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Regulation of Golgi function via phosphoinositide lipids

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Abstract

Phosphoinositides play important roles in Golgi traffic and structural integrity. Specific lipid kinases and phosphatases associate with the Golgi complex and regulate the multiplicity of trafficking routes from this organelle. Work in different model systems showed that the basic elements that regulate lipid signaling at the Golgi are conserved from yeast to humans. Many of the enzymes involved in Golgi phosphoinositide metabolism are essential for viability or cause severe human disease when malfunctioning. Phosphoinositide effectors at the Golgi control both non-vesicular transfer of lipids and sorting of secretory and membrane proteins. In addition, Golgi phosphoinositides were recently implicated in the metabolic and cell growth-dependent regulation of the secretory pathway.

Keywords

Golgi; phosphoinositides; lipid kinases; lipid phosphatases; secretory pathway

1. Introduction

Phosphoinositide signaling systems exist in all eukaryotes. Reversible phosphorylation of the inositol headgroup of phosphatidylinositol (PI) gives rise to seven distinct phosphoinositide species. For many years, phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂) has received the most attention because it is the precursor of various second messenger molecules involved in regulating intracellular calcium levels, protein kinase C signaling, and cell survival and propagation [1–3]. However, recent work established important signaling roles for all the other, initially less studied, phosphoinositides [4]. An important property of phosphoinositide lipids is their tightly regulated spatial distribution, which is highly relevant for their organelle-specific regulatory functions. Localized production of the different phosphoinositide molecules is coupled to the recruitment of specific phosphoinositide-binding factors [5]. In many cases, these effector proteins also bind selectively to small GTPases from the Rab and Arf families [6]. The coincidence detection of a particular phosphoinositide and a specific activated Arf or Rab allows the recognition of unique membrane-specific codes that spatially control multiple lines of intracellular events such as cytoskeletal organization, cell motility and vesicular membrane transport [6,7].

The finding that the yeast *VPS34* gene encodes a PI 3-kinase provided the first direct evidence that phosphoinositide regulate intracellular membrane traffic [8]. Production of endosomal pools of PI(3)P by Vps34p is involved in both endosomal membrane trafficking and vacuolar

protein sorting [9–12]. Later it was found that additional phosphoinositide species are constitutively enriched at other organellar membranes, which is important for controlling compartment-specific membrane trafficking and maintaining organelle identity [6,7]. The Golgi complex represents the central organelle of the secretory pathway. The continuous exchange of proteins and lipids between the Golgi and other intracellular membranes requires high fidelity in the spatial and temporal regulation of individual trafficking steps. Golgi phosphoinositides play a paramount role in these processes. This review summarizes the main pathways of phosphoinositide metabolism at the Golgi and the downstream processes that are governed by these lipid signals.

2. Golgi phosphoinositides and their downstream effectors

PI(4)P and PI(4,5)P₂ are the most important phosphoinositide regulators at the Golgi complex. Golgi membranes are a major site for PI(4)P generation [13]. Localization studies using fluorescent lipid binding probes or specific antibodies indicated that a substantial proportion of intracellular PI(4)P localizes to Golgi membranes [14–16]. In both yeast and mammals it was found that PI(4)P regulates forward trafficking from the Golgi to the cell periphery [17–21]. In yeast, generation of Golgi PI(4)P is essential for anterograde trafficking of a variety of cargo proteins, including invertase, Hsp150 and chitin synthases [17,19,20]. In addition, PI(4)P has been implicated in anterograde trafficking from the ER, although the precise mechanism of this regulation remains to be determined [22]. Mutations in PI 4-kinases cause also actin cytoskeletal defects and impaired vacuolar morphology suggesting that additional functional pools of PI(4)P exist [17,23]. Only little PI(4,5)P₂ can be detected in membranes other than the plasma membrane, yet a small proportion of this lipid appears to be localized to Golgi membranes [24]. Golgi PI(4,5)P₂ is mainly localized at stacked cisternal regions [24]. PI(4,5)P₂ was reported to stimulate anterograde trafficking of apical cargo in polarized cells [25]. In addition, PI(4,5)P₂ may contribute to the structural organization of the Golgi by interacting with cytoskeletal elements [26–28].

PI(4)P is essential for proper Golgi function. Two functionally distinct types of Golgi PI(4)P effectors have been identified, clathrin adaptors and lipid transfer proteins (Fig. 1). The first group consists of clathrin adaptor protein complex 1 (AP-1) and γ -ear-containing, ADP-ribosylation factor-binding proteins (GGAs). Clathrin-mediated transport from the Golgi is important for sorting of proteins to the endosomal and lysosomal compartments. AP-1 binds to the trans-Golgi network (TGN) by simultaneously interacting with PI(4)P and Arf1 [29]. The crystal structure of the AP-1 core was recently resolved [30]. Mutations of amino acids at a specific region of the gamma chain within the core impaired PI(4)P binding [30]. These results indicate that AP-1 gamma chain contains a novel phosphoinositide-binding motif that is essential for PI(4)P recognition and recruitment to the TGN.

Both studies in yeast and mammals have shown that PI(4)P is involved in recruitment of GGA proteins to the Golgi. A genetic screen for PI(4)P effector proteins in yeast identified the gene *GGA2* [31]. Recruitment of Gga2p to the TGN is required for Golgi-to-endosome trafficking [31]. Gga2p membrane binding is controlled by both PI(4)P and activated Arf1p [31]. Gga2p contains a phosphoinositide-binding motif within its N-terminal domain, which is essential for interacting with PI(4)P [31]. PI(4)P is also a key regulator of the recruitment of mammalian GGA proteins to the TGN [32]. Mammalian GGAs bind PI(4)P through their GAT domain, which also binds ubiquitin but not Arf1 [32]. Interestingly, PI(4)P increased the affinity of ubiquitin for GAT domain binding, indicating an additional role of this phosphoinositide for cargo recognition at the TGN [32]. Both, AP-1 and GGAs function in clathrin-dependent sorting of proteins between the TGN and endosomes [33]. However, recent evidence from yeast suggests that GGA proteins and AP-1 control distinct trafficking pathways from the TGN involving either late or early endosomal compartments [34]. In both cases, recruitment of the

clathrin adaptors to the Golgi requires coincidence detection of activated Arf1 and PI(4)P, which ultimately controls the formation of clathrin-coated vesicles at the TGN.

The second class of PI(4)P effectors at the Golgi consists of soluble lipid transfer proteins characterized by specific lipid-binding sites (Fig. 1). Ceramide transfer protein (CERT), oxysterol-binding protein (OSBP) and four-phosphate adaptor protein (FAPP2), all contain phosphoinositide-specific PH-domains that are required for Golgi targeting. There is significant *in vitro* data that these proteins are capable of facilitating non-vesicular transport and exchange of lipids between two distinct membranes [35]. It is also possible that the lipid-binding properties of specific lipid transfer proteins is involved in lipid-sensing or in extracting lipids from the bilayer for subsequent modification by suitable enzymes [35].

CERT is a ceramide-specific transfer protein, which was originally identified in the genetic analysis of a cell line with impaired sphingomyelin levels [36]. Ceramide is an essential precursor of Golgi sphingolipid biosynthesis but is itself synthesized at the ER and then transferred to appropriate sites within the Golgi [37]. Ceramide is converted to both sphingomyelin and glucosylceramide, which may occur at distinct regions of the Golgi [38]. Ablation of the CERT gene in mice induces ceramide accumulation in the ER and mislocalization of ceramide to mitochondria leading to mitochondrial degeneration [39]. These defects cause heart anomalies and early embryonic death [39]. CERT contains two functional domains that allow specific interactions with both ER and Golgi membranes [40]. A FFAT motif is required for CERT interaction with VAP proteins, which serve as transmembrane adaptors at the ER [41,42]. A PI(4)P-specific PH domain is required for Golgi recruitment of CERT [36]. Protein phosphatase 2C- ϵ (PP2C) interacts with VAP-A at the ER and dephosphorylates CERT in a VAP-A-dependent manner [43]. Dephosphorylation triggers steady-state redistribution of CERT from the cytoplasm to the Golgi apparatus [43]. At the Golgi, phosphorylation of CERT by protein kinase D (PKD) decreases the binding affinity of CERT towards PI(4)P thus triggering shuttling back to the ER [44]. PI(4)P biosynthesis at the Golgi is also stimulated by PKD via phosphorylation of a specific site in PI 4-kinase-III β (PI4KIII β) [45,46], indicating that ceramide transfer to the Golgi undergoes cycles of negative and positive regulation.

The oxysterol binding protein OSBP possesses similar structural elements as CERT, consisting of a N-terminal PH domain and a FFAT motif [47]. In addition, OSBP contains a C-terminal oxysterol/cholesterol binding domain [47]. There are 12 genes encoding OSBP-related proteins (ORPs) within the human genome, while the genome of the yeast *Saccharomyces cerevisiae* encodes seven OSBP homologues termed Osh1p-Osh7p [47]. The sterol-binding core of Osh4p consists of 19 anti-parallel beta-sheets and forms an almost perfect beta-barrel structure [48]. The exact cellular function of OSBP and its closely related family members is not entirely clear. Several reports indicate a role for OSBP and the yeast Osh proteins in the regulation of vesicular transport [49,50]. There is evidence that these proteins catalyze the non-vesicular transport of sterols in a phosphoinositide-dependent manner, but it is unclear whether these reactions involve the Golgi complex [51]. A recent study showed that ORP9, a protein closely related to the family of oxysterol-binding proteins, partitions between ER and Golgi membranes and is required for Golgi morphology and anterograde traffic [52]. Organelle-specific targeting of OSBP and ORP9 requires the VAP proteins at the ER and sufficient levels of PI(4)P at the Golgi [52,53]. In addition, the small GTPase Arf1 binds to the PH domain of OSBP, which contributes to Golgi targeting [54]. There is recent evidence that both CERT and OSBP function in a common pathway required for coordinated lipid transfer between the ER and the Golgi complex, which is important for Golgi trafficking [53,55]. Thus, proper regulation of intracellular cholesterol gradients via OSBP and ORPs seems to be highly relevant for proper Golgi function.

FAPP2 belongs to a separate family of lipid transfer proteins. FAPP2 contains a N-terminal PH domain with high affinity towards PI(4)P and a C-terminal glycosphingolipid binding domain that is similar to the one found in the glycosphingolipid transfer protein (GLTP) [35]. A crystal structure of apo-GLTP was reported in which the bound glycosphingolipid is sandwiched within an unconventional alpha-helical topology that forms two boundary layers [56]. FAPP2 was discovered independently as a factor required for glycosphingolipid biosynthesis and for anterograde trafficking from the Golgi [21,57–59]. FAPP2 is also involved in the transport of apical cargo in polarized cells and in cilium formation [57,60]. The PH domain of FAPP2 binds simultaneously to PI(4)P and to Arf1 [21]. The exact intracellular localization of glycosphingolipid transfer by FAPP2 is somewhat controversial and it appears that this protein can deliver glucosylceramide to both the ER and the TGN [58,59]. It is clear, however, that glucosylceramide transfer activity of FAPP2 is essential for proper glycosphingolipid biosynthesis [58,59]. In addition, FAPP2 depletion impairs the formation of condensed apical membrane domains in polarized cells [60]. Thus, FAPP2 is an important factor required for biogenesis and organization of the plasma membrane by coupling the synthesis of glycosphingolipids with the export of proteins and lipids to the cell surface.

3. Lipid kinases at the Golgi

Distinct members of various lipid kinase families are present at Golgi membranes [61]. Several PI 4-kinases are important for Golgi function because they generate the PI(4)P that is critical for anterograde trafficking. In mammalian cells, both PI4KII α and PI4KIII β contribute to the biosynthesis of Golgi PI(4)P. PI4KII α also associates with several other organelles including endosomes, the ER and the plasma membrane [62–64]. A study in polarized MDCK cells suggested that PI4KII α and PI4KIII β populate distinct regions within the Golgi [65]. It is therefore possible that these kinases control separate Golgi PI(4)P pools that function in individual trafficking pathways. For example it was shown that PI4KII α is required for sorting via the AP-1/clathrin-dependent pathway, whereas PI4KIII β functions in PI(4)P-dependent transfer of ceramide from the ER to the Golgi [29,66]. It remains unknown, however, how the recruitment of PI4KII α to specific Golgi regions is achieved. It was recently reported that the activity of this enzyme responds to cholesterol levels [67] and it is possible that PI4KII α localizes preferentially to cholesterol-rich regions within the Golgi, though this remains to be further analyzed.

Several factors controlling the Golgi association of PI4KIII β have been characterized. The GTP-bound form of Arf1 interacts directly with PI4KIII β and recruits the lipid kinase to the Golgi [68]. Both, Arf1 and PI4KIII β interact with the N-myristoylated, Ca²⁺-binding protein neuronal calcium sensor-1 (NCS-1) [69,70]. This complex formation seems to be essential for efficient membrane association of PI4KIII β [70,71]. A similar PI 4-kinase complex is also found in yeast [17]. Golgi recruitment of PI 4-kinase Pik1p requires the N-myristoylated cofactor Frq1, which is highly homologous to NCS-1 [72,73]. Human NCS-1 forms a functional complex with yeast Pik1p and rescues the inviability of *frq1* Δ cells [74]. PI4KIII β is also a target of protein kinase D (PKD). PI4KIII β can be phosphorylated by PKD1 and PKD2 at a single motif, which is then recognized by 14-3-3 proteins, which in turn stabilize the activated form of PI4KIII β [45,46]. PKD also regulates the fission of vesicles originating from the trans-Golgi network [75] and it is possible that activation of PI4KIII β contributes to this mechanism. Although there is no PKD homologue in yeast, Pik1p binds to 14-3-3 proteins upon phosphorylation by an unknown kinase [76]. Different from mammalian cells, however, 14-3-3 binding does not stimulate kinase activity but regulates the distribution of Pik1p between nucleus and Golgi membranes [76].

As mentioned above, there is evidence that a minor pool of PI(4,5)P₂ localizes to Golgi membranes. Though the majority of PI(4,5)P₂ is associated with the plasma membrane,

immunoelectron microscopy detected some PI(4,5)P₂ at the Golgi [24]. Arf1-dependent recruitment of PIP5K activity to the Golgi was reported [77], yet the molecular identity of the PIP5K isoform that is responsible for generating Golgi PI(4,5)P₂ has not been resolved.

4. Golgi lipid phosphatases

Several lipid phosphatases associate with the Golgi complex. Among these enzymes, the polyphosphoinositide phosphatase SAC1 is a major regulator of Golgi PI(4)P, whereas INPP4B, INPP5B, and OCRL1 display enzymatic activity towards PI(4,5)P₂.

OCRL1 is a PI(4,5)P₂-specific 5-phosphatase located at Golgi and endosomal membranes. Mutations in OCRL1 cause Lowe syndrome, also called oculocerebrorenal (OCRL) syndrome. First described by Charles Lowe and coworkers in 1952 [78], the syndrome was later portrayed as a triad of congenital cataracts, Fanconi syndrome of the proximal renal tubules, and mental retardation [79]. The *OCRL1* gene is composed of 24 exons on the X-chromosome [80]. Two distinct splice variants of OCRL1 exist, named OCRL1-a and OCRL1-b. In most tissues both isoforms are expressed, with the exception of the brain where only the longer form (OCRL1-a) is present [81]. While the significance of these different isoforms is not entirely clear, a recent report showed that OCRL1-a and OCRL1-b show distinct clathrin binding properties and display differences in their association with clathrin-coated vesicles [82]. Both OCRL1 splice variants contain two conserved domains, an inositol polyphosphate 5-phosphatase domain and a C-terminal Rho-GAP-like domain. The 5-phosphatase domain structure of OCRL1 resembles the organization of Mg²⁺-dependent nucleases [83]. Initially, it was found that OCRL1 localizes to the Golgi in a variety of tissue culture cells [84–86]. Subsequent studies have found additional pools of OCRL1 at endosomal membranes [87]. It was recently shown that OCRL1 interacts with several members of the Rab family of small GTPases [88–90]. The strongest interaction was reported for Golgi-associated Rab1, Rab6 and endosomal Rab5 [90]. The Rho-GAP domain of OCRL1 is also important for binding to the Rab5 effector APPL1 on peripheral early endosomes [87]. APPL1 and Rab5 independently contribute to recruit OCRL to endosomes and it was demonstrated that OCRL1 interacts directly with clathrin heavy chain [82,87–89,91]. Together, this data indicates that interactions with Rab GTPases and clathrin regulate recruitment of OCRL1 to endosomal and Golgi membranes. However, the exact physiological function of OCRL1 in either Golgi or endosomal membrane trafficking has yet to be determined and the molecular mechanism underlying Lowe syndrome remains unresolved.

Mammals possess a Golgi-localized type II inositol polyphosphate 5-phosphatase (INPP5B) [92]. This enzyme displays 45% amino acid sequence identity to OCRL1 and has a similar domain structure [93,94]. A portion of INPP5B interacts with Rab5 and is targeted to early endosomes [95]. However, INPP5B interacts also with several additional Rab proteins such as Rab1, Rab2, Rab6 and Rab9, which are localized to the ER-Golgi Intermediate Compartment (ERGIC), the Golgi stack and the TGN [96]. Different from OCRL1, INPP5B does not interact with clathrin and is largely absent from clathrin-coated structures and it was speculated that INPP5B plays a role in retrograde traffic from the ERGIC to the ER that is distinct from the function of OCRL1 [96]. However, in some organisms the cellular functions of OCRL1 and INPP5B appear to be partially redundant, which could explain why disruption of the OCRL1 gene in mouse failed to produce any signs of Lowe syndrome-specific phenotypes [97]. Mammals also have a type II inositol polyphosphate 4-phosphatase (INPP4B) and murine α and β isoforms of INPP4B were recently described [98]. These enzymes contain a C2 domain with preferential affinity towards phosphatidic acid and PI(3,4,5)P₃ [98]. Mouse INPP4B- β localizes to the Golgi whereas INPP4B- α is mainly cytosolic [98], but neither enzymology nor cellular functions of these proteins have been examined.

SAC1 is a transmembrane lipid phosphatase, originally identified in a suppressor screen in yeast actin mutants [99,100]. Yeast *sac1* null strains are viable but display pleiotropic phenotypes related to inositol metabolism, cytoskeletal organization and intracellular membrane traffic [20,99–102]. Disruption of the SAC1 gene in fruit flies and in mice causes early embryonic lethality [103,104]. All SAC1 orthologues have a cytosolic domain containing a phosphatase domain that is characterized by the motif CX₅R(T/S), which is also present in metal-independent protein and lipid phosphatases [105]. *In vitro* studies showed that SAC1 has catalytic activity towards PI(3)P, PI(4)P and PI(3,5)P₂, but SAC1 appears to function predominantly as a regulator of PI(4)P *in vivo* [23,106,107]. Topology analysis showed that yeast Sac1p is a type-II transmembrane protein containing two separate transmembrane segments [102,106]. A SAC1-like phosphatase domain is also found in phosphatases related to the yeast Fig 4 protein and in the phosphoinositide 5-phosphatase synaptojanin and its various splice variants, [108,109]. Yeast Sac1p forms a complex with dolicholphosphatemannose synthase Dpm1p at the ER [110]. The enzymatic product of Dpm1p, dolicholphosphate-mannose, is an essential mannosyl donor for glycosylation reactions in the ER. The interaction with Dpm1p is essential for the ER localization of Sac1p [110]. During exponential growth of yeast cells, Sac1p lipid phosphatase activity at the ER is required for efficient oligosaccharide biosynthesis [110]. However, the exact mechanism by which Sac1p regulates glycosylation is unknown. The starvation-induced reduction of yeast cell proliferation is accompanied by rapid and reversible translocation of Sac1p to Golgi membranes and during this condition ER-based glycosylation becomes independent from Sac1p function [110]. Both yeast and mammalian SAC1 orthologues are also important elements in a regulatory circuit that synchronizes the secretory pathway with cell growth during active proliferation, which is summarized below.

5. Golgi phosphoinositides and cell growth

With a few exceptions, such as in muscle cells and neurons, cell division requires concomitant cell growth. The dependency of the cell cycle on growth is established in specific cell size thresholds critical for cell cycle transitions [111]. One of the fundamental determinants of the growth rate in dividing cells is their translational capacity. Mitogenic stimulation of quiescent cells induces a rapid increase in translation rates and ribosome biosynthesis [112]. Properly controlled ER targeting of translating ribosomes is required for secretion, biosynthesis of proteins at intracellular membranes, and cell growth [113]. Plasma membrane proteins and phospholipids are transported to the cell periphery via the secretory pathway [114]. An upregulated secretion couples cell surface expansion to an overall increase in cell volume during cell growth. The capacity of exocytic transport is therefore an important determinant of overall cell proliferation rates. Analysis of yeast secretory mutants showed directly that impaired delivery of exocytic vesicles causes defects in cell surface growth and cell division [115].

Whereas the pathways that link growth signaling to stimulation of translation and ribosome biosynthesis are well studied [112,116], a mechanistic link between growth and secretion has remained elusive. Elements of a regulatory network that coordinates cell growth and Golgi traffic have been identified only recently. This novel growth-dependent pathway for coordinating cell growth and secretion operates at the level of Golgi phosphoinositides (Fig. 2). The SAC1 lipid phosphatase is a central factor in this regulation. Genetic and functional analysis of *sac1* mutants in yeast provided surprising evidence that this phosphatase plays a stimulating role in ER function but is a negative regulator of Golgi traffic [20,100,102,106]. First insight into the mechanism, by which SAC1 phosphatases regulate secretion, was obtained in a series of experiments in yeast showing that Sac1p shuttles between ER and Golgi in response to nutrient levels (Fig. 2A) [110]. During nutrient sufficiency in the presence of high glucose, yeast cells proliferate exponentially and Sac1p is mainly concentrated at the ER

[110]. In a not completely understood mechanism, ER-localized Sac1p is required to restrict a pool of PI(4)P that is synthesized by PI 4-kinase Stt4p to the plasma membrane, possibly at specific ER-plasma membrane contact sites [23,117]. When cells grow into late log phase, nutrients become limiting for proliferation [118]. Under these conditions, Sac1p accumulates at Golgi membranes and downregulates a Golgi-specific pool of PI(4)P synthesized by the PI 4-kinase Pik1p [110]. This effect can also be induced by glucose starvation [110]. The starvation-induced change in the intracellular localization of Sac1p is completely reversible. Upon addition of glucose to glucose-deprived cells, Sac1p translocates rapidly back to the ER. Two recent studies showed that localization of yeast PI 4-kinase Pik1p also responds to nutrient levels and cell growth (Fig. 2A) [76,117]. In exponentially growing yeast, Pik1p localizes largely to Golgi membranes and to the nucleus [76,117,119]. Pik1p association with the Golgi requires the non-catalytic cofactor Frq1p [72]. Glucose starvation induces the release of the Pik1p/Frq1p complex from the Golgi [76,117]. Binding of 14-3-3 proteins to the Pik1p/Frq1p complex stabilizes this complex in the cytosol and is thus involved in regulating the distribution of active Pik1p kinase between the Golgi and the nucleus [76]. Together, Sac1p and Pik1p are required for reciprocal metabolic control of Golgi PI(4)P, which is important for coordinating the secretory capacities of ER and Golgi membranes during cell growth (Fig. 2A).

Growth-dependent translocation of SAC1 between ER and Golgi is evolutionary conserved. However, in mammals this mechanism responds rather to mitogens than to nutrients (Fig. 2B). Human SAC1 accumulates at the Golgi in quiescent cells in response to serum starvation and rapidly shuttles back to the ER when cell growth is stimulated with growth factors [15]. The translocation of human SAC1 between ER and Golgi requires reversible oligomerization, which in turn regulates recruitment into coat protein complex II (COP-II) or coatamer protein complex I (COP-I) vesicles, respectively (Fig 2B). All mammalian SAC1 orthologues contain a N-terminal leucine zipper (LZ) motif required for oligomerization and COP-II binding, and a C-terminal di-lysine motif essential for binding to COP-I. Golgi-localized SAC1 down-regulates PI(4)P and constitutive secretion during quiescence [15]. Conversely, growth-stimulated cells rapidly establish a concentrated pool of PI(4)P at the Golgi, which promotes anterograde traffic. [15]. Whether the Golgi association of mammalian PI 4-kinases responds to mitogens and cell growth is unknown. Interestingly, the p38 mitogen-activated protein kinase (MAPK) that is also activated by cell stress is required for the rapid translocation of SAC1 from the Golgi back to the ER after growth factor stimulation [15]. Signaling through the extracellular signal-regulated kinase 1/2 (ERK) pathway, which has previously been implicated in regulating Golgi disassembly during mitosis [120–122], also affects mitogen-dependent shuttling of SAC1 [15]. While the signaling pathways and factors that regulate Golgi PI(4)P remain to be further analyzed, it is clear that SAC1 is not only a spatial regulator of intracellular PI(4)P but also an important component in linking growth signaling to Golgi function and secretion.

6. Concluding remarks

Phosphoinositides have emerged as essential factors in organizing the dynamic stability of the Golgi. PI(4)P appears to be the key player because this lipid marks the identity of Golgi membranes and directs lipid and protein traffic at this organelle. The Golgi levels of PI(4)P are regulated in a growth-dependent manner, which allows proper coordination of secretion and cell proliferation. Many of the effector proteins that bind to Golgi PI(4)P also bind to activated Arf1 and this coincidence detection is required for the assembly of proper trafficking structures. It is therefore crucial that PI(4)P is not uniformly distributed across the Golgi complex but forms functionally distinct pools that trigger concentration of specific lipids and cargo proteins at certain sites. How the lipid kinases and phosphatases that regulate PI(4)P metabolism establish these local phosphoinositide gradients remains a challenging question that waits to be addressed.

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Abbreviations

AP-1	clathrin adaptor protein 1 complex
CERT	ceramide transfer protein
ERGIC	ER-Golgi Intermediate Compartment
FAPP2	four-phosphate adaptor protein 2
GGA	γ -ear-containing, ADP-ribosylation factor-binding protein
OSBP	oxysterol-binding protein
PI	phosphatidylinositol
PI(4)P	phosphatidylinositol-4-phosphate
PI(4,5)P₂	phosphatidylinositol-4,5-bisphosphate
PI4K	phosphatidylinositol 4-kinase
TGN	trans-Golgi network

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Fig. 1. PI(4)P effectors at the Golgi

Two functionally distinct types of PI(4)P effectors are recruited to the Golgi. The first group consists of the clathrin adaptors AP-1 and the GGA proteins, which function in clathrin-mediated sorting to the endosomal/lysosomal system. The second group of effectors contains the lipid transfer proteins, OSBP, CERT, and FAPP2, which are important for non-vesicular transfer of sterols, ceramide and glucosylceramide. These lipid transfer proteins are implicated in sphingolipid biosynthesis and protein traffic from the TGN. AP-1, GGAs, OSBP and FAPP2 also bind to activated Arf1. The coincidence detection of GTP-bound Arf1 and PI(4)P controls the interaction of these factors with Golgi membranes.

A. Metabolic control of Golgi PI(4)P in yeast

B. Mitogen-dependent control of Golgi PI(4)P in mammalian cells

Fig. 2. Control of Golgi PI(4)P during cell growth

(A): Metabolic control of Golgi PI(4)P in yeast. In the presence of sufficient glucose, exponentially growing yeast exhibit high Golgi PI(4)P levels generated by Pik1p/Frq1p. Under these conditions, the lipid phosphatase Sac1p localizes to the ER in a complex with Dpm1p. Glucose starvation triggers translocation of Sac1p to the Golgi and release of Pik1p/Frq1p from Golgi membranes. The cytosolic Pik1p/Frq1p complex binds to 14-3-3 proteins encoded by *BMH1* and *BMH2*. (B): Mitogen-dependent control of PI(4)P in mammalian cells. In mitogen stimulated cells, SAC1 lipid phosphatase remains largely localized at the ER, which allows for increased levels of PI(4)P at the Golgi synthesized by PI4KIIα and PI4KIIIβ. During serum starvation and in quiescent cells, SAC1 oligomerizes and accumulates in the Golgi, which in turn down-regulates Golgi PI(4)P and slows constitutive secretion. Whether PI4KIIα and PI4KIIIβ remain associated with the Golgi under these conditions is unknown.