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Golgi Membrane Dynamics and Lipid Metabolism Review

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The striking morphology of the Golgi complex has fascinated cell biologists since its discovery over 100 years ago. Yet, despite intense efforts to understand how membrane flow relates to Golgi form and function, this organelle continues to baffle cell biologists and biochemists alike. Fundamental questions regarding Golgi function, while hotly debated, remain unresolved. Historically, Golgi function has been described from a protein-centric point of view, but we now appreciate that conceptual frameworks for how lipid metabolism is integrated with Golgi biogenesis and function are essential for a mechanistic understanding of this fascinating organelle. It is from a lipid-centric perspective that we discuss the larger question of Golgi dynamics and membrane trafficking. We review the growing body of evidence for how lipid metabolism is integrally written into the engineering of the Golgi system and highlight questions for future study.

Introduction

The Golgi apparatus is a central station for the sorting and transport of proteins and lipids that transit the secretory pathway. This organelle also serves as a biochemical factory where anterograde cargo is subject to serial post-translational modifications before being sorted at the *trans*-Golgi network (TGN) for delivery to the appropriate destinations. As such, the Golgi system plays a central role in eukaryotic cell biology. At steady state, Golgi membranes are typically organized in a stack of flattened cisternae with dilated rims [1]. Such an organization has been argued to reflect the logic for ordering the biochemical activities of the system. That is, one simply generates stable compartments in the context of the cisternal arrangement. This steady-state morphology is deceptive, however. The Golgi complex is a dynamic organelle subject to enormous membrane flux in its capacity as an intermediate station between the endoplasmic reticulum (ER) and the distal compartments of the secretory pathway. These fluxes are bidirectional because the Golgi system directs retrograde trafficking pathways for purposes of retrieval and recycling of Golgi and ER components and receives cargo from the plasma membrane and endosomes [1].

The structural plasticity of the Golgi system is evident at multiple levels. In mammalian cells, this organelle disassembles in mitosis and subsequently reassembles into a functional unit upon completion of cell division [2]. Golgi structural plasticity is also evident when the system is subjected to a variety of perturbations [3]. Disruption of Golgi morphology interferes with the modification, sorting and delivery of proteins and with wider cellular processes, such as ciliogenesis, cell polarity, cell migration, stress responses and apoptosis [4–6]. Thus, the forces that shape Golgi morphology exert unexpectedly broad effects on cell physiology. Perhaps reflective of these wider cellular functions, individual Golgi stacks are often laterally interconnected to form a reticular ribbon positioned in the perinuclear region of the cell in proximity to the vertebrate centrosome.

Yet, the Golgi system is resilient. It exhibits remarkable capacities for self-organization that allow it to recover from catastrophic structural derangements. As an example, induction of the collapse of the Golgi system into the ER by treatment with the drug brefeldin A is followed by reformation of a functional organelle upon drug removal [7]. Thus, the steady-state form of the Golgi system portrays an illusion of compartmental stability. The very existence of the organelle is balanced on a knife's edge of competing forces that create it and consume it. It is the remarkable dynamics of the Golgi system that have, over the past decade, fueled a re-evaluation of the fundamental nature of this organelle, and maturation models now supplant stable compartment models as favored mechanisms for Golgi biogenesis and function [8].

Initial studies of Golgi membrane trafficking and dynamics were exclusively protein-centric [9,10]. It is now appreciated that lipid metabolism is integrally written into the fabric of the transport carrier cycle and of Golgi function. Since the first demonstrations to this effect in permeabilized cell systems and in yeast [11–14], we now understand that the interface of lipid metabolism with membrane trafficking is complex. This interface is a major factor in controlling Golgi morphology and dynamics. It also involves a large cast of interesting proteins and enzymes, including lipid transfer proteins [11,12,15–18], lipid kinases and phosphatases [19–22], phospholipase D (PLD) and phospholipase A_2 (PLA_2) [23–25], phospholipid acyl-transferases [26,27], and amino-phospholipid flippases that harness their ATPase activities for topological control of lipid distribution between bilayer leaflets [28–30].

Lipid metabolism interfaces with membrane trafficking in several general ways. First, it helps to create platforms for protein recruitment to, and activation at, appropriate sites on membrane surfaces. The reduced dimensionality achieved by recruiting soluble factors to a surface has a powerful concentrating effect that promotes effective biochemistry in systems governed by modest affinities. In these capacities, lipid metabolism has a signaling role. Second, it facilitates the structural deformations of membranes that accompany vesicle budding, fusion and tubulation. Third, it effects a lateral segregation of molecules, and this partitioning contributes to Golgi function. For example, regulation by lateral segregation is the underlying principle of a rapid partitioning model proposed to account for cargo export kinetics from the Golgi complex [31]. The model is based on a continuous two-phase system; one that can readily be generated by lipid segregation into fluid and relatively less fluid domains. While the continuous two-phase partitioning model is overly simplistic, and some of its basic tenets are at odds with known properties of the Golgi complex [32], the concept illustrates how self-organizing principles linked to lipid metabolism/composition might give rise to complex Golgi functions. Studies

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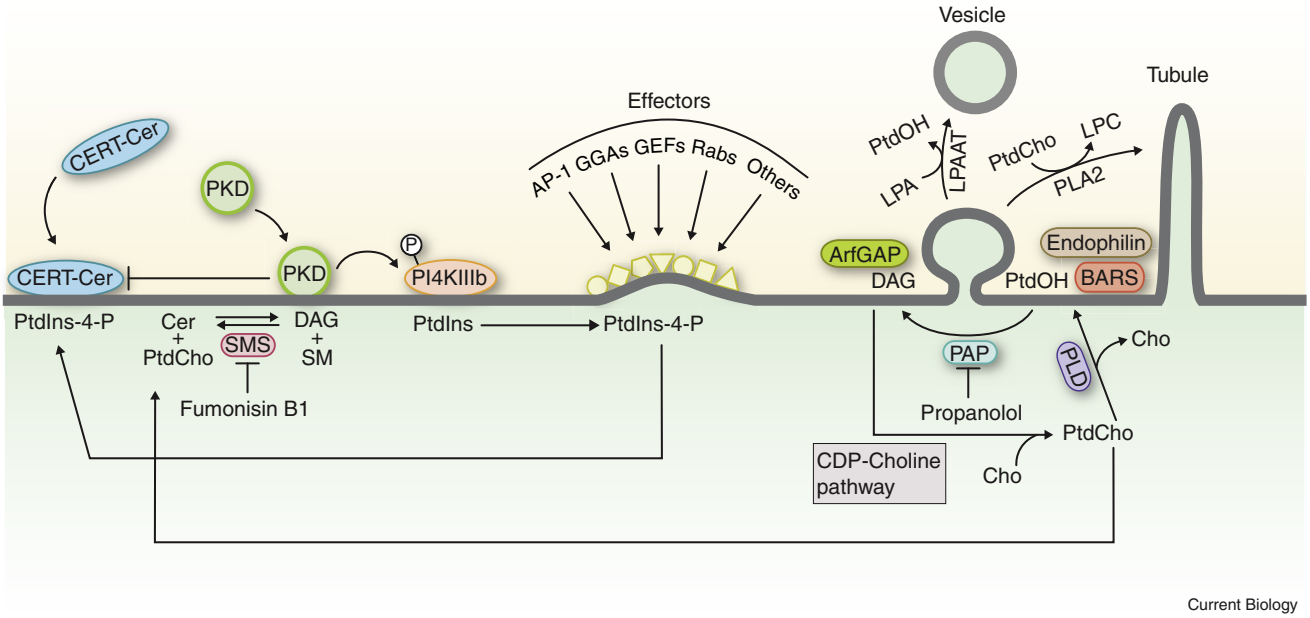

Figure 1. Lipid metabolism and formation of transport carriers.

This diagram highlights existing views of the interface between lipid metabolism and components of the protein machinery that drives formation of vesicles or tubular transport carriers in mammals. No obvious ceramide transfer protein (CERT) or protein kinase D (PKD) activities are present in yeast, and yeast do not have obvious counterparts for BARS, endophilin or lysoPtdOH acyltransferases (LPAATs). The dissonance between this diagram and *in vivo* readouts for PLD, BARS and CERT function in mammals is discussed in the text. (Cer, ceramide; LPC, lyso-PtdCho; PAP, PtdOH phosphatase; SM, sphingomyelin; SMS, sphingomyelin synthase.)

suggesting that the transmembrane domains of resident proteins are matched to the physical properties of the membranes in which these reside also support partitioning concepts [33]. In this review, we describe the impact of lipid metabolism on Golgi dynamics and organize the discussion from the perspective of classes of lipids and how these molecules modulate Golgi functions.

PtdIns-4-phosphate and TGN Function

Involvements of phosphatidylinositol (PtdIns), and its phosphorylated derivatives (the phosphoinositides), were the first established examples of lipids having active roles in regulating membrane trafficking [11–14,34,35]. PtdIns-4-phosphate (PtdIns-4-P) is an important phosphoinositide in the operation of the Golgi system [36]. That biologically sufficient production of PtdIns-4-P is integrated with phosphatidylcholine (PtdCho) metabolism provides a striking demonstration of the cross-talk between lipid metabolism and Golgi secretory function [11,12,37,38] (the issue of cross-talk is discussed below in the context of lipid transfer protein function).

Mammalian Golgi membranes harbor two types of PtdIns 4-kinases — PI4KIIIβ and PI4KIIα. Their respective yeast cognates are Pik1 and Lsb6, and Pik1 localizes to yeast Golgi membranes [39]. The PI4KIIIβ enzymes are the best understood and function as heterodimers with a myristoylated Ca^{2+}-binding non-catalytic subunit [40,41]. These PtdIns 4-kinases also engage in direct interactions with the vesicle biogenic machinery; mammalian PI4KIIIβ homes to Golgi membranes by binding to the GTP-bound form of the small GTPase Arf1 [42,43], whereas the yeast ortholog Pik1 targets to Golgi membranes by binding to a guanine nucleotide exchange factor (GEF) for Arf GTPases [44].

PtdIns 4-kinase catalytic activity is clearly important for Golgi function. Acute inactivation of yeast Pik1 kinase activity [21,22], or induction of the degradation of PtdIns-4-P to PtdIns in mammalian Golgi [45], results in trafficking defects. Inactivation of PtdIns-binding proteins, such as Sec14, which potentiate PtdIns 4-kinase activities by presenting PtdIns to the enzyme for efficient modification, also compromises Golgi membrane trafficking [11,38]. The lipid kinase activity is not the sole essential property of PtdIns 4-kinase with respect to Golgi function, however; *Drosophila* PI4KIIIβ binds to the small GTPase Rab11 in the TGN and executes a scaffolding function independent of its catalytic activity [46].

How does PtdIns-4-P potentiate Golgi secretory functions? First, PtdIns-4-P contributes to the recruitment of peripheral membrane proteins important for transport carrier biogenesis (Figure 1). These include Golgi adaptors for clathrin binding, such as AP-1 [47,48], and Arf1-GTP effectors, such as GGA proteins [49,50], Rabs and Rab-GEFs [51,52], and the Arf-GEF GBF1 [53]. Oxysterol binding-related proteins (OSBPs) interface with PtdIns-4-P signaling [16–18], and other lipid binding/transfer proteins that further remodel Golgi membrane lipid composition are also PtdIns-4-P effectors (see below).

Second, PtdIns-4-P modulates protein activities by direct binding mechanisms. One example is the yeast amino-phospholipid flippase Drs2, a type-IV integral membrane ATPase, which translocates phosphatidylserine (PtdSer) and phosphatidylethanolamine (PtdEtn) from the lumenal to the cytosolic leaflets of TGN/endosomal membranes [28–30]. Drs2 flippase activity is stimulated by binding both to PtdIns-4-P and to an Arf1-GEF [54]. Another example of the regulation of the activity of a protein component of the trafficking

machinery by PtdIns-4-P binding is described by the coincidence-detection mechanism for the function of yeast Sec2, a GEF for the Rab GTPase Sec4. PtdIns-4-P binding quenches the ability of Sec2 to nucleate assembly of the exocyst complex, which is required for the interaction of secretory vesicles with the plasma membrane [54,55]. By discouraging premature Sec2-mediated recruitment of the exocyst to TGN membranes, PtdIns-4-P helps to preserve the compartmental distinction between transport intermediates and the Golgi system.

Recent studies of the yeast PtdIns-binding protein Sec14 and PtdIns-4-P-binding protein Vps74 indicate that retrograde membrane flow from endosomes to the TGN and retention of glycosyltransferases within the Golgi system, respectively, is PtdIns-4-P dependent [56,57]. PtdIns-4-P binding by Vps74 coordinates the interactions of this protein with the cytosolic tails of glycosyltransferases and with the coatomer complex, which coats vesicles [57]. Vps74 is an ortholog of the mammalian protein GOLPH3, which collaborates with the non-conventional myosin MYO18A to control Golgi morphology [57]. In addition, GOLPH3 interacts with the retromer complex, which potentiates retrograde membrane trafficking from endosomes [58]. These data suggest that GOLPH3 also functions in cargo sorting and retrieval in mammals.

The Yin and Yang of Lipid Transfer Proteins and TGN Functions

Extensive involvement of lipids in regulating Golgi function demands close coordination of lipid metabolism with PtdIns-4-P signaling. Lipid transfer proteins are the coupling devices through which this coordination is executed, and PtdIns transfer proteins (PITPs) provide outstanding examples. The major yeast PITP, Sec14, coordinates PtdIns-4-P function in the TGN with the activity of a pathway that generates PtdCho from diacylglycerol (DAG) [11–13,59,60]. The remarkable structural design for how Sec14 differentially binds PtdIns and PtdCho is central to how Sec14 is posited to use heterotypic phospholipid exchange to effect a 'PtdCho-primed' presentation of PtdIns to PtdIns 4-kinases [37,38]. Such an elaborate presentation mechanism is essential for phosphoinositide homeostasis *in vivo* because PtdIns 4-kinases are biologically inadequate interfacial enzymes when asked to modify membrane-incorporated PtdIns, i.e. the presumed natural mode of presentation. By this view, Sec14-stimulated PtdIns-4-P synthesis is primed in response to PtdCho metabolic cues [37,38]. Indeed, analysis of the effects of mammalian disease-associated mutations in Sec14-like proteins suggests that such presentation functions are general properties of these proteins [37,61]. A physical interaction between Sec14 and PtdIns 4-kinases has not been reported, and such an interaction may not be necessary for presentation of PtdIns to these kinases [15,16]. Details of the PtdIns-presentation mechanism remain to be elucidated, and other evidence suggests that some mammalian multidomain Sec14-like proteins involved in vesicle trafficking bind both lipids and proteins [62].

The pro-secretory activities of yeast Sec14 are opposed by Kes1 (also known as Osh4), a member of an unrelated class of lipid transfer proteins (the oxysterol binding related proteins — ORPs) [15,16]. Kes1 binds to PtdIns-4-P and this activity is essential for Kes1's biological function as a trafficking 'brake' [15,16]. The Kes1–Sec14 antagonism plays itself out in the context of PtdIns-4-P signaling [16,18,63], but how this occurs is not clear. Some evidence suggests that ORPs stimulate phosphoinositide phosphatases that degrade PtdIns-4-P (such as Sac1, see below [18]). Other data indicate that Kes1 competes with pro-secretory factors for PtdIns-4-P binding [64]. Kes1 has two PtdIns-4-P-binding sites — one on the protein surface [16], and the other involving the hydrophobic cavity and overlapping with the sterol-binding site [65]. PtdIns-4-P binding is essential for Kes1 localization to TGN/endosomal membranes. Missense substitutions in either of the two PtdIns-4-P binding sites render Kes1 incompetent for targeting to TGN/endosomes [16,64].

With regard to sterol binding, it is now clear that sterol-binding defects enhance Kes1 biological activity as a TGN/endosomal trafficking brake [64,66]. These findings are in direct contradiction to a prominent claim that sterol binding is required for Kes1 function *in vivo* [67]. The dual PtdIns-4-P and sterol-binding activities of Kes1 cooperate in a rheostat mechanism where the interplay between sterol- and PtdIns-4-P-binding controls the amplitude of the Kes1-imposed PtdIns-4-P clamp on TGN/endosomal trafficking [64] (Figure 2). The discovery that the Kes1 sterol-binding site overlaps with a PtdIns-4-P-binding site neatly accounts for how sterol tunes Kes1-mediated inhibition of PtdIns-4-P signaling. When coupled with the demonstration that Kes1 and other ORPs are collectively dispensable for non-vesicular sterol transfer in yeast [68], the data indicate that Kes1 is not a sterol transfer protein *in vivo* as has been previously argued [69,70].

The Kes1 rheostat has broader physiological roles as it sets the gain of endosomal sphingolipid signaling, modulates activation of the serine/threonine kinase TOR by amino acids, regulates the nuclear activity of the major transcriptional activator of the general amino acid control pathway (Gcn4 in yeast and Atf4 in mammals), and administrates a coherent exit from proliferative programs to quiescent states (Figure 2). The transcriptional arm of this novel endosomal–nuclear axis involves the cyclin-dependent kinase module of the Mediator transcriptional coactivator complex [64]. Whether mammalian PITPs and ORPs play similarly opposing functions is a question for future inquiry. The idea that a PITP–ORP 'tug-of war' fine-tunes cell growth regulation and metabolic control as a function of TGN/endosomal trafficking flux has interesting implications for cell entry into post-mitotic fates and for tissue biogenesis [64].

Metazoan Lipid Transfer Proteins and the Golgi System

The steroidogenic acute regulatory protein-related lipid transfer (StART)-like mammalian PITPs are structurally unrelated to Sec14-like PITPs, and functional depletion of specific isoforms, such as PITPβ, has been reported to compromise retrograde Golgi-to-ER transport mediated by the COP1 coat complex [71]. This defect purportedly comes without compromising anterograde ER-to-Golgi trafficking — a curious result given that retrograde transport is essential for recycling of the v-SNARE vesicle fusion protein required for anterograde ER-to-Golgi transport. With regard to vertebrate PITPβ, zebrafish with strongly reduced PITPβ levels develop normally, but exhibit defects in outer segment biogenesis and/or maintenance in double cone photoreceptor cells [72]. These data argue against housekeeping roles for PITPβ in retrograde Golgi-to-ER trafficking,

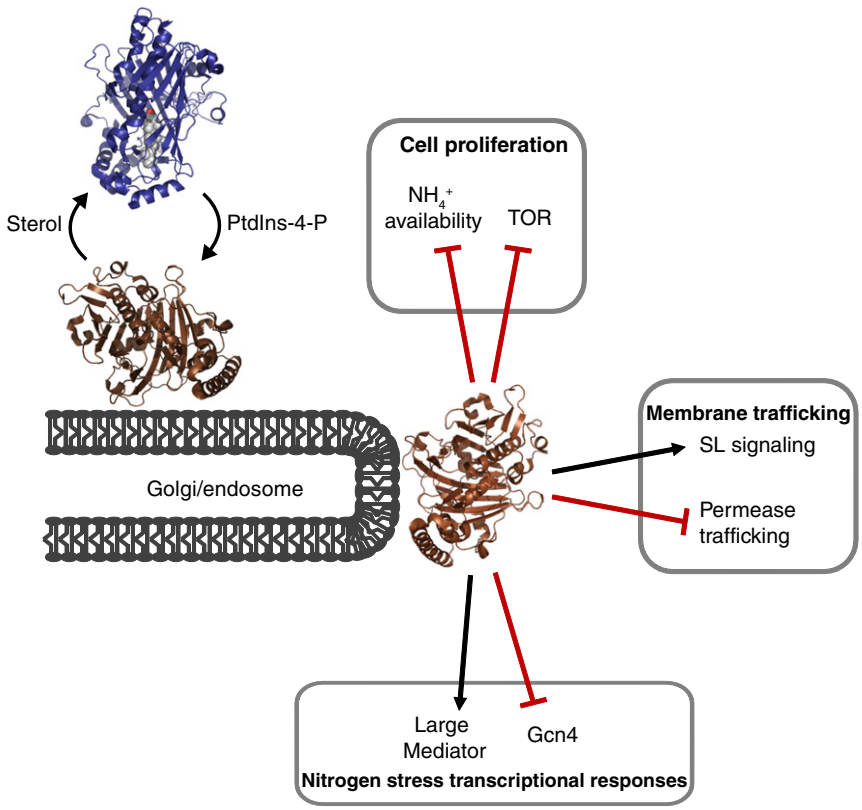

Figure 2. Kes1 integrates PtdIns-4-P signaling, sterols, TGN trafficking control, and larger cellular physiological responses.

Kes1 is recruited to Golgi membranes by virtue of its ability to bind PtdIns-4-P where it clamps the availability of this phosphoinositide and functions as a trafficking 'brake' [64]. Sterol binding at the TGN releases Kes1 from the membrane, thereby releasing the trafficking brake. Broader consequences of this negative regulation of membrane trafficking include the control of cell proliferation by TGN/endosomal sphingolipid metabolism, TOR signaling, and execution of nitrogen stress transcriptional responses.

although such functions might be important in specialized contexts that require high-capacity membrane flux.

Other StART-like PITPs are reported to play roles similar to yeast Sec14 in coordinating Golgi PtdCho and DAG metabolism with PtdIns-4-P signaling [17]. Nir2 is a multidomain PITP reported to collaborate with two other StART-domain lipid transfer proteins, an OSBP and a ceramide transfer protein (CERT), in forming a membrane contact site. The membrane contact site is hypothesized to bridge TGN and ER membranes — a concept that couples TGN activities with those of the ER [73]. For the purposes of discussion, the hypothetical membrane contact site is illustrated in Figure 3. Both OSBP and CERT are PtdIns-4-P-binding proteins and both are substrates of protein kinase D (PKD) [74]. In this model, CERT supplies the TGN with ceramide via a mechanism in which PtdIns-4-P binding mediates CERT interaction with TGN membranes, and CERT phosphorylation by PKD releases CERT from the TGN [75] (Figure 3). Current models propose that CERT fuels DAG production by sphingomyelin synthase in the Golgi at the expense of ceramide. DAG recruits PKD which then activates PI4KIIIβ [73]. Nir2 is posited to co-assemble into the CERT–OSBP membrane contact site for the purpose of transferring PtdIns from the ER to the Golgi to sustain PI4KIIIβ activity [73]. In sum, this specific membrane contact site model assigns essential roles for CERT, Nir2 and OSBPs in promoting membrane trafficking from the Golgi complex [76].

CERT and Nir2 gene ablation data fail to support the basic hypotheses, however. *Drosophila* lacking CERT develop normally and reach adulthood. Although these *cert*$^{0/0}$ flies age prematurely due to oxidative plasma membrane damage, they are otherwise remarkably unaffected [77]. By contrast, *cert*$^{0/0}$ mice expire at embryonic day 11.5 from a failure in cardiac organogenesis. *cert*$^{0/0}$ embryonic fibroblasts are viable, although these too are prone to accelerated senescence [78]. Thus, the evidence consistently highlights a role for CERT in oxidative stress management. While a requirement for CERT in the trafficking of unusual cargos required for organogenesis remains a formal possibility, genetic data argue against an involvement of CERT in core Golgi trafficking functions. It is also yet to be demonstrated that CERT bridges ER and Golgi membranes *in vivo*, a critical tenet of the membrane contact site model (Figure 3).

Are Nir2-like PITPs obligatorily required for the function of metazoan Golgi, as proposed [73]? Available evidence does not support this hypothesis either. *Drosophila* mutants ablated for their single Nir2 ortholog (RdgB) survive through adulthood, but do suffer a rapid light-accelerated retinal degeneration caused by an inability of photoreceptors to terminate the photoresponse. This degeneration is cured by expression of the isolated RdgB PITP domain, which presumably does not efficiently assemble into a membrane contact site, and is associated with a restored photoresponse, even under saturating light conditions [79]. This is an impressive outcome given the enormous phosphoinositide flux demanded by this signaling cascade. Mammals employ Nir2 differently from flies, however, as evidenced by the demonstration that *nir2*$^{0/0}$ mice suffer pre-implantation lethality [80]. The terminal *nir2*$^{0/0}$ phenotype in mice is uncharacterized, so it is unknown whether the lethality stems from Golgi trafficking defects, or not.

There is also no direct evidence to indicate that Nir2 functions in a PtdIns-supply capacity, even though this is the common interpretation. It is difficult to determine in vertebrate systems whether StART-like PITPs operate in cells as PtdIns-presenting scaffolds or as PtdIns carriers. These two modes of action differ in fundamental respects and have recently been discussed from alternative points of view [73,81]. However, vertebrate StART-like PITPs resemble their yeast and plant Sec14-like PITP counterparts in having the intrinsic capacity to potentiate PtdIns 4-kinase

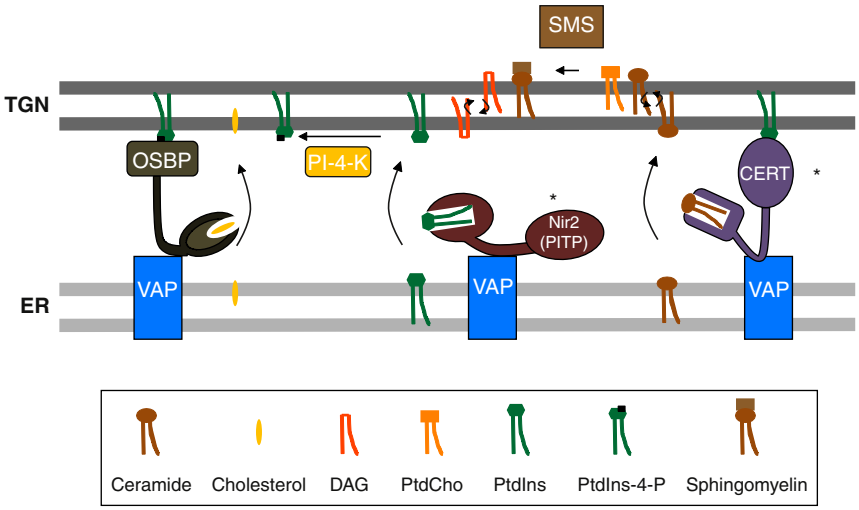

Figure 3. Lipid transfer proteins and a hypothetical membrane contact site.

CERT, OSBP and Nir2 are proposed to co-assemble into a TGN–ER membrane contact site where these proteins catalyze ER-to-TGN trafficking of ceramide, sterol and PtdIns-4-P, respectively. CERT and OSBP interact with the TGN by virtue of their PtdIns-4-P-binding activities. Nir2 is proposed to supply the TGN with PtdIns from the ER for sustained PtdIns-4-P production. The membrane contact site is envisioned to be held together by integral membrane proteins of the ER (VAPs) that bind the FFAT motifs of CERT, OSBP and Nir2, and the lipid transfer activities are proposed to be essential for membrane trafficking from the Golgi complex. The concepts highlighted by asterisks are not supported by available *in vivo* data (see text).

activities under conditions in which PtdIns-supply requirements are moot [72]. Moreover, the discovery that PtdIns synthase mobilizes from the ER to sites adjacent to plasma membrane and other organelles to replenish phosphoinositide signaling pools in those membranes challenges the fundamental assumption for why cells would even require *bona fide* PITPs [82].

Sac1 Phosphoinositide Phosphatases and the Golgi System

Maintenance of phosphoinositide homeostasis requires a balanced control over the biosynthetic and the degradative aspects of their metabolism. Phosphoinositide turnover is achieved by lipid phosphatases, such as synaptojanins, the oculocerebrorenal Lowe's Syndrome OCRL protein, PTEN, and myotubularins. These enzymes are particularly interesting because of their association with a variety of diseases [83–86]. The mixed-specificity phosphoinositide phosphatase Sac1 localizes to ER and Golgi membranes and is unique amongst the inositol lipid phosphatases in that it is an integral membrane protein [19,87]. This enzyme is incapable of utilizing phosphoinositides with vicinal phosphate groups as substrates [20]. Sac1 executes wider physiological roles on the basis of its modulation of PtdIns-4-P signaling in both yeast and mammals. Under conditions of extreme nutrient or growth factor insufficiency, the Sac1 PtdIns-4-P phosphatase redistributes from the ER to the Golgi complex [88]. Presumably, regulated trafficking of the phosphatase discourages cell proliferation by clamping the activity of the distal secretory pathway (via degradation of Golgi PtdIns-4-P) under suboptimal growth conditions.

Sac1 dysfunction also alleviates the normally essential requirement for PITP activity for Golgi secretory function in yeast [19,87], reflecting the fact that Sac1 constitutes the major PtdIns-4-P degradation activity in yeast [20,60]. Paradoxically, Sac1 specifically consumes the PtdIns-4-P produced by the plasma-membrane-localized PtdIns 4-kinase Stt4 and not the Golgi-localized PtdIns 4-kinase Pik in yeast [89,90]. It is unknown whether this specificity of Sac1 for particular pools of PtdIns-4-P translates to mammals. While Sac1 insufficiencies do not evoke large derangements in bulk PtdIns-4-P mass in mammalian cells, they do result in pre-implantation lethality, morphological derangements of the Golgi, defects in mitotic spindle organization, mis-sorting of Golgi glycosyltransferases, and aberrant protein glycosylation [91,92].

Diacylglycerol and the Transport Carrier Cycle

In addition to PtdIns-4-P, to which we assign primarily a signaling role, a number of other lipids also have key involvements in Golgi functions. Some of these lipids likely play both signaling and structural roles; DAG, a neutral lipid with unusual physical properties, is one of these. The extreme inverted cone shapes assumed by DAG (due to its small headgroup to acyl chain axial area ratio) facilitate adoption of the non-bilayer configurations that lipid molecules assume in strongly deformed membrane regions. Such deformations accompany both vesicle budding and scission [93,94]. Accordingly, DAG regulates vesicle budding at multiple steps in the exocytic pathway. These include transport from the yeast and mammalian TGN [17,59,95–97], and formation of mammalian COP1-bound vesicles for retrograde trafficking from early Golgi cisternae to the ER [65,66].

In some cases, DAG directly regulates the activity of protein components of the trafficking machinery. For example, DAG potentiates Arf GTPase-activating protein (ArfGAP) function in both yeast and mammals [95,97], and DAG exhibits at least two points of action in mammalian COP1-dependent vesicle biogenesis. One is at an early step in formation of buds/tubules when the membrane is first deformed, and another at the scission step where the nascent vesicle is released from its donor membrane [98,99] (Figure 1). DAG involvement in scission requires ArfGAP1 activity, suggesting that DAG potentiates scission both by activating ArfGAP1 and by facilitating the formation of non-bilayer membrane structures that characterize fission intermediates [98]. DAG-activated PKD also promotes vesicle scission in the TGN [100,101]. Although there is as yet no evidence for obligate DAG involvements in early stages of the yeast secretory pathway, roles for DAG in the yeast TGN have been documented [98].

Compartment-specific requirements imply that DAG primarily functions in a signaling capacity. In that regard, DAG recruits PKD isoforms to mammalian TGN membranes [96]. PKD activation serves as the nexus of a larger signaling

hub which connects DAG metabolism to downstream lipid metabolic events required for optimal membrane trafficking from the TGN [100–102] (Figure 1). This larger hub includes the recruitment of PI4KIIIβ, which generates PtdIns-4-P in the TGN with the pro-trafficking sequelae detailed above. DAG recruits PKC, Ras guanine nucleotide release proteins [103–106], and PKCη (which phosphorylates PKD and activates the enzyme) to TGN membranes as well [107].

Phosphatidic Acid Metabolism and Golgi Membrane Trafficking

Pools of DAG generated from phosphatidic acid (PtdOH) by the action of PtdOH phosphatases are required for membrane trafficking through the yeast and mammalian Golgi [98,99]. That PtdOH itself executes pro-secretory functions was suggested by demonstrations that PLD, which hydrolyzes PtdCho to PtdOH and choline, is activated by PtdIns-4,5-P_2 and Arf-GTP [23,25]. Mammals express two PLD isoforms — PLD1 and PLD2 — and numerous studies claim obligatory PLD1 and/or PLD2 involvements in producing PtdOH pools that are essential for membrane trafficking through the mammalian Golgi system [108,109]. Biochemical studies suggest PtdOH acts in concert with its binding proteins endophilin and BARS in the scission of COP1 vesicles, which form in an Arf-GTP-dependent manner [110,111] (Figure 1). *In vitro* studies suggest that endophilin and BARS resolve fission intermediates by physically deforming membranes and that a PtdOH pool generated by PLD2 is required for execution of these functions [112]. The *in vivo* relevance of these findings is suggested by experiments that show that PLD2 depletion affects *cis*-Golgi maintenance and retrieval of KDEL receptors from early Golgi cisternae back to the ER [113]. DAG kinases, which produce PtdOH from DAG, cannot function as PLD2 surrogates in this system [113], suggesting direct roles for PLD-generated PtdOH pools in Golgi function. Whether PtdOH serves as a DAG precursor in these contexts is unresolved.

PtdOH Remodeling Enzymes and Golgi Dynamics

Phospholipids are subject to two-stage remodeling reactions that convert one molecular species of a particular phospholipid to another. The first reaction involves removal of the *sn*-2 fatty acid from the glycerol backbone of a phospholipid by a PLA_2 to form a lyso-phospholipid with a single acyl chain. The lyso-phospholipid is a substrate for acyltransferases that incorporate another fatty acid at *sn*-2 to regenerate the original phospholipid, albeit a different molecular species. PtdOH-remodeling enzymes have been suggested to contribute to Golgi dynamics and trafficking in the light of reports showing that endophilin and BARS are lyso-PtdOH acyltransferases (LPAATs) [110,111]. More detailed analyses showed that these proteins have no such activity, however [114]. The evidence indicates a PLA_2–LPAAT cycle regulates tubulation events that potentiate membrane trafficking and cargo sorting in mammalian Golgi [24,26,27,115]. PLA_2-induced Golgi tubulations are enhanced by secretory cargo, and these tubules consolidate what would otherwise be individual Golgi stacks into a Golgi ribbon [24,26,27,115]. Connecting tubules are suggested to represent the portals through which anterograde cargo passes as it transits from one Golgi cisterna to the next.

COP1 or BARS initiates the formation of both tubules and vesicles from mammalian Golgi membranes *in vitro* [116]. Growing tubules are stabilized by cytosolic PLA2 (cPLA$_2$-α) activity on the one hand, and resolved into vesicles by LPAAT-γ on the other. In these assays, tubules score as non-concentrative cargo carriers, while vesicles score as concentrative carriers, suggesting that anterograde trafficking is a passive process while retrograde trafficking is an active one [116]. Because the formation of both tubules and vesicles is dependent on COP1, this idea offers a resolution to the debate of whether COP1-coated membranes define anterograde or retrograde carriers by conceptualizing how COP1 might participate in both pathways [32]. How general cPLA$_2$–LPAAT mechanisms are as a core Golgi trafficking strategy is unclear given that yeast and worms do not have obvious LPAATs. However, yeast have a naturally vesiculated Golgi — a feature that might obviate an LPAAT requirement. Also, some organisms might employ monoacylglycerol-acyltransferases, rather than LPAATs, for vesicle scission.

Genetic Models for PLD Function

Given the pharmacological and biochemical evidence for PLD-generated PtdOH pools in driving multiple aspects of membrane trafficking, it is surprising that mice nullizygous for either *PLD1* or *PLD2* are developmentally normal [117,118]. The PLD1 null model does reveal a *PLD1* requirement in both starvation-induced expansion of autophagosomes and clearance of protein aggregates in brain tissue by macroautophagy [98]. The enzyme relocalizes from endosomes to the outer membrane of autophagosomes in the face of nutrient stress via a mechanism that requires PtdIns 3-kinase activity [98]. *PLD2*-nullizygous mice, while also overtly normal, present enhanced resistance to the neurotoxic effects of amyloid β-peptide [98]. Perhaps most surprising is that PLD1 and PLD2 activities fail to cross-compensate to any significant degree because *pld1*$^{0/0}$ *pld2*$^{0/0}$ double mutant mice do not appear to exhibit enhanced phenotypes relative to the respective single mutants (G. DiPaolo, personal communication).

Does DAG-kinase-mediated conversion of DAG to PtdOH compensate for PLD in nullizygous mice and cells? While an unresolved question, compensation by DAG kinases would necessarily operate in the absence of the numerous physical interactions reported between PLD isoforms and membrane trafficking components and proteins involved in lipid signaling [98]. Such functional compensation would also be inconsistent with the conclusions of *in vitro* experiments that contend that PtdOH pools produced by DAG kinases cannot substitute for those generated by PLD2, at least not for COP1 vesicle budding [113]. Why the dissonance? *In vitro* reconstitutions, their power notwithstanding, are inefficient systems. Consequently, these might exhibit non-physiological dependencies on particular lipid metabolic pathways for basic operation (i.e. those that preserve a relative robustness in cell-free preparations), even when the *in vitro* system faithfully reconstitutes a specific lipid requirement.

PLD is also non-essential for core trafficking functions in fungi because the single PtdCho-specific yeast PLD is dispensable in vegetative cells [119]; however, PLD catalytic activity is required for membrane trafficking in mutants lacking certain lipid transfer proteins [120]. A physiological role for PLD is demonstrated by the developmental reorientation of membrane trafficking from the TGN to the nuclear envelope during sporulation. PLD produces a PtdOH pool that recruits and activates a sporulation-specific t-SNARE

of the Sec9/SNAP-25 family (Spo20). This t-SNARE redirects post-Golgi trafficking to the forming nuclear envelopes at the expense of the plasma membrane, and PLD defects prevent post-Golgi vesicle fusion with nascent nuclear envelopes [121]. Thus, PLD-generated PtdOH generated by PLD is required for a developmentally-regulated vesicle fusion process, but not vesicle formation or vesicle scission.

Genetic Models for BARS Function

Are *in vivo* models consistent with *in vitro* data regarding a role for endophilin and BARS as PtdOH effectors in membrane trafficking? In the case of endophilin there is good agreement as this protein is indeed essential for fission and uncoating of clathrin-coated vesicles in neurons [122–124]. BARS is reported to be essential for fragmentation of the Golgi ribbon in cultured cells, and ribbon scission is required for cells to negotiate the G2/M boundary. Interestingly, only cells with Golgi ribbons exhibit BARS-regulated Golgi mitotic checkpoints [125]. These findings emphasize the link between Golgi structure, lipid metabolism, and cell-cycle control.

BARS has a curious history, however, as it was first described as a member of the CtBP protein family of transcriptional co-repressors and is a spliceoform of CtBP1 [126]. BARS/CtBP null (*ctbp1*$^{0/0}$) mice exhibit various developmental phenotypes associated with defects in body size, vascularization and body patterning. These phenotypes primarily reflect the transcriptional functions of BARS/CtBP. The *ctbp1*$^{0/0}$ phenotypes, and the viability of *ctbp1*$^{0/0}$ embryonic fibroblasts, are not consistent with essential roles for BARS in Golgi housekeeping functions or obligate requirements for BARS in progression through the G2/M Golgi checkpoint [126]. It has been noted that the Golgi system of *ctbp1*$^{0/0}$ embryonic fibroblasts differs from that of wild-type fibroblasts in that it is not organized as an intact ribbon, and this morphological derangement is argued to relieve *ctbp1*$^{0/0}$ fibroblasts of a requirement for BARS in cell-cycle progression [125]. This argument begs the question of what activities are responsible for fragmentation of the Golgi ribbon in cells that lack BARS. Also, what activity (if any) compensates for BARS in *ctbp1*$^{0/0}$ cells? Non-neuronal endophilins are candidates, and *in vitro* data support this notion [127]. But, given the dissonance between *in vitro* and *in vivo* readouts, this hypothesis must be tested in a suitable *in vivo* context.

Amino-Phospholipids and Membrane Trafficking

Functional involvements of glycerophospholipids in Golgi secretory function are not limited to PtdIns, phosphoinositides, PtdCho, and PtdOH. Roles for amino-phospholipids, such as PtdEtn and PtdSer, in membrane trafficking is amply demonstrated by the important roles that amino-phospholipid flippases, such as Drs2, play in controlling membrane trafficking through the yeast TGN/endosomal system [28–30]. These P4-type ATPases translocate PtdSer and PtdEtn from cytosolic to lumenal membrane leaflets, and these activities interface with PtdIns-4-P signaling and the Arf pathway because yeast Drs2 flippase activity is stimulated by binding both to PtdIns-4-P and to an Arf1-GEF [54]. Yeast P4-type ATPases are also indirectly subject to regulation by sphingolipids via the Fpk protein kinases that phosphorylate (and activate) the flippases [128].

The complexity of the amino-phospholipid flippase involvement in yeast membrane trafficking is emphasized by the overlapping functional redundancies of multiple Drs2-like flippases [28–30]. A long-standing idea is that amino-phospholipid flippases promote positive membrane curvature (and therefore vesicle budding) by driving local leaflet asymmetries, both in terms of phospholipid composition and phospholipid distribution between the cytosolic and lumenal TGN/endosomal leaflets [28–30]. While the evidence identifies an interface of Drs2 (and Drs2-like flippases) with Arf and clathrin-dependent functions in yeast [36], it remains to be determined how flippase activities potentiate membrane trafficking. The functional significance of removal of PtdEtn or PtdSer from the cytosolic leaflet, and/or enrichment of the lumenal leaflet with PtdEtn or PtdSer, also needs to be explored.

Trafficking functions for PtdSer on the cytosolic leaflets of endosomal membranes are also recognized. This amino-phospholipid is required for retrograde membrane trafficking from mammalian recycling endosomes [129]. The primary, and perhaps exclusive, PtdSer effector in this system is evectin-2. This protein harbors a pleckstrin homology domain that displays an exquisite specificity for PtdSer and does not bind phosphoinositides. PtdSer binding is required for evectin-2 localization to recycling endosomes and for protein function in cells [129], but how evectin-2 regulates trafficking remains to be elucidated.

Sterols and the Golgi Complex

Membrane sterol content increases progressively through the compartments of the secretory pathway, and this gradient facilitates membrane protein sorting [130]. Sterols organize plasma membrane microdomains that modulate endocytosis and receptor activation and regulate membrane trafficking from the TGN. Biosynthetic trafficking of a subset of yeast plasma membrane proteins is disrupted by defects in sterol biosynthesis [131–133]. A common property of the affected cargos is their incorporation into ergosterol-containing detergent-resistant membranes [134]. Interestingly, compromising late steps in sterol biosynthesis results in mis-sorting of these cargos; although bulk sterol levels are unchanged under these conditions, the chemical profile of the accumulated sterols is altered [135]. These accumulated sterols, while chemically distinct from ergosterol, support the formation of detergent-resistant membrane microdomains. Yet, integral membrane proteins destined for the plasma membrane are mis-sorted, indicating that subtle differences in sterol structure influence trafficking fidelity.

Budding of anterograde vesicles from the TGN is proposed to be driven by the phase separation of sterol and sphingolipids into microdomains where the immiscibility of two liquid phases in lipid bilayers promotes the membrane bending necessary for vesicle budding [136]. Indeed, sterols and sphingolipids are enriched in TGN-derived vesicles relative to the bulk composition of the donor organelle [134,137]. The data suggest that a single lipid-driven sorting process drives biogenesis of TGN-derived vesicles bound for the plasma membrane [134]. This mechanism diverges from that which governs COP1 vesicle budding from bulk Golgi membranes *in vitro*. Those vesicles have a reduced sphingomyelin and cholesterol content relative to the bulk Golgi membranes from which they were formed [138]. Yet, sphingomyelin has an important role as a cofactor in COP1 vesicle formation: Brügger, Wieland and colleagues [139] have

reported the remarkable discovery that a single molecule of a specific molecular species of sphingomyelin binds to the transmembrane domain of a COP1 coat subunit (p24), modulates the oligomeric state of p24, and thereby regulates COP1 coat biogenesis.

Glycolipid Transfer Proteins

Glycolipid transfer proteins bind both sphingoid- and glycerol-based glycolipids and mobilize these lipids between membrane bilayers *in vitro* [140]. The glucosylceramide (GlcCer) transfer protein FAPP2 is recruited to Golgi membranes in a PtdIns-4-P-dependent manner, and is required for the production of complex glycosphingolipids for which GlcCer is a precursor. Although FAPP2 is suggested to deliver GlcCer to distal Golgi compartments as a lipid carrier [141], others report that FAPP2 promotes retrograde transport of GlcCer from Golgi to the ER [142]. The rationale for the retrograde pathway is that newly synthesized GlcCer, which resides in the cytosolic leaflet of Golgi membranes, is mobilized to the ER for the purpose of being flipped into the lumenal ER leaflet (Figure 3). Vesicular trafficking from the ER to the Golgi subsequently introduces the lumenally disposed GlcCer to Golgi-localized glycosyltransferases for maturation into complex glycosphingolipids [142]. A GlcCer-independent role for FAPP2 in TGN trafficking has also been suggested by the finding that FAPP2 forms a curved dimer that tubulates membranes in a PtdIns-4-P-dependent manner [143]. These studies describe a mechanism for how FAPP2 potentiates cargo transport from the TGN to apical surfaces of polarized epithelial cells [144].

Concluding Thoughts

Much progress has been made in understanding the mechanisms that control Golgi dynamics and architecture since the discovery of this organelle more than 100 years ago. Lipids, lipid-binding proteins, and lipid metabolism are major contributors to plasticity of the Golgi system. However, we have only a rudimentary understanding of the cross-talk between different arms of the Golgi lipid metabolome. 'Systems' approaches to model the landscape of Golgi lipid metabolism will be necessary for a detailed description of crosstalk mechanisms. These approaches also hold the ultimate promise of unifying lipid biochemical principles with Golgi function.

It still remains unclear why the Golgi adopts its characteristic morphology, given that secretory activity can be insensitive to dramatic structural derangements of this organelle. The answer must lie in unappreciated levels of physiological regulation associated with the organization of the Golgi, or with the maturation process itself. Insights to this effect are offered by tunable PITP–ORP rheostats, as these suggest mechanisms for integrating TGN/endosomal maturation (and lipid signaling) with the control of cell proliferation and nuclear responses to stress [64]. These circuits reveal an unappreciated physiological plasticity of Golgi/endosomal maturation programs and identify involvements of such rheostats in modulating Golgi plasticity. Such circuits seem ideally suited for chaperoning cell entry into post-mitotic states or in maintaining post-mitotic cell physiology. Perhaps maturation mechanisms for membrane trafficking evolved, in part, because these afford superior instruments for fine-tuning cell-growth regulation and metabolic control than do stable compartment mechanisms. In this regard, the fidelity of mitotic spindle formation and function is also influenced by Golgi organization, and evidence is building that lipid metabolism has a hand in this circuit as well [92,98,141,142]. We anticipate that studies of Golgi lipid metabolism in the developmental context of multicellular organisms will prove a major contributor to the future of Golgi research. The fruits of those studies will undoubtedly yield more surprises from an organelle that has already produced its share.

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