the Golgi apparatus and the plasma membrane harbor the highest steady state concentrations of PtdIns4*P*, with lesser amounts also detected on the ER and on endosomes [3,4].

The principal PI4Ks of the Golgi apparatus are PI4KIIα and PI4KIIIβ and their targeting and regulation largely determines the sites and amounts of PtdIns4*P* produced within Golgi membranes (Fig. 1a). Association of PI4KIIα with Golgi membranes is conferred by palmitoylation, but the basis of specific Golgi targeting and regulation of its activity are poorly understood [5,6]. Targeting of PI4KIIIβ to the Golgi is mediated principally by binding to GTP-bound Arf1 [7], a major regulator of protein trafficking, but PI4KIIIβ also binds a myristoylated calcium-binding protein called neuronal calcium sensor-1 (NCS-1) that also binds Arf1 [8–10]. Localization of yeast Pik1 to the TGN requires an NCS-1 homolog called Frq1 [11], but there is no known requirement for Arf. However, Pik1 may associate with Sec7, the guanine nucleotide exchange factor (GEF) that activates Arf1 [12], and thus, human PI4KIIIβ and yeast Pik1 should localize to regions of the Golgi containing activated Arf1. The activity of PI4KIIIβ is strongly activated by protein kinase D (PKD)-mediated phosphorylation [13] and PKD is itself targeted to the Golgi by binding to Arf1 [14].

Yeast Pik1 is phosphorylated by an as yet unidentified protein kinase, however, phosphorylation promotes dissociation of Pik1 from Golgi membranes and association with the 14-3-3 proteins Bmh1 and Bmh2 in the cytosol [15]. Other upstream factors control PtdIns4*P* levels at the TGN, including the PtdIns transfer proteins (PITPs) Nir2 in mammalian cells and Sec14 in yeast that are proposed to channel PtdIns to PI4Ks, either by ensuring an adequate PtdIns supply in the Golgi or by directly presenting PtdIns to the kinase [16,17]. Kes1 (also called Osh4) is a yeast oxysterol binding protein (OSBP) family member that represses PtdIns4*P* levels in the Golgi, although it isn't known if Kes1 carries out this task by inhibiting Pik1, stimulating phosphatase activity, or simply competing with other effectors for PtdIns4*P* interaction (Box 2) [18–20]. Collectively, these data indicate that PtdIns4*P* production is tightly coordinately with lipid dynamics and Arf activation.

Box 2

Potential functions for lipid transfer proteins in nonvesicular lipid transport or membrane sensing

An important unresolved issue is whether lipid transfer proteins (LTPs) mediate the nonvesicular transfer of lipid between organelles at contact sites or if they "sense" lipid composition locally and respond by controlling signaling cascades or enzymatic pathways [17,41,46,88]. The figure depicts these two models in the context of the mammalian (Figure IIa) and yeast (Figure IIb) Golgi, although the models are not necessarily mutually exclusive. In (Figure IIa), the LTPs CERT and OSBP1 transfer ceramide and cholesterol, respectively, from the ER to the Golgi via non-vesicular transport. This model assumes that CERT and OSBP1 interact simultaneously with Golgi and ER membranes by binding Golgi PtdIns4*P* via their PH domains and with VAP (VAP-A or VAP-B) in the ER membrane via their FFAT motifs, so that their lipid-binding domains are free to exchange ceramide and cholesterol between closely apposed Golgi and ER membranes. The PITP Nir2 binds VAP and is proposed to mediate exchange of PtdIns and PtdCho between the ER and Golgi [89]. Also in human cells, the LTP FAPP2 is proposed to mediate non-vesicular retrograde (Golgi to ER) delivery of GlcCer, a precursor of complex glycosphingolipids, so that it can be flipped to the luminal leaflet of the ER and returned by vesicular transport to the Golgi for conversion to complex glycosphingolipids [38]. In this model, PtdIns4*P* is used by LTPs as an identifying feature of Golgi membranes in ER-Golgi contacts, but it has yet to be established if these LTPs localize to sites of close apposition between specialized regions of the ER (*trans*-ER) and *trans* Golgi cisternae that have been observed in electron microscopy studies [90,91]. In addition, the role of CERT in non-vesicular ceramide

transport is compelling, but the evidence that FAPP2, OSBP and Nir2 actually transfer lipid between organelles is less clear [17,46].

In yeast, Golgi-ER contact points have not been observed and a complementary model of LTP function has emerged from studies of an OSBP, Kes1, and a PITP, Sec14. These LTPs are proposed to regulate the lipid composition of the TGN in support of vesicle-mediated trafficking by directly regulating the amount of PtdIns4*P* in the Golgi (Figure IIb). Sec14 maintains a Golgi pool of DAG by repressing PtdCho synthesis and coordinately promotes synthesis of PtdIns4*P*, possibly by channeling PtdIns to Pik1. Increased PtdIns4*P* promotes Golgi recruitment of Kes1, which is proposed to control the ergosterol (the major yeast sterol) content of the TGN either by directly mediating its transfer from the ER to the TGN, as shown in (Figure IIa), or by signaling to other factors that down-regulate PtdIns4*P* levels, as shown in (Figure IIb). In the latter model, the ratio of sterol-bound to sterol-free Kes1 on Golgi membranes serves as a measure of ergosterol content, and PtdIns4*P* levels are adjusted to coordinate vesicle budding with formation of the appropriate ergosterol and sphingolipid enriched membrane structure [92].

A curious aspect of PI4KIIIβ and Pik1 is that they shuttle through the nucleus [21–23]. No specific function for PI4Ks in the nucleus have been identified, however, engineered forms of yeast Pik1 that are restricted to the nucleus or to the cytoplasm fail to support cell viability, indicating that Pik1 has essential functions in both compartments [23]. Nuclear accumulation of Pik1 is promoted by nutrient (e.g., glucose) deprivation, a condition which also results in the release of PI4K effectors from Golgi membranes and slowing of the rate of secretion [15]. The upstream signaling pathways that regulate Golgi residence and enzymatic activity of Pik1, as well as the mechanism by which Pik1 is displaced from the Golgi upon nutrient deprivation, are not yet known.

A major determinant of PtdIns4*P* pools in membranes of the early secretory pathway is Sac1, an integral membrane phosphoinositide phosphatase that primarily resides in the ER and early cisternae of the Golgi apparatus of growing cells (Fig. 1a) [24–26]. Although mammalian PI4Ks have been localized to all Golgi cisternae, most Golgi PtdIns4*P* effectors are enriched in late Golgi compartments, suggesting that a gradient of PtdIns4*P* exists across the Golgi stack, with *cis* Golgi cisternae harboring the smallest amount of PtdIns4*P* and the TGN harboring the most (Fig. 1). Trafficking of Sac1 through early Golgi compartments is predicted to establish and maintain this gradient. Interestingly, nutrient status is a key determinant of Sac1 localization; when nutrients are abundant, Sac1 is predominantly localized to the ER, but in quiescent human cells and or glucose-deprived yeast cells, it is rapidly exported from the ER to the Golgi apparatus and secretion is attenuated [27–29]. That the attenuation of secretion is due to depletion of Golgi PtdIns4*P* is supported by experiments that artificially targeted the Sac1 catalytic domain to the Golgi in rapidly growing human cells and observed a similar decrease in secretion [30]. Taken together, the results indicate that the abundance and distribution of PtdIns4*P* on Golgi cisternae results from the opposing activities of PI4Ks and Sac1, whose activities are tightly regulated by metabolic cues and a challenge for future studies is to identify the upstream regulatory factors that control these activities. The relative enrichment of PtdIns4*P* on late Golgi cisternae results in the enrichment of PI4K effectors on these compartments where they regulate lipid composition and control budding of vesicles.

Functions of PtdIns4*P* at the Golgi

Membrane biogenesis and lipid homeostasis

A major function of PtdIns4*P* in animal cells is to control sphingolipid biosynthesis through the recruitment of PtdIns4*P*-binding proteins to the Golgi apparatus that modulate this pathway. Sphingolipid production initiates in the ER with the condensation of serine with palmitoyl-CoA and proceeds through a series of reactions to generate ceramide. The ceramide transfer protein CERT must then shuttle ceramide from the ER to the late Golgi where sphingomyelin synthase transfers the phosphocholine headgroup from phosphatidylcholine (PtdCho) to ceramide, a reaction that produces sphingomyelin and DAG [31] (Box 1 and Box 2). The function of CERT in SM synthesis depends on its PtdIns4*P*-binding PH domain and an FFAT (two phenylalanines in an acidic tract) motif that binds to VAP-A and VAP-B on the ER membrane [32,33]. These interactions may take place simultaneously at membrane contact sites where the ER is juxtaposed to a *trans*-Golgi cisterna, which should provide a more efficient lipid transfer mechanism than diffusion of CERT through the cytosol (Box 2) [31]. CERT is a PKD substrate and phosphorylation reduces its Golgi localization, thereby reducing ceramide transport and synthesis of sphingomyelin (Fig. 1b) [34]. This process could provide a mechanism for fine-tuning sphingomyelin and DAG levels in the Golgi as CERT activity stimulates DAG production, which feeds back to inhibit CERT through activation of PKD. Other potentially important sources of DAG in this regulatory network come from phospholipase C-mediated cleavage of PtdIns(4,5)P_2, which is activated by Golgi-associated heterotrimeric Gβγ subunits [35], or the Arf-stimulated cleavage of PtdCho by phospholipase D followed by breakdown of phosphatidic acid to DAG [36]. Surprisingly, knockdown of CERT disrupts sphingomyelin synthesis but does not impair glycosphingolipid synthesis because a different lipid transfer protein feeds substrate into this pathway [33,37].

The glucosylceramide transfer protein FAPP2 is a PtdIns4*P* effector required for glycosphingolipid synthesis in the Golgi [37]. Another fate for ceramide in the Golgi is conversion to glucosylceramide (GlcCer), a reaction catalyzed by GlcCer synthase on the cytosolic leaflet of early Golgi compartments. FAPP2 facilitates delivery of GlcCer to glycosyltransferases on the luminal face of later compartments that catalyze glycosphingolipid maturation. Why FAPP2 is needed for movement of GlcCer a few cisternae down the Golgi stack and how it facilitates translocation of GlcCer across the bilayer to the luminal side is mysterious. Work from Sprong and colleagues suggest that FAPP2 actually mediates retrograde transport of GlcCer from the Golgi to the ER [38] where it could easily access the ER lumen via an undefined flippase in the ER membrane (Box 2) [39]. GlcCer would then be delivered by vesicular transport to the luminal glycosyltransferases of the *trans*-Golgi [38]. Another interesting possibility is suggested by the observation that the rate of GlcCer spontaneous flip-flop in artificial membranes is substantially slowed in the presence of cholesterol, indicating that GlcCer interaction with cholesterol can prevent transbilayer movement [40]. Thus, it is possible that FAPP2 could stimulate spontaneous GlcCer translocation to the luminal leaflet of the Golgi by simply preventing its interaction with cholesterol in the cytosolic leaflet. In this model, FAPP2 would bind and release GlcCer on the same membrane surface containing PtdIns4*P* and serve as an emulsifying agent that prevents premature lateral segregation of GlcCer and cholesterol into condensed complexes.

Sterols can influence sphingolipid synthesis through interaction with OSBPs, of which several are PtdIns4*P* effectors [41]. OSBP1 translocates from the cytosol to the TGN upon oxysterol (e.g. 25-hydroxycholesterol) binding or cholesterol depletion [42], where it stimulates the CERT-dependent synthesis of SM [43]. Yet phosphorylation of OSBP1 and CERT by PKD reduces their association with the TGN, thereby blunting the influence of

oxysterol on SM synthesis (Fig. 1b) [44]. Moreover, phosphorylation of OSBP1 induces TGN fragmentation and inefficient TGN to plasma membrane protein transport [44], hallmark phenotypes of cells with hyperactivated PKD [45]. The phenotypes that result from acute experimental activation of PKD or sterol depletion imply that coupling of sphingolipid and sterol metabolism through OSBP1 and CERT are critical downstream effects of PKD for its function in regulating exocytic carrier formation from the TGN. Under physiologic conditions, the opposing effects of PKD phosphorylation on Golgi accumulation of ceramide and sterol (via increased PtdIns4*P* synthesis) and displacement of CERT and OSBP1 from the Golgi may be the basis of a tightly coupled mechanism that enhances delivery of these lipids to the Golgi and prevents them from accumulating to toxic levels.

A lively debate has centered on whether OSBP1 and OSBP-related proteins (ORPs) mediate the nonvesicular transport of sterol or if they “sense” sterol content and respond by modulating signaling cascades (Box 2) [46]. Both OSBP1 and ORP9L are able to transfer cholesterol between liposomes in vitro and PtdIns4*P* stimulates sterol transport dramatically when present in the donor membrane [47]. In addition to the PtdIns4*P*-binding PH domains, both OSBP1 and ORP9L have FFAT motifs capable of binding VAP-A at the ER [48] and so it is possible that OSBP1 and ORP9L mediate sterol transport between the ER and TGN. Yeast Kes1 can also bind two membranes simultaneously and transfer cholesterol between them in a phosphoinositide-dependent fashion in vitro, even though it lacks a canonical PH domain or a FFAT motif [49] [50]. In addition, several of the yeast OSBPs (Osh proteins) localize to ER membrane sites closely apposed to the plasma membrane [50], perhaps representing membrane contact sites.

Recent studies indicate that a yeast phospholipid flippase called Drs2 is an important effector of PtdIns4*P*, Kes1 and an Arf guanine nucleotide exchange factor (Arf-GEF) at the Golgi (Fig 2) [51,52]. Drs2 is a type IV P-type ATPase that pumps PtdSer and PtdEth from the lumenal leaflet of the TGN to the cytosolic leaflet [53–55]. Flippases of this P-type ATPase family help generate membrane asymmetry by concentrating aminophospholipids in the cytosolic leaflet while sphingolipids are concentrated in the exofacial leaflet where they are synthesized. This asymmetric membrane organization appears to be established in late Golgi compartments and maintained at the plasma membrane [56]. A link between Drs2 and PtdIns4*P* was first suggested by a synthetic lethal genetic relationship between *drs2* and *pik1* alleles [57]. Drs2p does not influence PtdIns4*P* levels at the TGN; however, Drs2 flippase activity is strongly dependent on PtdIns4*P* synthesis by Pik1 [52]. In contrast to most PtdIns4*P* effectors, Drs2p is an integral membrane protein and it does not use PtdIns4*P* for localization to the TGN. Rather, it is the enzymatic activity of Drs2 that is regulated by PtdIns4*P*. A PtdIns4*P* binding site was mapped to a basic patch in the C-terminal cytosolic tail of Drs2 that overlaps a previously defined binding site for the ArfGEF Gea2 and this interaction also stimulates flippase activity [52]. Kes1 was found to be a potent inhibitor of Drs2 flippase activity, although this is not likely mediated through a direct protein-protein interaction because inhibition is conferred by vastly substoichiometric concentrations of recombinant Kes1 [51]. While the mode of flippase inhibition by Kes1p is not yet known, it is likely mediated through regulation of PtdIns4*P* levels as shown in Box 2.

Vesicle-mediated trafficking

The close relationship between PI4K signaling, lipid metabolism, and vesicle-mediated export from the TGN was originally established from work on Sec14, a PtdIns/PtdCho transfer protein required for budding of exocytic vesicles from the Golgi. Sec14 is a sensor of membrane lipid composition that regulates a Golgi pool of DAG and stimulates PtdIns4*P* synthesis by Pik1 [19,58,59]. Subsequent work showed that anterograde trafficking from the Golgi is blocked by inactivation of Pik1 and firmly established the requirement for PtdIns4*P* in export from the Golgi [21,60]. Accumulated evidence in human and yeast cells now

indicates that PtdIns4*P* recognition is crucial for at least two distinct aspects of vesicle biogenesis at the TGN, sorting into vesicles, and membrane rearrangements that drive vesicle budding. Sorting of cargo into vesicles bound for the endo-lysosomal system is mediated by the AP-1 and GGA clathrin coated vesicle (CCV) adapters, which are recruited to the TGN by coincident binding of PtdIns4*P* and Arf (Fig. 2) [61–63]. As yet, no TGN exocytic coat proteins that bind PtdIns4*P* have been identified, but there are several candidate vesicle budding factors that are regulated by PtdIns4*P*. One is yeast Drs2, which is proposed to impart curvature to the membrane to support vesicle budding by flippase driven displacement of phospholipid from the luminal to the cytosolic leaflet, as described earlier (Fig. 2) [56,64,65]. Recent work has also shown that binding of human FAPP1 and FAPP2 to membranes containing PtdIns4*P* elicits the formation of membrane tubules from immobilized membranes [66,67]. Structural studies suggest that recognition of PtdIns4*P* by the PH domain of FAPP1 and FAPP2 drives insertion of a wedge shaped loop into the cytosolic leaflet, thereby driving changes in membrane curvature induced by protein binding [66,67].

Data continues to emerge from a variety of experimental systems linking PI4K signaling to Rab GTPase signaling networks that coordinate vesicle budding and transport at the Golgi, a connection that was first suggested by the finding that Rab11 and PI4KIIIβ bind each other [68]. In *Drosophila melanogaster* spermatocytes, the PI4KIIIβ ortholog, *Four wheel drive* (Fwd), recruits Rab11 to the Golgi and this activity is independent of its enzymatic activity but, curiously, secretion in *fwd* mutant cells can be partially rescued by a catalytic null PI4KIIIβ mutant [68,69]. The Rab11 homologs in yeast are Ypt31 and Ypt32 (Ypt31/32) and genetic interaction studies imply an essential relationship between Pik1 and this pair of partially redundant late Golgi Rabs [20,57,70]. There is no evidence that Pik1 and Ypt31/32 physically associate; however, recent data suggests that PtdIns4*P* plays a crucial role in a "Rab cascade" that orders sequential events in the late secretory pathway [70]. PtdIns4*P* and Ypt32-GTP recruit Sec2, a GEF for the Rab protein Sec4, onto newly forming vesicles, where it subsequently recruits and activates Sec4, the Rab that regulates transport and fusion of vesicles with the PM (Fig. 2) [71]. Sec2 also binds Sec15, a subunit of the Sec4-GTP effector complex called 'exocyst', and this network is proposed to fuel a positive feedback loop that sustains Sec4-dependent recruitment of exocyst to secretory vesicles [72]. To ensure that exocyst is not recruited prematurely to the TGN via the Ypt32-Sec2 complex, this potent activation mechanism is tightly controlled by PtdIns4*P* recognition by Sec2; when simultaneously bound to PtdIns4*P* and Ypt32, Sec2 adopts a conformation that is unable to bind Sec15. In the absence of PtdIns4*P*, Sec2 undergoes an allosteric transition to a form that binds the exocyst [70]. Importantly, these results imply that secretory vesicles contain less PtdIns4*P* than the TGN membranes from which they budded, so that Sec4 can recruit exocyst to the vesicle, but the mechanism of PtdIns4*P* exclusion from, or turnover on, secretory vesicles is unknown.

The best characterized roles of Golgi PI4Ks are to promote anterograde transport of secretory cargo through the Golgi stack, but recent data suggests that they may also regulate retrograde trafficking of Golgi resident proteins. Two groups discovered that a yeast Golgi PtdIns4*P*-binding protein called Vps74 is required for retention of a subset of resident glycosyltransferases within the Golgi [73,74]. In *vps74* loss-of-function mutants these Golgi enzymes are trafficked via the anterograde pathway to the lysosome-like vacuole where they are degraded, and when *VPS74* is overproduced, these enzymes localize predominantly to the ER. Vps74 binds directly to the cytosolic portions of these Golgi enzymes and to coatamer (COPI), the vesicle coat complex that mediates retrograde trafficking, [73,74], so the data suggest that Vps74 is required for sorting of late Golgi glycosyltransferases into the retrograde pathway.

Vps74 is an ortholog of human GOLPH3, which was initially identified through proteomic studies of purified Golgi and shown to decorate Golgi cisternae, vesicles, and tubules that originate from the *trans* face of the Golgi [75–77]. Atomic resolution structures of Vps74 and GOLPH3 are very similar and led to the identification of a conserved pocket that is required for PtdIns4*P* binding and Golgi localization of each protein [73,78,79]. Despite these similarities, there are confusing results regarding their functions in each organism. In cultured human cells treated with RNAi to knock down GOLPH3 expression, localization of four different Golgi resident enzymes is not grossly perturbed, but the structure of the Golgi is more compact and Golgi-to-PM delivery of a reporter protein is slowed, suggesting that GOLPH3 is required for efficient Golgi export via the anterograde pathway [78]. A clue to the effect of GOLPH3 knockdown on Golgi structure came from the identification of an unusual myosin, MYO18A, as a GOLPH3 interacting protein. In MYO18A knockdown cells the structure of the Golgi is affected in a similar manner to GOLPH3 knockdown cells, leading to the proposal that GOLPH3 facilitates MYO18A-dependent flattening of Golgi cisternae by bridging PtdIns4*P* and MYO18A [78]. In contrast, yeast *vps74* mutants show no gross defects in Golgi structure or rate of secretion, and the yeast genome does not encode a MYO18A ortholog [73,74,79]. Although the data may seem to suggest that Vps74 and GOLPH3 have distinct functions in each organism, this is unlikely because GOLPH3 can partially substitute for Vps74 when expressed in yeast [74], so it may be that Vps74 and GOLPH3 serve a similar, as yet unidentified, function.

The importance of understanding the functions of GOLPH3 family proteins is underscored by the recent finding that human GOLPH3 is overexpressed in a wide variety of human tumors [80]. GOLPH3 overexpression or RNAi-mediated knockdown results in enhanced receptor tyrosine kinase (RTK) signaling which drives cellular transformation via hyper-activation of the target of rapamycin (mTOR) signaling pathway. The manner in which alteration of GOLPH3 expression enhances RTK signaling is unknown, though several possible mechanisms can be suggested from what is currently known about GOLPH3 family proteins [81]. It is possible that retention of RTKs in the Golgi enhances or alters autocrine signaling, or that aberrant processing (ie, glycosylation) of RTKs alters signaling. The GOLPH3-interacting protein MYO18A localizes to the cell cortex in addition to the Golgi [82], raising the possibility that GOLPH3 and MYO18A could have additional roles outside of the Golgi. Moreover, it is reported that GOLPH3 associates with a protein named Vps35, a component of an endocytic sorting device called retromer that functions to export cargo proteins from the endosomal pathway that leads to the lysosome [80]. This interaction could promote stability and recycling of RTKs, leading to increased abundance of RTKs on the PM and enhanced signaling.

Concluding remarks

PI4Ks are master regulators of Golgi function that coordinate the activities of coat protein-based and lipid-based transport pathways exiting the Golgi (Fig. 3). Coincidence detection of PtdIns4*P* and components of Arf and Rab GTPase networks has emerged as a core mechanism used to recruit and regulate the activities of Golgi vesicle budding and targeting factors. Newly identified Golgi PI4K effectors in yeast include Drs2, a phospholipid flippase required for the formation of vesicles from the TGN, and the Rab-GEF Sec2, which coordinates sequential activation of Rab GTPases that function to produce and target secretory vesicles with the plasma membrane. The conserved Vps74 and GOLPH3 proteins seem to be trafficking effectors, however it is currently unclear if these proteins function via a common, conserved mechanism. As GOLPH3 was recently implicated in diverse human cancers this is an important question for future studies to resolve.

Recent evidence continues to manifest functional links between PI4Ks and lipid dynamics in the mammalian Golgi. The levels of PtdIns4*P* and DAG control the overall content of sphingolipid and sterol of late Golgi compartments via recruitment and regulation of lipid homeostatic regulators including OSBP1, FAPP2 and CERT. PKD promotes sphingolipid and sterol accumulation indirectly through activation of PI4KIIIβ, but curiously, it also inhibits OSBP1 and CERT by phosphorylating them directly. This arrangement allows PKD to co-regulate sphingolipid and sterol content of Golgi membranes and may be key for coalescing sphingolipid, sterol and integral membrane proteins into microdomains, as proposed in the 'raft' hypothesis of Golgi protein sorting, [83,84]. The coordinate production of DAG with sphingolipids may also contribute to fission of carrier vesicles from the TGN by promoting negative curvature [85].

Surprisingly, CERT, FAPP2 and PKD are not present in yeast, even though sphingolipids, sterol and PI4K are critical for proper Golgi function. This reflects differences in the mechanisms that regulate Golgi PtdIns4*P* levels and lipid homeostasis in each species, but there are also conserved mechanisms that utilize PITPs (Sec14 and Nir2) and OSBPs (Kes1 and OSBP1). The PITPs function by stimulating PtdIns4*P* synthesis and maintaining a Golgi pool of DAG, and the OSBPs function to modulate sterol content by a still mysterious mechanism. OSBP1 may directly mediate transfer of sterol from the ER to the Golgi, and in the Golgi it may act downstream of PtdIns4*P* to promote concentration of sterol into domains within late Golgi cisternae and thereby promote vesicle formation. In this manner PI4Ks, PITPs and OSBPs appear to be the heart of a regulatory axis that couples control of TGN lipid composition to protein and lipid sorting processes to effect vesicle-mediated export of cargo from the TGN.

Acknowledgments

We thank Vytas Bankatis and Vivek Malhotra for helpful discussions. Research in the authors' laboratories is supported by grants from the United States National Institutes of Health.

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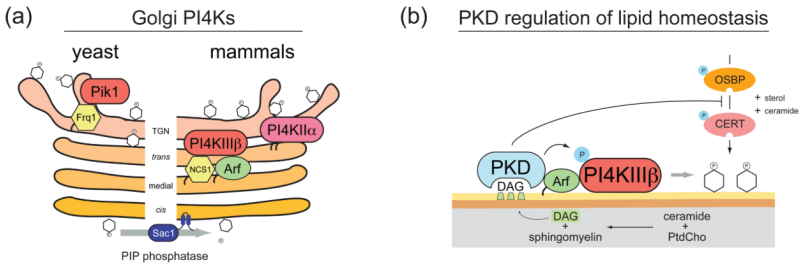

Figure 1. Control of PtdIns4*P* levels in the Golgi

(a) PtdIns 4-kinases and phosphatases of yeast and mammalian Golgi. In yeast, one PI4K, Pik1, localizes to *trans* cisternae of the Golgi apparatus. Membrane association of Pik1 requires a myristoylated protein, Frq1, which is constitutively bound to Pik1. There are two mammalian Golgi PI4Ks, PI4KIIα and PI4KIIIβ, and their distribution between Golgi cisternae has not yet been firmly established. Membrane association of PI4KIIα requires palmitoylation, but the basis of specific Golgi targeting is unknown. Targeting of PI4KIIIβ is mediated by binding to two myristoylated proteins, NCS1 (a Frq1 ortholog) and Arf1, which are reported to also associate with each other. Early Golgi cisternae are thought to be depleted of PtdIns4*P*, relative to late cisternae, by the Sac1 phosphoinositide phosphatase which traffics between the ER and early Golgi compartments. (b) Protein kinase D regulation of PI4KIIIβ and Golgi lipid homeostasis. Protein kinase D (PKD) is a master regulator of sphingolipid and sterol homeostasis through phosphorylation–dependent modulation of PI4KIIIβ, CERT, and OSBP1 activities. PKD is recruited to the Golgi by binding to diacylglycerol (DAG), which is produced during sphingomyelin synthesis and via other pathways (see text). PKD-mediated phosphorylation activates PI4KIIIβ, resulting in increased production of PtdIns4*P* on Golgi membranes, which should enhance recruitment of the PtdIns4*P*-binding proteins, CERT and OSBP1. CERT delivers ceramide to the Golgi from its site of synthesis in the endoplasmic reticulum to support sphingomyelin synthesis and OSBP1 is linked to sterol metabolism. Although DAG produced during SM synthesis favors recruitment of PKD and activation of PI4KIIIβ, phosphorylation of CERT and OSBP1 by PKD reduce their Golgi localization and inhibits SM synthesis. This regulatory loop balances flux through the SM synthetic pathway.

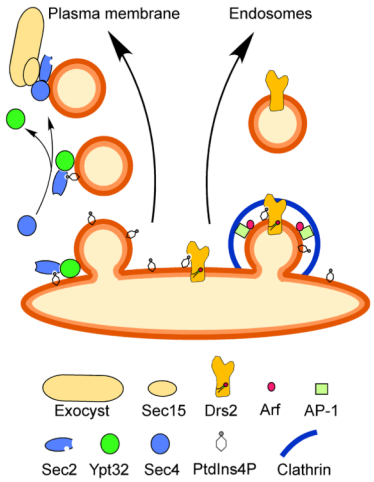

Figure 2. Golgi PI4K regulation of vesicle-mediated trafficking

In yeast, targeting of Golgi-derived secretory vesicles to the plasma membrane requires recruitment of a multi-protein complex called ‘exocyst’ to the secretory vesicle. Exocyst recruitment requires activation of the Sec4 Rab GTPase by the Rab-GEF, Sec2, and binding of vesicle-associated Sec2 to the exocyst subunit, Sec15. Initially, Sec2 is recruited to the Golgi by coincident binding of PtdIns4*P* and the GTP bound form of the Ypt32 Rab GTPase during vesicle budding. When bound to PtdIns4*P*, Sec2 adopts a conformation that does not bind Sec15, and this prevents premature recruitment of exocyst to the Golgi. Once a vesicle has budded from the Golgi and PtdIns4P levels diminish, Sec2 activates Sec4 and both proteins bind Sec15. PtdIns4*P* also stimulates the phospholipid flippase activity of Drs2, which helps establish the asymmetric lipid distribution between the two leaflets of the TGN membrane and is coupled to the formation of AP-1/clathrin-coated vesicles targeted to the endosomal system.

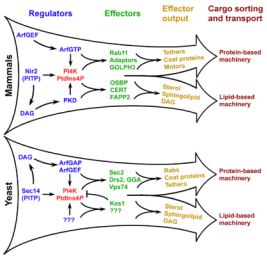

Figure 3. Regulators and effectors of Golgi PI4Ks

In mammals (top), ArfGEF, Arf-GTP, Nir2, DAG and PKD are upstream factors that regulate the localization or activity of PI4K (PI4KIIIβ). Downstream effectors of PI4K or PtdIns4*P* include Rab11 (*Drosophila melanogaster*), the clathrin adaptor proteins AP-1 and GGAs, which coincidently bind PtdIns4P and Arf, and GOLPH3. The effector outputs of this group are the protein-based trafficking machinery including a vesicle coat protein (clathrin), a motor protein (MYO18) and presumably tethers that facilitate vesicle targeting. The lipid transfer proteins OSBP1, CERT and FAPP2 are PtdIns4*P* effectors while CERT and OSBP1 are also direct substrates of PKD. The effector outputs of this group are sphingolipids and cholesterol, which may coalesce into microdomains driving a lipid-based protein sorting process, as well as DAG. In yeast (bottom), Sec14 stimulates PtdIns4*P* synthesis by PI4K (Pik1) and also modulates DAG levels in the Golgi. DAG stimulates an Arf GTPase activating protein (ArfGAP (Gcs1)) while an Arf-GEF (Sec7) binds to PI4K, perhaps to coordinate the Arf-GTP and PtdIns4*P* levels at the TGN. Known effectors of PtdIns4P linked to protein-based based trafficking machinery include Sec2 (Rab-GEF), Drs2 (flippase), GGA (clathrin adaptor) and Vps74 (GOLPH3 homolog). Kes1 (OSBP) requires Pik1 activity for recruitment to the TGN, but also appears to repress PtdIns4P levels. Yeast cells do not appear to have PKD, FAPP2 or CERT orthologs, but Kes1 has been implicated in the formation of sphingolipid/sterol-rich exocytic vesicles from the TGN.

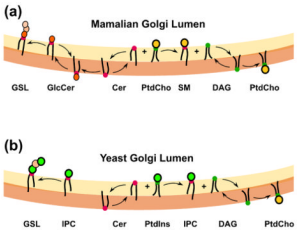

Figure I.

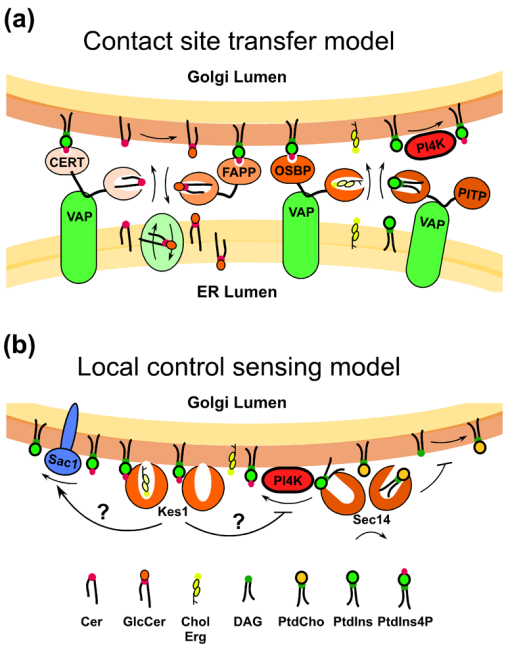

Figure II.