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Regulation of the Golgi Complex by Phospholipid Remodeling Enzymes

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Abstract

The mammalian Golgi complex is a highly dynamic organelle consisting of stacks of flattened cisternae with associated coated vesicles and membrane tubules that contribute to cargo import and export, intra-cisternal trafficking, and overall Golgi architecture. At the morphological level, all of these structures are continuously remodeled to carry out these trafficking functions. Recent advances have shown that continual phospholipid remodeling by phospholipase A (PLA) and lysophospholipid acyltransferase (LPAT) enzymes, which deacylate and reacylate Golgi phospholipids, respectively, contributes to this morphological remodeling. Here we review the identification and characterization of four cytoplasmic PLA enzymes and one integral membrane LPAT that participate in the dynamic functional organization of the Golgi complex, and how some of these enzymes are integrated to determine the relative abundance of COPI vesicle and membrane tubule formation.

Keywords

Golgi complex; Lands cycle; cytoplasmic PLA₂; Lysophospholipid acyltransferases; COPI coated vesicles; membrane tubules

Introduction

The mammalian Golgi complex is an iconic structure consisting of stacks of flattened membrane cisternae that are dynamically modified by the formation of coated vesicles and membrane tubules [1–5]. In addition, the cisternal stacks are often bridged together by membrane tubules into large ribbon-like structures, often forming a single interconnected organelle [6, 7]. The coated vesicles and membrane tubules mediate cargo import and export events, which by their combined activities help to shape the architecture and polarity of the Golgi complex, and contribute to bidirectional trafficking of cargo across the cisternal stacks. Although numerous structural, scaffold, fusion, and tethering proteins, e.g., COPI and AP-1 clathrin coats, GRASP, SNARE proteins, etc., have been shown to be crucial elements that regulate the functional organization of the Golgi complex, many fundamental questions about how the Golgi works remain unanswered, e.g., how secretory proteins move from the *cis* to the *trans* side of the Golgi stack [8]. Our lack of understanding is primarily due to the integrated and dynamic nature of the Golgi membranes, which makes it difficult

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to alter only a single aspect of Golgi trafficking without affecting every other. Recently, the dynamic functional organization of the mammalian Golgi complex has been reported to involve continual phospholipid remodeling by the opposing actions of phospholipase A (PLA) and lysophospholipid acyltransferase (LPAT) enzymes, a process in which acyl chains are continually removed and reincorporated (Fig. 1) [9, 10]. In other words, dynamic remodeling of Golgi membrane phospholipids is thought to play a role in shaping the structure of the Golgi complex itself. This review will focus on how these recently identified enzymes contribute to the dynamic nature of the mammalian Golgi complex.

In the 1960's Lands and colleagues established that after their synthesis, glycerol phospholipid acyl chains turnover in a cycle of deacylation and reacylation catalyzed by the opposing actions of PLA and LPAT enzymes, respectively, in what is now called the Lands cycle (Fig. 1) [11, 12]. The turnover of acyl chains serves to remodel phospholipids and thus alter their functional properties, although the full extent of the Lands cycle role in cells is unclear. Nevertheless, several functions of the Lands cycle have been established including the incorporation and release of arachidonic acid (AA) for the production of eicosanoids and prostaglandins [13], production of platelet activating factor (PAF) [14–16], and the incorporation of host-derived fatty acids into membrane phospholipids in *Giardia* and other intracellular parasites [17]. Here we describe recent studies which suggest that Lands cycle phospholipid remodeling might regulate the functional organization of the Golgi complex.

The mammalian Golgi complex, including the ER-Golgi-Intermediate Compartment (ERGIC) and *trans*-Golgi Network (TGN), produces various types of membrane cargo transporters such as COPI and AP-1 clathrin coated vesicles, regulated secretory granules, and constitutive secretory vesicles, whose function and biogenesis have been extensively studied (Fig. 2) [18–22]. For example, COPI coated vesicles mediate retrograde trafficking from the Golgi complex and ERGIC, whereas AP-1 coated vesicles mediate the sorting, packaging, and transport of newly synthesized lysosomal enzymes from the TGN to endosomes [19, 22, 23]. Membrane tubules of uniform diameter (60–80 nm) have also been observed to emanate from different regions of the Golgi complex (Fig. 2), but in contrast to coated vesicles, much less is known about their functions, and indeed, even their existence or prevalence under normal conditions is a matter of debate [4, 8, 24]. For example, although the ERGIC and TGN exhibit extensive membrane tubulation, the cisternal membranes of the Golgi stack proper generally have few tubules. When viewed by 3-D tomographic reconstructions, membrane tubules are observed to link cisternal stacks into intact ribbons, but they are often not prominently seen in static images to extend out into the cytoplasm [4, 25–28]. Rather, Golgi membrane tubules are most readily observed when coated vesicle formation is inhibited or when GFP-tagged proteins are overexpressed for live cell imaging experiments [29–33], leading to concerns that they are only seen under unusual conditions. However, recent studies have revived the idea that COPI (and perhaps AP-1) coated vesicles are intimately linked to the formation of membrane tubules [34], and that membrane tubules can perform trafficking functions independent of coated vesicles, both of which utilize Golgi-associated PLA₂ and LPAT remodeling enzymes [9].

The relationship between Golgi complex coated vesicles and membrane tubules has puzzled cell biologists since the discovery of the remarkable properties of brefeldin A (BFA) [30, 33, 35–37]. BFA is an inhibitor of guanine nucleotide exchange factors (GEFs) for certain ADP-ribosylation factor (Arf) GTPases [38–40], which prevents the recruitment of COPI coatomer and AP-1 subunits to Golgi and TGN membranes and the formation of COPI and AP-1 coated vesicles [38, 41, 42]. As a consequence, Golgi and TGN membranes form extensive numbers of membrane tubules [30, 33, 36, 37, 43]. Interestingly, BFA-induced membrane tubules move in the same directions as the coated vesicles that would normally form, i.e., retrograde back to the ER and anterograde from the TGN to endosomes. These

studies suggested that BFA-induced membrane tubules are exaggerated forms of membrane protrusions that would normally be used to produce coated vesicles [35]. These original observations on the effects of BFA were followed by a period when little progress was made on understanding the exact nature of Golgi membrane tubules. More recently, however, several studies have shown that RNAi knockdown of Golgi Arfs [44], some of their GEFs such as GBF1 [45], and AP-1-associated GGA1 (Golgi-localized, γ ear-containing, ADP ribosylation factor-binding) [46] also induced membrane tubule formation. These results are consistent with original ideas about the BFA effect, which are that membrane tubules reflect an inherent capacity of Golgi membranes to generate curvature, which if not used to make COPI or AP-1 coated vesicles, will continue to form abnormally long membrane tubules [35, 47]. Thus, the Golgi complex has an underlying COPI- and AP-1-independent mechanism capable of initiating membrane curvature, which recent studies report include PLA₂ enzymes that associate with the cytoplasmic side of Golgi membranes.

Although COPI vesicle formation has been extensively examined, there is no clear consensus on the exact molecular mechanisms [22, 48]. A variety of *in vitro* reconstitution studies have shown that COPI vesicles can be produced on artificial liposomes with purified coatamer subunits and Arf1-GTP [19, 48–50], indicating that these components represent the minimal machinery required for COPI vesicles biogenesis. In particular, Arf1-GTP, which is required for recruiting the coatamer subunits for vesicle budding, has also been reported to be sufficient to mediate the fission step for release of a free vesicle [50]. Arf1 could induce membrane fission by virtue of its energetically favorable interaction with positively curved membranes and its unfavorable interaction with the negatively curved bud neck, which makes resolution of the fission event energetically attractive [48]. In addition to this minimal system, a variety of other proteins have been reported to participate in COPI vesicle fission. For example, incubation with ArfGAP1, phospholipase D (PLD), and brefeldin A-ribosylated substrate (BARS) have been shown stimulate the completion of membrane fission [51–55]. It is not clear how ArfGAP1 promotes budding or if ArfGAP1 is a stoichiometric component of the budded vesicle because it was recently reported that *in vitro* COPI vesiculation in the presence of GTP but lacking Arf GEFs is inhibited by enzymatic concentrations of ArfGAP1 [56]. The Arf1-GTP stimulated action of PLD has also been reported to contribute to vesicle fission by hydrolyzing phosphatidylcholine (PC) to phosphatidic acid (PA), which could aid in producing negative curvature and recruiting BARS at the bud neck [57–59]. Although BARS and PLD may not be required in the minimal machinery for COPI vesicle formation, they could enhance the efficiency of COPI budding *in vivo* (discussed further below).

PLA and LPAT Enzymes Remodel Phospholipids and the Golgi Complex

PLA enzymes cleave acyl chains from phospholipids to yield a lysophospholipid (LPL) and a free fatty acid (Fig. 1). PLA enzymes are subdivided by their specificity to the *sn*-1 (PLA₁) and *sn*-2 (PLA₂) position of phospholipids; some cross-specificity has been observed [60]. Nine PLA₁ enzymes have been identified in mammals, six of which are extracellular proteins of the pancreatic lipase family; the remaining are intracellular lipases unrelated to pancreatic lipase [61]. The mammalian PLA₂ superfamily comprises 14 groups of enzymes (Group I, II, etc.), which are distinguished by their cellular locations, substrate specificities, and Ca²⁺ dependence [13]. The *sn*-2 position of phospholipids in mammalian cells can be enriched in AA, which plays critical roles in many signal transduction pathways ranging from inflammation to mitogenesis [62]. Indeed, the diverse PLA₂ superfamily plays important roles in myriad physiological processes such as reproduction, inflammation, heart disease, Alzheimer's disease, and cancer [63–65]. Although most current literature primarily cite PLA₂ enzymes for their roles in inflammation, support for other functions is provided by recent studies showing that cytoplasmic PLA₂ enzymes regulate membrane trafficking.

The first indication that cytoplasmic PLA₂ enzymes play a role in vesicle and membrane tubule biogenesis came from studies of pharmacological PLA₂ inhibitors in cultured mammalian cells [9, 66, 67]. PLA₂ inhibitors block intra-Golgi protein trafficking in cell-free reconstitution systems, thus implicating the requirement for PLA₂ activity at the Golgi complex [68]. More specifically, cytoplasmic PLA₂ enzymes appear to play critical roles in forming membrane tubules, as inhibitory PLA₂ drugs block BFA-induced membrane tubulation and trafficking in these organelles [67, 69]. Conversely, PLA₂ stimulating peptides melittin and a peptide from a mammalian protein containing a melittin homology domain, PLA₂ activating protein peptide (PLA₂Ap), enhance cytosol-dependent Golgi membrane tubulation *in vitro* [70], possibly by embedding within the hydrocarbon region of membranes to facilitate PLA₂ activity. A potential link between the catalytic lipase activities of PLA₂ enzymes and membrane tubulation is supported by a hypothesis that the conversion of cylindrically-shaped phospholipids to conically shaped products in local regions of a single leaflet of a lipid bilayer forces the membrane to adopt a curved structure, thus facilitating the formation of membrane tubules (Fig. 1) [66]. Indeed, diverse lipid components alone can induce membrane curvature as observed in fluorescent, giant unilamellar vesicles [71].

In the context of the Lands cycle, LPATs oppose the action of PLA enzymes (Fig. 1). LPATs catalyze the transfer of a fatty acid from an acyl-CoA donor to a LPL, most commonly at the *sn*-2 position, to yield a phospholipid. Mammalian LPATs comprise two gene families of transmembrane proteins, 1-acylglycerol-3-phosphate acyltransferase (AGPAT) and membrane bound O-acyltransferases (MBOAT) [72–74]. The catalytic activities and substrate specificities of their gene products are not entirely characterized; however, 14 or more members are expected to have more than nominal LPAT activity. These enzymes are also categorized, and sometimes named, according to substrate preferences, e.g., enzymes that produce PA from lysophosphatidic acid (LPA) are referred to as LPAATs; enzymes that produce PC are referred to as LPCATs [75]. Most LPATs are ER or mitochondria localized; AGPAT3/LPAAT3 is exceptional and also localizes to the Golgi complex [76].

The individual activities of PLA and LPAT enzymes to remodel membrane lipids has been extensively studied in terms of lipid homeostasis, inflammation, and signal transduction. Emerging evidence, from multiple independent studies, support an additional role for lipid remodeling in membrane trafficking of the Golgi complex, as well as other organelles. The exact molecular mechanisms responsible for LPAT mediated trafficking are still unknown. One possibility is that the interconversion of phospholipids and LPLs confers negative and positive curvature respectively, directly to membranes. This curvature promotes the formation of tubular and/or vesicular membrane carriers (Figs. 3 and 4) [66, 71, 77]. Another possibility is that compartment-specific PLA and LPAT activity produces a concentration gradient of lipid species that recruit effector proteins, such as COPI and clathrin coats (discussed further below). This recruitment model has many parallels to the extensively studied phosphoinositide phosphate (PIP) remodeling system [78]. It should be noted that lipid curvature and effector recruitment models are not mutually exclusive.

The localized synthesis, remodeling, and selective partitioning of phospholipids, as well as cholesterol, sphingolipids, and plasmalogens throughout the secretory system create tremendous potential for membrane heterogeneity and complexity [79]. The maintenance of a gradient of lipid species between membrane compartments is crucial to and assisted by the selective partitioning of protein and lipid cargoes into anterograde- and retrograde-bound vesicles and membrane tubules [80]. Curved membranes tend to exclude sphingolipids and are enriched in phospholipids. For instance, *in vitro* reconstitution of COPI budding has shown that phase transition of phospholipid enriched domains is prevented at bud sites [49].

Phospholipid transfer proteins, flippases, PIPs, and, DAG also play important roles in the dynamic organization of the Golgi complex but are beyond the scope of this review [55, 78, 81–87].

Adding to this cast of phospholipid remodelers, the past two years have seen the identification of four specific cytoplasmic PLA and one integral membrane LPAT enzyme that play roles in the dynamic functional organization of the Golgi complex (Table). Although these enzymes can individually catalyze changes in membrane phospholipids, it has not yet been directly demonstrated that any pair of PLA and LPAT enzymes are functionally coupled in a Lands cycle of phospholipid remodeling. In any case, it is likely that these discoveries herald many more reports of PLA and LPAT enzyme involvement in Golgi membrane trafficking, and other compartments of the secretory and endocytic pathways.

PAFAH1b

One of the first PLA₂ members discovered to be directly involved in membrane tubulation and trafficking are the two members of Group VIII PLA₂s, also known as platelet activating factor acetylhydrolase (PAFAH) 1b 2 and 3. These two PLA₂ enzymes, called $\alpha 1$ (gene name PAFAH1b2) and $\alpha 2$ (gene name PAFAH1b3), are Ca²⁺-independent and may form either homo- or hetero-dimers to form a catalytic pocket for the phospholipid substrate (Table) [88]. Both subunits within the dimer harbor a conserved GX SXG sequence and contribute critical residues to the catalytic pocket, where the loss of the serine is sufficient to abolish the lipase activity [89]. The catalytic dimers also associate with a non-catalytic, regulatory β -subunit also known as Lis1 (gene name PAFAH1b1), which is the causative gene for a neurodegenerative disorder called Miller-Dieker lissencephaly [90]. Lis1 is better known for its role in regulating dynein-mediated, microtubule-dependent transport [91, 92]. Lis1 appears to inhibit minus-end directed movement of dynein, and with the help of kinesin, the Lis1/dynein complex undergoes anterograde transport to the plus-end of microtubules, where the dynein-associated proteins, Nde1 or Ndel1, bind to the Lis1/dynein complex and mediate cargo transport back towards the minus ends of microtubules [93]. Nde1 and Ndel1 thus permit the compact accumulation of Lis1 in a juxtanuclear region where the minus ends of microtubules converge near centrosomes and indeed, mouse cells lacking Ndel1 show a cytoplasmic dispersed distribution of Lis1 [94]. Thus, Lis1 has dual, but not completely separate, roles in Golgi membrane dynamics via PAFAH1b-dependent phospholipid modification and dynein-mediated, microtubule-dependent transport.

Interestingly, the various possible combinations of catalytic dimers between $\alpha 1$ and $\alpha 2$ exhibit different substrate specificities and are differentially regulated by Lis1 [95]. Differences between $\alpha 1$ and $\alpha 2$ are punctuated by the observation that in rats, $\alpha 1$ is only expressed in developing, neonatal neurons and $\alpha 2$ is conversely expressed in both neo- and post-natal stages and is not limited to neural tissues [96].

All three members of the PAFAH1b group contribute to the maintenance of Golgi architecture (Fig. 2, steps 1 and 2) [97]. This was first shown when biochemical fractionation of bovine brain cytosol identified all three subunits as cofractionating with PLA₂ activity and Golgi membrane tubulation in an *in vitro* reconstitution assay. Importantly, purified catalytically active, but not inactive $\alpha 1$ and $\alpha 2$ can induce Golgi membrane tubulation *in vitro*, and both $\alpha 1$ and $\alpha 2$ partially localize to the Golgi cisternae and the TGN *in vivo*. PAFAH1b $\alpha 1$ and $\alpha 2$ recruitment to Golgi membranes may be PIP dependent, as $\alpha 1$ appears to have a strong affinity for PI(4)P in protein-lipid overlay assays [98], and PI(4)P is known to be enriched within Golgi membranes [78]. Knockdown and over-expression studies demonstrated that both Lis1 binding and the PLA₂ activities of $\alpha 1$

and $\alpha 2$ are important for regulating Golgi structure and function. For example, knockdown of $\alpha 1$ and $\alpha 2$ caused the Golgi ribbon to fragment into separated mini-stacks, which could result from both loss of PLA₂ activity that is required to generate membrane tubules and Lis1-regulated, dynein-mediated movement of Golgi mini-stacks to the cell center [99, 100]. This idea is supported by the finding that Golgi mini-stacks produced following Lis1 knockdown are still able to generate membrane tubules, whereas mini-stacks in $\alpha 1/\alpha 2$ knockdown cells cannot [97]. Likewise, the loss of either Nde1 or Ndel1 also leads to the dispersal of the Golgi complex [101]. Although it is tempting to hypothesize that a compact, juxtanuclear Golgi structure can be achieved by $\alpha 1/\alpha 2$ establishing a link between Golgi membrane tubules and Lis1/dynein/Nde1/Ndel1, other reports demonstrate that $\alpha 1/\alpha 2$ directly compete with Ndel1 and dynein for Lis1 binding (Fig. 5) [102]. Indeed, Lis1 undergoes stark, conformational changes when it switches from a complex with a $\alpha 2/\alpha 2$ homodimer to one with Ndel1 [103].

In addition to regulating membrane tubulation from the Golgi complex, PAFAH1b has been shown to have similar activity on early endosomes and the endocytic recycling compartment [98]. Moreover, PAFAH1b contributes to efficient endocytic recycling of transferrin and transferrin receptors. Thus, PAFAH1b functions at multiple intracellular compartments [9].

The discovery that PAFAH1b regulates Golgi structure and function is somewhat surprising given that it has a very strong substrate preference for PAF, which has an acetyl group in the *sn*-2 position and is best known as a potent extracellular mediator of platelet activation and inflammation [104, 105]. How then could PAFAH1b be involved in Golgi dynamics? First, PAFAH1b is a cytoplasmic enzyme, which due to its location is unavailable to hydrolyze extracellular PAF, and, moreover, mice knockout studies show that it is not the key enzyme responsible for turnover of extracellular PAF, a function most likely carried out by an unrelated, secreted PAFAH [104–107]. Second, PAFs possibly have other roles in cells because they are ubiquitously found in intracellular membranes, including those of *Saccharomyces cerevisiae* [108]. The relevant question for Golgi structure/function is how can PAFAH1b change membrane shape since PAF is structurally similar to LPLs? One possibility is that *in vivo* PAFAH1b hydrolyzes unidentified substrates. Another possibility is that PAF is nevertheless sufficiently different from LPLs to help generate outward membrane curvature when hydrolyzed on the cytoplasmic leaflet of Golgi membranes. Reconstitution experiments using defined phospholipid compositions will be required to definitively address these issues.

cPLA₂ α

Another PLA₂ member, a cytoplasmic Group IV PLA₂ named cPLA₂ α (gene name PLA2G4A) also exerts effects on membrane dynamics at the Golgi complex (Table). cPLA₂ α is one of the most studied PLA₂ members for its roles in the generation of AA and its subsequent signaling pathways [109]. cPLA₂ α possess a C2 domain that binds to intracellular Ca²⁺, leading to its translocation from the cytosol to both ER and Golgi membranes [110, 111], where it regulates both Golgi structure and membrane trafficking.

Similar to the membrane tubulating activities of PAFAH1b, San Pietro et al. found that cPLA₂ α is capable of forming membrane tubules between Golgi cisternae [112], which provide direct continuities between *cis*, *medial*, and *trans* Golgi cisternae for anterograde intra-Golgi protein and membrane transport (Fig. 2, Step 3) [27, 28, 113]. These intra-cisternal membrane tubules are induced during times of rapidly increased secretory load [114], but it is unclear how stable they are and if they function under other conditions. Indeed, the same study goes on to demonstrate that cells from cPLA₂ α knockout mice are still capable of transporting VSV from the ER through the Golgi complex to the plasma

membrane but the protein becomes sequestered within the Golgi when PAFAH1b $\alpha 1$ is knocked down. This clearly establishes redundancy of multiple PLA₂ members in membrane trafficking. However, it remains possible that cPLA₂ α may serve to provide distinct trafficking and sorting services than that of PAFAH1b, and this is demonstrated through experiments showing that cPLA₂ α is specifically involved in transport of various transmembrane junction proteins such as VE-cadherin, occludin, and claudin-5 from the Golgi complex to cell-cell contacts [115]. Also, in Purkinje neurons, cPLA₂ α relies on Ca²⁺ influxes to translocate from the cytosol to the Golgi in order to regulate AMPA receptor levels at the plasma membrane [116]. Finally, other recent studies have provided evidence that cPLA₂, in essence, negatively regulates COPI coated vesicle formation (Fig. 2, Step 4), likely by shifting the balance toward membrane tubule formation (discussed further below) [34].

Overexpression of cPLA₂ α in kidney epithelial cells leads to fragmentation of the Golgi ribbon and prevents anterograde transport as protein cargo sequesters within the ER, while leaving the cytoskeletal and microtubule networks intact [117]. It is possible that membrane tubules formed by cPLA₂ α may serve as precursors to membrane vesicles and this claim is buttressed by observations that cholesterol-induced vesiculation of the Golgi membranes requires cPLA₂ α recruitment to the Golgi and that this is inhibited by the cPLA₂ inhibitor, methyl arachidonyl fluorophosphonate (MAFP) [118]. Interestingly, this same report goes on to show that this vesiculation is independent of clathrin coat complexes and requires dynamin 1 and 2, thus supporting the view that vesicles can possibly be pinched off of nascent membrane tubules.

The activation of cPLA₂ α and its targeting to membranes have been extensively examined. cPLA₂ α can be activated through three distinct phosphorylation sites by PKC, MAPK family member ERK1/2, and CaM kinase, but the phosphorylation of cPLA₂ α does not always lead to AA release [119–121]. This observation clearly implicates cPLA₂ α to be involved in other processes that do not involve liberating AA and may also explain a possible route in uncoupling the functions of releasing AA from Golgi phospholipids and of regulating Golgi structure. The relationship between phosphorylation and Ca²⁺-binding in regulating cPLA₂ α translocation to membranes remains unclear. However, one report notes that AA can possibly mimic Ca²⁺ ionophores in stimulating cPLA₂ α translocation to ER but not Golgi membranes in a Ca²⁺-independent manner [122]. The targeting of cPLA₂ α to Golgi membranes may be similar to PAFAH1b in that PIPs play a facilitative role. Indeed, reports show that PI(4,5)P₂ enhances both cPLA₂ α activity and membrane-binding affinity *in vitro* through interactions with cationic residues within the membrane-binding surface of the protein [123, 124]. However, another study raises conflicting results by showing that PI(4,5)P₂ only enhances cPLA₂ α activity and not its Ca²⁺-dependent membrane binding affinity [125]. Incidentally, Golgi membranes contain PI(4,5)P₂ in addition to PI(4)P [126].

Both membrane tubulation and vesiculation may be facilitated through enhanced membrane fluidity, and PLA₂ activity may be involved in increasing membrane fluidity, as the addition of palmitoyl trifluoromethylketone (PACOCF₃), a PLA₂ inhibitor, reduces membrane fluidity in rat hippocampal tissue [127, 128]. Enhanced membrane fluidity may facilitate lateral segregation of conically shaped LPLs generated by PLA₂ enzymes into regions of high curvature such as membrane tubules [129]. Membrane fluidity also facilitates the formation of lipid rafts as exemplified by the observation that PLA₂ activity is specifically responsible for the budding and fission of liquid ordered (L_o) phase rafts from liquid disordered (L_d) giant unilamellar vesicles (GUVs) [130]. This is potentially explained by the hypothesis that PLA₂ enzymes produce conically-shaped LPLs within L_o rafts, which in turn lead to the budding of rafts into membrane vesicles and/or tubules and is overall energetically favorable because the protrusion of L_o rafts into vesicles/tubules away from

GUVs decrease the free energy that is confined within L_o/L_d phase boundary [130]. In another twist, lipid phases can additionally lead to inhibition of cPLA $_2\alpha$. One study shows that although sphingomyelin (SPH) cannot be hydrolyzed by cPLA $_2\alpha$, L_o phases enriched in SPH can bind to and sequester cPLA $_2\alpha$ from L_d phases that contain hydrolysable glycerophospholipid substrates [131]. The same study showed that such inhibition is specifically due to sequestering cPLA $_2\alpha$ within phases apart from substrate phases because the addition of cholesterol or ceramide leads to the abolishment of separated phases and the reestablishment of cPLA $_2\alpha$ activity.

PLA2G6/iPLA $_2\beta$

A Group VIA-2 PLA $_2$, PLA2G6/iPLA $_2\beta$ (also known as iPLA2B, PNPLA9), is a third PLA $_2$ member that regulates Golgi trafficking indirectly by regulating the ERGIC (Table) [132]. The ERGIC is an independent compartment located between the ER and the *cis*-face of the Golgi complex, facilitates bidirectional trafficking between the two organelles, and may serve as a precursor to the *cis* aspect of the Golgi stack [133, 134]. The ERGIC is comprised of tubulovesicular membrane structures and potentially serves roles in cargo protein quality, sorting, and concentration [133]. PLA2G6/iPLA $_2\beta$ has multiple splice variants, of which the full length variant, called L-iPLA $_2$, is catalytically active while its shorter splice variants, ankyrin-iPLA $_2$ -1 and -2 are inactive but thought to negatively regulate L-iPLA $_2$ via forming oligomers through their ankyrin repeats [135, 136]. PLA $_2$ involvement at the ERGIC is first implicated through the use of PLA $_2$ drug inhibitors that prevent the recycling of ERGIC-localized protein ERGIC-53 in addition to the formation of ERGIC membrane tubules [137]. Recent work shows that Arf1 and Arf4 knockdown can generate ERGIC membrane tubules that are dependent on PLA2G6/iPLA $_2\beta$ as knockdown of this protein also leads to inhibition of membrane tubule formation [132]. The same study noted that PLA2G6/iPLA $_2\beta$ -induced membrane tubules may be viable alternatives to vesicles in quickly and efficiently directing cargo between ERGIC clusters and cargo containing COPI vesicles (Fig. 2, Step 5). ERGIC tubulation and mobility are also regulated by microtubules and their associated motor proteins and whether these tubule-inducing functions are independent or shared with PLA2G6/iPLA $_2\beta$ remains unknown [138].

Aside from basic cell biological interest in understanding how phospholipid modifying enzymes and membrane tubules influence intracellular membrane trafficking, recent studies have revealed that the function of these enzymes and membrane structures might be linked to pathological conditions. For example, mutations in human PLA2G6A gene (and in mouse models) cause a spectrum of autosomal recessive neurodegenerative disorders, characterized by severe cognitive and motor regression, now referred to as PLA2G6-Associated Neurodegeneration (PLAN) [139–142]. The connection between PLA2G6's function at the ERGIC and disease manifestations in PLAN is not understood but could involve specific defects in secretory trafficking.

iPLA $_1\gamma$

The final cytoplasmic PLA protein that has effects on the Golgi complex is a PLA $_1$ family member, iPLA $_1\gamma$ (gene name DDH2 or KIAA0725p) (Table) [143, 144]. The other two cytoplasmic PLA $_1$ family members include iPLA $_1\alpha$ (PA-PLA $_1$) and iPLA $_1\beta$ (p125) [145]. While iPLA $_1\gamma$ shares a common characteristic with iPLA $_1\beta$ in that the overexpression of either results in structural disruption of both the ERGIC and the Golgi, only the overexpression of iPLA $_1\gamma$ lead to mislocalization of Golgi resident proteins such as p115 and GM130 [146]. One of the first iPLA $_1\gamma$ studies showed that the protein is localized to both Golgi and ERGIC membranes (Fig. 2) [143]. The same study indicated that siRNA knockdown of iPLA $_1\gamma$ disrupted COPI- and Rab6-independent, i.e., BFA stimulated, Golgi-

to-ER retrograde trafficking, whereas VSVG-ts045-GFP anterograde transport was unaffected. However, a more recent study conflicts with these results, finding that iPLA₁γ knockdown did not affect Golgi-to-ER retrograde trafficking but did inhibit VSVG-ts045-GFP anterograde transport [144]. These discrepancies can be explained by the observation that the original knockdown studies had an unintended off-target knockdown of Rab6 [144], which regulates Golgi retrograde trafficking [147]. In mammals, the physiological roles of the PLA₁ family are unclear but it is known that iPLA₁α is highly expressed in brain and testis and thus possibly linked to neural development and spermatogenesis, similar to PAFAH1b [106, 107, 148, 149]. In addition, the molecular mechanisms by which iPLA₁γ influences trafficking through the Golgi complex are unknown.

Lysophospholipid Acyltransferases

The fruitful studies of PLA₂ enzymes in Golgi membrane trafficking stimulated an interest in LPAT activity as a potential mediator of Golgi membrane trafficking, perhaps by counteracting the tubulating activity of PLA₂ enzymes (Fig. 3). Given that in model membrane systems PLA₂ and LPAT lipid products stabilize positive and negative curvature, respectively, and that PLA₂ activity stimulates membrane tubulation in cells, it was hypothesized that LPAT activity might prevent tubule formation in cells [66]. It had also been hypothesized that the negative curvature at the neck of a budding vesicle could be stabilized by LPAT lipid products [150]. Finally, it was suggested that cytoplasmic PLA₂ and LPAT enzymes could work in concert: cytoplasmic PLA₂ enzymes could promote outward curvature or budding that would subsequently be acted on by LPATs to produce negative curvature to facilitate coated vesicle production [66].

The first two reports of LPAT activity being involved in membrane trafficking were from studies of BARS and endophilin [151–153]. BARS is a mediator of Golgi membrane tubule fission [53], and endophilin is an N-BAR domain containing protein that was recently shown to be required for uncoating of coated vesicles at the plasma membrane but not fission per se [154]. Both of these proteins were reported to have low levels of acyltransferase activity; however, this activity was later determined to be associated with LPAAT enzyme contaminants [155].

The first solid indication that LPATs were involved in membrane trafficking came from studies that utilized a powerful new tool, the small molecule CI-976. CI-976 (2,2-methyl-N-(2,4,6-trimethoxyphenyl)dodecanamide) was originally synthesized as a fatty acid anilide derivative designed to mimic fatty acyl-CoA for inhibition of acyl-CoA:cholesterol acyltransferase (ACAT) [156, 157]. It is a small hydrophobic, membrane permeant, and competitive ACAT inhibitor [158]. While CI-976 possesses only weak inhibitor activity against ACAT, a screen for inhibition of Golgi associated LPAT activity showed strong inhibition of LPC and lysophosphatidylethanolamine (LPE) acyltransferase activities [159–162]. With this potent inhibitor, the study of LPAT enzymes in mammalian membrane trafficking immediately progressed. Most intriguingly, treatment of mammalian cells with CI-976 stimulated a remarkable enhancement of Golgi membrane tubulation and retrograde trafficking [160, 161]. This tubulation activity was phenotypically similar to BFA induced tubulation, although comparatively delayed. Additionally, CI-976-induced Golgi tubulation was inhibited by concomitant PLA₂ inhibition, indicating for the first time that both PLA₂ and LPATs work in concert to regulate the dynamic structure of the Golgi complex [161].

Golgi-associated LPAT enzyme(s) could regulate membrane trafficking by one of, or a combination of, five mechanisms (Fig. 3 and 4): 1) production of conical phospholipids that resist the high positive curvature present in tubules; 2) conversion of inverted-cone shaped LPLs that stabilize positive curvature to phospholipids that are less stabilizing; 3) production

of conical phospholipids that stabilize the high negative curvature at the neck of budding vesicles; 4) production of phospholipids that recruit vesicle-budding factors; and, 5) production of phospholipids that affect lipid and protein partitioning. There is evidence for all of these mechanisms and deciphering the relative contribution of each is a major challenge in the field.

Further studies identified a PC- and PE-forming LPAT tightly associated with the Golgi complex [159]. The connection between this enzyme and CI-976 stimulated tubulation of the Golgi complex and Golgi-to-ER retrograde trafficking is unclear [161], and the exact molecular identity of the LPC/LPEAT activity is still unknown. In fact, recent studies strongly indicate that the observed CI-976 effect on the Golgi could be due to inhibition of LPAAT mediated acylation of LPA to PA, which is catalyzed by AGPAT3/LPAAT3 (also called LPAAT γ), a Golgi localized LPA specific acyltransferase (Table) [76].

AGPAT3/LPAAT3 was the first specific LPAT shown to be involved in the dynamic function of the Golgi complex. It is an integral membrane protein with two transmembrane domains and is found in both the Golgi complex and ER [163]. Overexpression of AGPAT3/LPAAT3 was shown to slow ERGIC-53 recycling from the cis-Golgi to the ER/ERGIC and to reduce BFA-stimulated membrane tubulation. Conversely, AGPAT3/LPAAT3 knockdown accelerated ERGIC-53 recycling. Importantly, overexpression of AGPAT3/LPAAT3 counteracted the tubule-inducing effects CI-976, suggesting that AGPAT3/LPAAT3 is a direct target of the drug. However, this result does not rule out the possibility that the CI-976-inhibitable Golgi LPC/LPEAT activity also contributes to Golgi membrane remodeling. For example, a Golgi LPC/LPEAT enzyme could come into play because AGPAT3/LPAAT3 generated PA can be hydrolyzed to DAG by PA phosphatase and then converted to PC or PE, followed by Lands Cycle remodeling via cPLA $_2\alpha$ (Fig. 6). In addition, PC and PE can be generated via Golgi localized cholinephosphotransferase (CPT1) or a phosphoethanolamine transferase, respectively [164].

Like PLA $_2$ enzymes, LPATs are also involved in other secretory and endocytic trafficking steps. For example, CI-976 reversibly inhibited a very late step in COPII vesicle budding, resulting in the accumulation of secretory cargo at ER exit sites (ERESs) [165]. Interestingly, ERESs underwent Sar1p-dependent membrane tubulation in the presence of CI-976, suggesting the involvement of LPAT activity in the fission of COPII budding elements *in vivo*. In addition to secretory trafficking, CI-976 was also found to stimulate membrane tubules from endosomes and to inhibit recycling of transferrin and transferrin receptors from the endocytic recycling compartment, suggesting a role for LPATs in the budding of vesicles from endosome tubules [160]. Currently, the precise LPATs involved in COPII budding or endocytic recycling have yet to be identified.

Integration of cPLA $_2\alpha$ and AGPAT3/LPAAT3 to Influence Golgi COPI Vesicle and Membrane Tubule Formation

The above studies on Golgi-associated PLA $_2$ and LPAT enzymes suggest an intimate association between the formation of membrane tubules and coated vesicles. Support for this idea came from previous studies showing that CI-976 inhibited COPI vesicle formation [53]. This association was recently directly tested by Hsu and colleagues who discovered that cPLA $_2\alpha$ and AGPAT3/LPAAT3 function to regulate the relative abundance of COPI vesicle and membrane tubule formation (Fig. 4) [34]. Using purified Golgi complex membranes in an *in vitro* COPI budding assay, they first found that COPI coatomer subunits and Arf1 are required for both vesicle and membrane tubule formation. They surmised that Arf1 and coatomer initiates the budding events that become vesicles and membrane tubules. When examining endogenous cPLA $_2\alpha$ and AGPAT/LPAAT3 by immunofluorescence

microscopy, they were shown to colocalize with endogenous coatamer proteins. The coatamer subunit α -COP was shown by immuno-EM to localize to the base and tip, but not the stem, of membrane tubules. In this assay, AGPAT3/LPAAT3 activity increased COPI vesicle formation, which was shown by the addition of CI-976 or AGPAT3/LPAAT3 antibody to budding reactions. The authors suspect that AGPAT3/LPAAT3-generated PA, a known recruiter of COPI coatamer subunits and BARS, a COPI vesicle fission protein [53], as well as a negative curve-inducing phospholipid [77, 166], are responsible for stimulating COPI coating of the nascent vesicle. In support of this idea, inhibition of AGPAT3/LPAAT3, by CI-976 or specific antibody, stimulated membrane tubulation at the expense of vesicle production. The final step of COPI vesicle fission required the conversion of PC to PA by PLD2, but not PLD1.

The addition of pharmacological PLA₂ inhibitors or anti-cPLA₂ α antibody to cytosol in their reconstitution assay increased vesicle formation and concomitantly decreased tubule formation, which was reversed by the addition of purified cPLA₂ α . Their model posits that Arf1 and COPI coatamer recruitment initiates bud formation and that buds then mature into either vesicles or membrane tubules, depending on the balance of cPLA₂ α and AGPAT3/LPAAT3 activity (Fig. 2, Step 5; Fig. 4B–E). It is still a matter of debate as to whether generation of curve-inducing PA or recruitment of effector proteins, is most important in AGPAT3/LPAAT3-mediated vesicle biogenesis. They further suggest that AGPAT3/LPAAT3 generated PA initiates negative membrane curvature but is insufficient for complete vesicle budding, a process that is finalized by the additional generation of PA by PLD2 (Fig. 4F). The generation of PA by both enzymes could aid in the recruitment of the fission protein BARS [53]. Hsu and colleagues also assert that the biogenesis of Golgi membrane tubules, both intercisternal and intracisternal, which facilitate anterograde trafficking and Golgi ribbon formation respectively, are initiated as COPI buds (Fig. 4D). These results provide important insights into how Lands cycle phospholipid remodeling might influence the dynamic behavior of Golgi membranes. Interestingly, however, this model of membrane curvature initiation solely by COPI/Arfs cannot explain enhanced Golgi membrane tubule formation by BFA or knockdown of Arf GEFs, and underscores the notion that cytoplasmic PLA₂ enzymes can induce membrane tubules on their own (Fig. 4G). In addition, because COPI vesicle budding and fission can be reconstituted using minimal components coatamer and Arf-GTP [50], these phospholipid modifying enzymes likely function to enhance the efficiency of the COPI budding machinery.

The central roles of DAG and PA in Golgi membrane tubule and vesicle formation have been extensively examined, and their participation raises important points for the analysis of how lipid/phospholipid changes affect membrane trafficking. Both DAG and PA have been shown to be required for membrane tubule and vesicle formation from the Golgi and TGN [59, 167–170], likely by generating both curvature-altering phospholipids and platforms for effector protein binding. How the Golgi complex controls the numerous pathways that lead to and from PA and DAG is still a mystery (Fig. 6). In addition, understanding how lipids/phospholipids can exert dramatic local effects in the face of rapid lateral diffusion and conversion to other products is a challenge. Nevertheless, it is becoming increasingly clear that remodeling of Golgi membrane phospholipids by PLA₂ and LPAT enzymes adds a new layer of complexity to an already complex organelle. Moreover, it is likely that the full complement of PLA and LPAT enzymes involved in secretory (and endocytic) trafficking has not yet been revealed.

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Listing of abbreviations

AA	Arachidonic acid
ACAT	acyl-CoA cholesterol acyltransferase
AGPAT	1-acylglycerol-3-phosphate acyltransferase
Arf	ADP-ribosylating factor
BARS	BFA-inhibited, ADP-ribosylated substrate
BFA	Brefeldin A
CPT1	Cholinephosphotransferase
DAG	Diacylglycerol
DAGK	Diacylglycerol kinase
ER	Endoplasmic reticulum
ERES	ER exit site
ERGIC	ER-Golgi-intermediate complex
GEF	Guanine nucleotide exchange factor
GUV	Giant unilamellar vesicle
L_o	Liquid ordered
L_d	Liquid disordered
LPA	Lysophosphatidic acid
LPAAT	Lysophosphatidic acid acyltransferase
LPAT	Lysophospholipid acyltransferase
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolamine
LPL	Lysophospholipid
MAFP	Methyl arachidonyl fluorophosphonate
MBOAT	Membrane bound O-acyltransferase
MT	Microtubule
MTOC	Microtubule organizing center
PA	Phosphatidic acid
PACOCF₃	palmitoyl trifluoromethylketone
PAFAH	Platelet activating factor acetylhydrolase
PC	Phosphatidylcholine
PIP	Phosphoinositide phosphate
PLA	Phospholipase A
PLA_{Ap}	PLA ₂ activating protein peptide
PLD	Phospholipase D
SM	Sphingomyelin

SMS	Sphingomyelin synthase
SPH	Sphingomyelin
TGN	<i>trans</i> -Golgi network

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Highlights

- Membrane remodeling by PLA₂ and LPAT enzymes influence mammalian Golgi complex structure and function
- The phospholipases PAFAH1b, cPLA₂α, PLA2G6/iPLA₂-β, and iPLA₁γ localize to the Golgi complex
- PAFAH1b, cPLA₂α, and PLA2G6/iPLA₂-β induce Golgi membrane tubule formation
- The transmembrane lysophospholipid acyltransferase AGPAT3/LPAAT3 influences Golgi structure and function
- AGPAT3/LPAAT3 and cPLA₂α regulate COPI vesicle and membrane tubule formation

Figure 1.

A simple model of Lands cycle phospholipid remodeling by PLA₂ and LPAT enzymes and how their phospholipid products can generate membrane curvature based on their physical shapes.

Figure 2.

Membrane trafficking pathways from and within the mammalian Golgi complex. Several studies have recently reported that four cytoplasmic PLA₂ enzymes are associated with the Golgi complex and regulate trafficking in various ways. As shown in Step 1, the cytoplasmic PLA₂ enzyme PAFAHIB induces membrane tubules from the Golgi complex that contribute to the formation of an intact ribbon structure. In addition, PAFAHIB also appears to influence anterograde trafficking from the TGN (Step 2). A second cytoplasmic enzyme, cPLA₂α, mediates the formation of intra-cisternal membrane tubules that appear to facilitate anterograde transport when the Golgi complex receives a large bolus of secretory cargo (Step 3). In addition, cPLA₂ may also negatively regulate COPI coated vesicle formation (Fig. 2, Step 4), perhaps by shifting the balance toward membrane tubule formation. A third cytoplasmic PLA₂, PLA2G6, was shown to mediate the formation of membrane tubules from the ERGIC, which may be important for connecting regions involved in COPI vesicle budding (Step 5). The final PLA enzyme recently found associated with ERGIC and Golgi membranes is iPLA₁γ, which appears to contribute to anterograde transport through the Golgi complex by an unknown mechanism.

Figure 3.

Possible routes for the individual and concerted actions of PLA₂ and LPAT enzymes to influence the formation of membrane tubules and coated vesicles. (A) Membrane tubules could be generated and regulated by the opposing actions of PLA₂ and LPAT enzymes. Hydrolysis of membrane phospholipids by cytoplasmic PLA₂ enzymes could generate positive curvature inducing LPLs, ultimately resulting membrane tubule formation. The LPLs could be reacylated by LPAT enzymes back to phospholipids, thus negatively regulating the formation of membrane tubules. (B) The hydrolytic activity of cytoplasmic PLA₂ enzymes could generate membrane tubules that are consumed through coated vesicle budding, which is facilitated by LPAAT activity for membrane fission. In this generic

model, the coated vesicles could be either COPI vesicles budding from the ERGIC or AP-1 clathrin coated vesicles budding from the TGN. The consequences of inhibition by PLA₂ and LPAT antagonists are shown, which have been supported by several studies (9, 66).

Figure 4.

Models for cPLA₂α and AGPAT3/LPAAT3 activities contributing to COPI vesicle and Golgi membrane tubule formation. (A) Flat membrane that will be modified by curve producing proteins; (B) Arf1 and coatomer binding deforms membranes, initiating the first steps of vesiculation or tubulation; (B to C) AGPAT3/LPAAT3 activity produces unsaturated PA, a lipid that resists positive curvature; (B to D) cPLA₂α activity produces LPC, a positive curvature stabilizing and tubule inducing lipid; (C and D to E) LPC stabilizes positive curvature of the bud and unsaturated PA stabilizes negative curvature at the bud neck; (F) The concerted PA production activities of AGPAT3/LPAAT3 and PLD2

at the bud neck help to recruit BARS and aide vesicle fission; (G) PLA₂ activity can induce membrane curvature and tubulation in the absence of Arf1 and coatomer.

Adapted from Ref. 93 (Yamada et al., 2008)

Figure 5.

Model integrating membrane curvature produced by the PLA₂ activity of PAFAHib $\alpha 1$ and $\alpha 2$ with Lis1-mediated dynein transport along microtubules. PAFAHib initiates outward membrane curvature to generate a membrane tubule, which can be pulled/extended along microtubules (MT) by Lis1 and Ndel interactions with dynein. Dynein may be able to carry the membrane tubule towards the minus end of microtubules, facilitating the convergence of the Golgi stack and positioning at the microtubule organizing center (MTOC, minus end of the microtubules).

Figure 6.

Enzymatic pathways, several involved in Lands cycle reactions, which lead to the modification and interconversion of lipid species. Lysophosphatidic acid (LPA); phosphatidic acid (PA); diacylglycerol (DAG); phosphatidylcholine (PC); lysophosphatidylcholine (LPC); sphingomyelin (SM); ceramide (Cer); phosphatidylinositol 4-phosphate (PI4P); phosphatidylinositol 4, 5-phosphate (PI4,5P); 1-acylglycerol-3-phosphate acyltransferase 3 (AGPAT3); lysophosphatidic acid acyltransferase 3 (LPAAT3); phospholipase A₂ (PLA₂); diacylglycerol kinase (DAGK); phosphatidic acid phosphatase (PA P'tase); phospholipase D (PLD); PC specific phospholipase C (PC-PLC); phosphatidylinositol specific PLC (PI-PLC); sphingomyelin synthase (SMS).

Table 1

of Golgi-Remodeling Enzymes.

Class	Gene Name	Features
PLA Enzymes	PAFAH1b	Has two Ca^{2+} -independent PLA_2 subunits, potentially links membrane tubules to microtubules, affects Golgi structure and trafficking, also regulates endocytic recycling
	c $\text{PLA}_2\alpha$	Ca^{2+} -dependent PLA_2 , maintains Golgi structure, provides intra-Golgi connections
	$\text{PLA}_2\text{G6/iPLA}_2\beta$	A cytoplasmic PLA_2 associated with the ERGIC and regulates its structure/function
	i $\text{PLA}_1\gamma$	A cytoplasmic PLA_1 enzyme located to cis/medial Golgi cisternal and influences anterograde trafficking
LPATs	AGPAT3/LPAAT3	An integral membrane LPAT of both the ER and Golgi complex that regulates both Golgi structure and trafficking