



## Mini-review

Localization and function of cytosolic phospholipase A<sub>2</sub>α at the GolgiChristina C. Leslie<sup>a,\*</sup>, Todd A. Gangelhoff<sup>a</sup>, Michael H. Gelb<sup>b,c</sup><sup>a</sup> Department of Pediatrics, National Jewish Health, Denver, CO 80206, USA<sup>b</sup> Department of Chemistry, University of Washington, Seattle, WA 98195, USA<sup>c</sup> Department of Biochemistry, University of Washington, Seattle, WA 98195, USA

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## ABSTRACT

Cytosolic phospholipase A<sub>2</sub>α (cPLA<sub>2</sub>α, Group IVA phospholipase A<sub>2</sub>) is a central mediator of arachidonate release from cellular phospholipids for the biosynthesis of eicosanoids. cPLA<sub>2</sub>α translocates to intracellular membranes including the Golgi in response to a rise in intracellular calcium level. The enzyme's calcium-dependent phospholipid-binding C2 domain provides the targeting specificity for cPLA<sub>2</sub>α translocation to the Golgi. However, other features of cPLA<sub>2</sub>α regulation are incompletely understood such as the role of phosphorylation of serine residues in the catalytic domain and the function of basic residues in the cPLA<sub>2</sub>α C2 and catalytic domains that are proposed to interact with anionic phospholipids in the membrane to which cPLA<sub>2</sub>α is targeted. Increasing evidence strongly suggests that cPLA<sub>2</sub>α plays a role in regulating Golgi structure, tubule formation and intra-Golgi transport. For example, recent data suggests that cPLA<sub>2</sub>α regulates the transport of tight junction and adherens junction proteins through the Golgi to cell–cell contacts in confluent endothelial cells. However, there are now examples where data based on knockdown using siRNA or pharmacological inhibition of enzymatic activity of cPLA<sub>2</sub>α affects fundamental cellular processes yet these phenotypes are not observed in cells from cPLA<sub>2</sub>α deficient mice. These results suggest that in some cases there may be compensation for the lack of cPLA<sub>2</sub>α. Thus, there is continued need for studies employing highly specific cPLA<sub>2</sub>α antagonists in addition to genetic deletion of cPLA<sub>2</sub>α in mice.

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## 1. Introduction

Since the discovery of a high molecular weight, arachidonic acid preferring cytosolic phospholipase A<sub>2</sub> (cPLA<sub>2</sub>), now classified as Group IVA cPLA<sub>2</sub> (cPLA<sub>2</sub>α), there has been a great deal of progress in elucidating its biochemical properties, regulation and function [1–8]. The interest in cPLA<sub>2</sub>α stems from its role as the first regulatory enzyme in the pathway for the production of oxygenated metabolites of arachidonic acid [9].

Among the 20 enzymes classified as PLA<sub>2</sub>s in mammals, cPLA<sub>2</sub>α is the only member that exhibits preference for the *sn*-2 arachidonyl group [6,10–13]. cPLA<sub>2</sub>α is expressed in many cell types and is highly regulated in order to control the cellular levels of free arachidonic acid. The function of cPLA<sub>2</sub>α in mediating the regulated release of arachidonic acid for the production of eicosanoids is well established [14–18]. These potent lipid mediators have diverse functions in regulating normal processes and contributing to disease pathogenesis [9]. In cells involved in promoting acute inflammation in

response to tissue injury such as neutrophils, macrophages and mast cells, cPLA<sub>2</sub>α activation results in the production of numerous lipid mediators including leukotrienes, prostanoids and platelet-activating factor [14–18]. These mediators have diverse functions in promoting inflammation and contributing to its resolution. The coordinated action of secreted lipid mediators is regulated in a cell type specific manner by the differential expression of specific G-protein coupled receptors that initiate diverse signal transduction pathways and biological responses [19–21].

In addition to its role in initiating eicosanoid production, there is data indicating that cPLA<sub>2</sub>α plays a role in regulating the structure and trafficking through the Golgi apparatus. Evidence is emerging that phospholipases and acyltransferases regulate trafficking and membrane tubulation by modification of the local lipid composition that affects membrane curvature [22–27]. This review will highlight new information about the localization and functional role of cPLA<sub>2</sub>α at the Golgi.

2. Calcium-dependent translocation of cPLA<sub>2</sub>α to the Golgi

cPLA<sub>2</sub>α is regulated by post-translational mechanisms such as increases in levels of intracellular calcium [Ca<sup>2+</sup>]<sub>i</sub>, phosphorylation

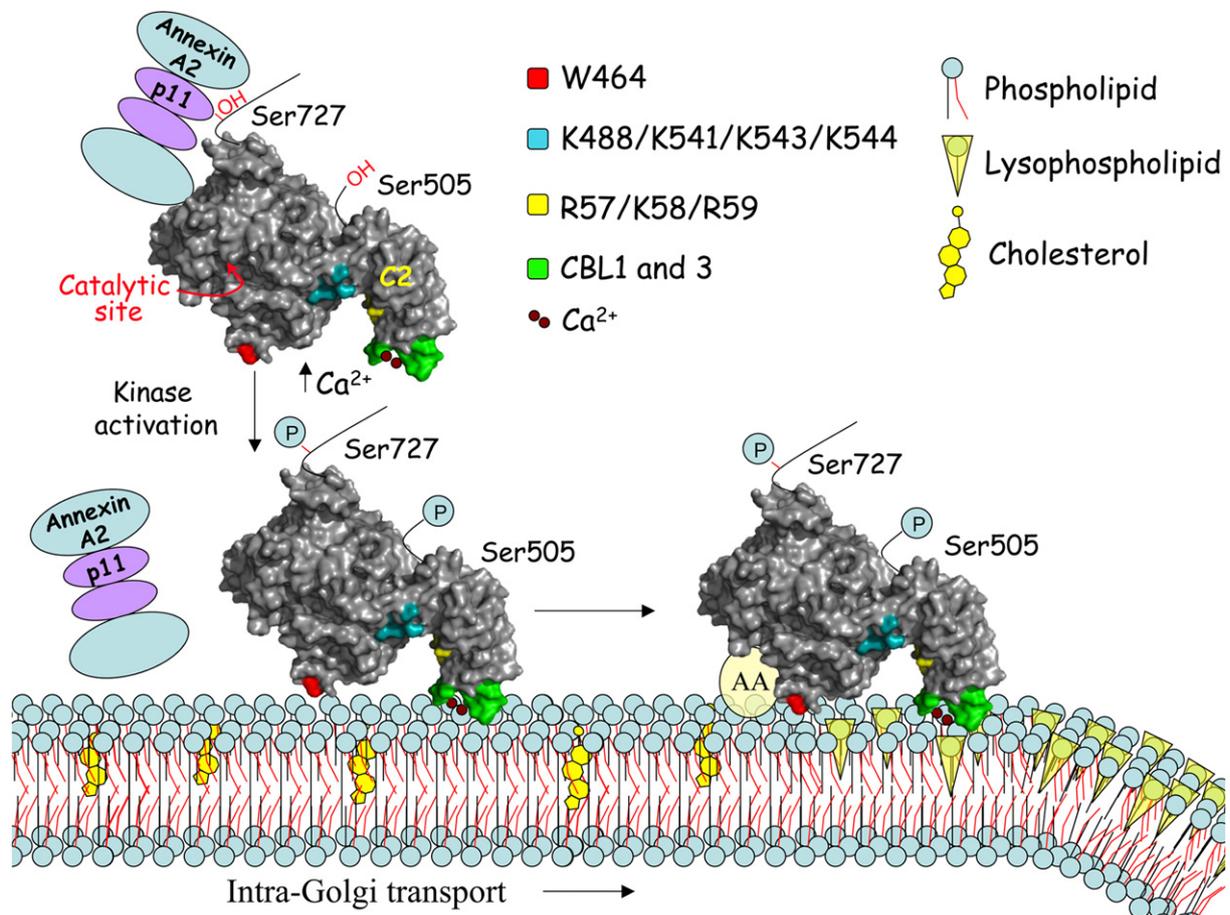
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and interaction of basic residues with anionic membrane components (Fig. 1) [4,5]. Agonists that increase  $[Ca^{2+}]_i$  stimulate cPLA<sub>2</sub>α-dependent release of arachidonic acid by promoting translocation of cPLA<sub>2</sub>α from the cytosol to membrane [28–32]. This is a critical regulatory step necessary for cPLA<sub>2</sub>α to access phospholipid substrate. The cPLA<sub>2</sub>α C2 domain has a high affinity for calcium, which binds to anionic residues in the calcium binding loops (CBLs) [33–36]. Calcium binding reduces the negative electrostatic potential of the surface exposed CBLs allowing the surrounding hydrophobic residues in CBL1 and CBL3 to penetrate membrane (Fig. 1) [37–40]. In contrast, calcium binding to other C2 domains, such as the protein kinase C alpha (PKCα) C2 domain, induces an “electrostatic switch” from electronegative to electropositive promoting the interaction of basic residues with anionic phospholipids [41–45]. Differences in the properties of C2 domains determines the lipid binding specificity *in vitro* and the membrane targeting specificity in cells [46–50]. The cPLA<sub>2</sub>α C2 domain preferentially binds to phosphatidylcholine (PC) and mediates the calcium-dependent

translocation of cPLA<sub>2</sub>α to the Golgi, endoplasmic reticulum and nuclear envelope (Fig. 1) [31,34,38,51,52]. The PKCα C2 domain exhibits calcium-dependent binding to anionic phospholipids and translocates to the inner leaflet of the plasma membrane, which is enriched in the negatively charged phospholipids phosphatidylserine and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P<sub>2</sub>) [44,46–50].

In early studies it was observed that cPLA<sub>2</sub>α translocates to the perinuclear region including the nuclear envelope in a variety of cells in response to increases in  $[Ca^{2+}]_i$  [29,30,53]. This was particularly interesting in light of work showing localization of 5-lipoxygenase, 5-lipoxygenase activating protein and leukotriene C<sub>4</sub> synthase to the nuclear envelope suggesting that this may be a site for production of leukotrienes [54–56]. Subsequent work using organelle markers and Golgi disrupting agents clearly demonstrated that cPLA<sub>2</sub>α also translocates to the Golgi apparatus, which in most cells is located adjacent to the nucleus [31]. Translocation of cPLA<sub>2</sub>α is regulated by both the amplitude and duration of  $[Ca^{2+}]_i$ . The concentration of calcium required for binding to



**Fig. 1.** Proposed mechanism of cPLA<sub>2</sub>α localization and function on the Golgi. The N-terminal C2 domain of cPLA<sub>2</sub>α is attached to a large catalytic domain that contains the catalytic site Ser/Asp dyad, and the sites phosphorylated by MAPKs (Ser505) and MAPK-interacting kinases (Ser727). The hydroxyl group of Ser727 interacts with p11/Annexin A2 complexes maintaining cPLA<sub>2</sub>α in an inactive state. Phosphorylation of Ser727 causes disassociation of the bulky p11/Annexin A2 complex allowing the calcium-dependent interaction of cPLA<sub>2</sub>α with the Golgi membrane. Calcium binding to the cPLA<sub>2</sub>α C2 domain reduces the negative electrostatic potential of the surface exposed CBLs allowing the surrounding hydrophobic residues in CBL1 and CBL3 to penetrate the membrane. The basic residues (R57/K58/R59) (yellow) in the C2 domain of cPLA<sub>2</sub>α form the proposed site for interaction with C-1-P. Calcium-dependent binding of cPLA<sub>2</sub>α to the Golgi positions the catalytic domain on the membrane, which is stabilized by interaction of Trp464 (red) in the catalytic domain with the membrane. There is evidence that association of cPLA<sub>2</sub>α with the Golgi is influenced by changes in cholesterol content. Phosphorylation at Ser505 increases the hydrolytic activity of cPLA<sub>2</sub>α on the membrane perhaps by promoting a conformational change due to its proximity to the flexible linker that connects the catalytic and C2 domains. A patch of basic residues (K488/K541/K543/K544) (teal) in the catalytic domain also regulates the ability of cPLA<sub>2</sub>α to release arachidonic acid from the Golgi. These residues are necessary for activation of cPLA<sub>2</sub>α *in vitro* by polyphosphoinositides, however, the endogenous anionic components in the Golgi that interact with this basic site have not been identified. Therefore the ability of cPLA<sub>2</sub>α to release arachidonic acid (AA) and form lysophospholipids in the Golgi involves increases in calcium, phosphorylation and interaction of basic residues with anionic components in the membrane. Lysophospholipids generated at the rims of the Golgi cisternae by cPLA<sub>2</sub>α are thought to induce positive membrane curvature for formation of tubules that connect the Golgi stacks and promote intra-Golgi transport. Surface representation of the x-ray crystal structure of cPLA<sub>2</sub>α (PDB: 1CJY) was made using PYMOL.

Golgi is lower than for association with the endoplasmic reticulum (ER)/nuclear envelope [31,32]. This is consistent with data showing that cPLA<sub>2</sub>α preferentially translocates to the Golgi in response to physiological increases in [Ca<sup>2+</sup>]<sub>i</sub> that occurs with agonists such as ATP and serum, and to Golgi and the ER/nuclear envelope in response to calcium ionophores, which cause a supra-physiological rise in [Ca<sup>2+</sup>]<sub>i</sub> [31]. Serum induces a typical capacitative increase in [Ca<sup>2+</sup>]<sub>i</sub> and potentially stimulates arachidonic acid release. The initial transient increase in calcium from intracellular stores promotes the rapid translocation of cPLA<sub>2</sub>α to Golgi [57]. However the subsequent influx of extracellular calcium due to store depletion is essential for stable binding of cPLA<sub>2</sub>α to Golgi and for arachidonic acid release [57]. This is evident from results showing that chelating extracellular calcium, which blocks calcium influx but not the initial calcium transient from intracellular stores, prevents serum-stimulated arachidonic acid release. Therefore a rapid transient increase from intracellular stores in this model in itself is not sufficient for cPLA<sub>2</sub>α to release arachidonic acid [57]. However a low amplitude sustained calcium increase due to influx of extracellular calcium that occurs in the absence of a rapid calcium transient from intracellular stores, results in a slow rate of cPLA<sub>2</sub>α translocation to Golgi and low level arachidonic acid release [57]. Collectively, studies have established the importance of extracellular calcium influx and the duration of calcium elevation for stable binding of cPLA<sub>2</sub>α to Golgi and arachidonic acid release [32,57–59].

### 3. Role of the phospholipid-binding specificity in targeting cPLA<sub>2</sub>α to Golgi

Most C2 domains are calcium-dependent phospholipid-binding domains that function to promote interaction of proteins to membrane. In several cell models, cPLA<sub>2</sub>α C2 domain has been shown to localize to the same intracellular membranes as full-length cPLA<sub>2</sub>α in response to calcium increases indicating that it is responsible for the membrane targeting specificity [22,31,48]. Hydrophobic residues in the CBLs in the cPLA<sub>2</sub>α C2 domain are required for calcium-dependent binding to PC rich vesicles *in vitro* and localization to intracellular membranes in cells (Fig. 1) [38,39,51,60,61]. The sites of cPLA<sub>2</sub>α localization, Golgi, ER and nuclear envelope, are the sites for synthesis of PC [62,63]. It has been suggested that a high local concentration of PC on internal membranes functions to lower the [Ca<sup>2+</sup>]<sub>i</sub> necessary for cPLA<sub>2</sub>α C2 domain to bind to these membrane [47,48].

The enrichment of PC in intracellular membranes may be sufficient to provide the targeting specificity of cPLA<sub>2</sub>α to internal membranes. However, the role for an additional lipid or protein in regulating cPLA<sub>2</sub>α targeting to internal membranes cannot be ruled out. There are studies suggesting that ceramide-1-phosphate (C-1-P), produced from ceramide kinase (CERK), is an endogenous regulator of cPLA<sub>2</sub>α that acts by enhancing calcium-dependent membrane binding and activity [64–66]. *In vitro* studies demonstrated that C-1-P acts through the cPLA<sub>2</sub>α C2 domain to increase the calcium-dependent binding of cPLA<sub>2</sub>α to PC-containing mixed micelles and acts as an allosteric activator by decreasing the dissociation constant [67]. C-1-P is a much better activator of cPLA<sub>2</sub>α *in vitro* when a mixed micelle assay consisting of PC in Triton X-100 detergent micelles is used versus PC liposomes. For example the presence of 5 mol% C-1-P in Triton X-100/PC mixed micelles leads to a ~15-fold activation of cPLA<sub>2</sub>α [67], whereas with PC liposomes the activation is ~2-fold (M. Gelb and C. Leslie, unpublished) [65]. This is reminiscent of the activation reported by PI(4,5)P<sub>2</sub>, where the activation is much more pronounced with mixed micelles than with liposomes [57,68]. This tends to suggest that when the PC is at low surface packing density (mixed micelles), cPLA<sub>2</sub>α is more dependent on anionic lipid additives for binding to membranes and activation. This creates

controversy for the role of these anionic lipids in regulating the action of cPLA<sub>2</sub>α in cells. Although a PC liposome is only a model of a cell membrane, it is arguably a better model than a PC-detergent mixed particle containing mostly Triton X-100.

The interaction of cPLA<sub>2</sub>α with C-1-P *in vitro* is mediated in part by basic residues (R57/K58/R59) in the C2 domain, and mutations in these residues impair agonist-induced translocation of cPLA<sub>2</sub>α (Fig. 1) [69,70]. The major route of C-1-P production is the phosphorylation of ceramide by CERK. Knockdown of CERK by siRNA results in a partial reduction in C-1-P levels and reduces agonist-induced arachidonic acid release and translocation of cPLA<sub>2</sub>α in A549 cells [64,66]. Collectively these studies have led to the hypothesis that C-1-P produced from CERK is a proximal activator of cPLA<sub>2</sub>α, necessary for its C2 domain-dependent membrane binding and ability to release arachidonic acid. This is an attractive hypothesis since CERK localizes to the Golgi for production of C-1-P [66,71]. However, results of recent studies do not corroborate these conclusions. Generation of CERK<sup>-/-</sup> mice confirmed that CERK plays a major role in production of *N*-palmitoyl-C-1-P in mast cells, macrophages and cerebellum although in some cells (neutrophils) CERK is not the major pathway for C-1-P production [70,72]. However, cPLA<sub>2</sub>α-dependent release of arachidonic acid and eicosanoid production is identical in wild type and CERK<sup>-/-</sup> macrophages and kidney fibroblasts despite a greater reduction in C-1-P compared to cells treated with siRNA [70,72]. In addition, no differences are observed in wild type and CERK<sup>-/-</sup> mice in a cPLA<sub>2</sub>α-dependent model of rheumatoid arthritis [72]. Additional results using a specific CERK inhibitor suggest that the lack of effect on cPLA<sub>2</sub>α regulation in CERK<sup>-/-</sup> cells and mice is not due to compensatory mechanisms. The specific CERK inhibitor NVP-231 significantly reduces cellular levels of C-1-P but has no effect on arachidonic acid release or PGE<sub>2</sub> production in several cell types stimulated with a variety of agonists [73]. These results do not support a role for the CERK-dependent production of C-1-P as a generalized mechanism for regulating cPLA<sub>2</sub>α and eicosanoid production. The results raise the question of why siRNA knockdown of CERK inhibits arachidonic acid release but this is not observed in CERK<sup>-/-</sup> cells or with a specific CERK inhibitor. It also raises questions about the basis for the translocation defect observed with cPLA<sub>2</sub>α mutated at the putative C-1-P binding site in the C2 domain [70]. It is possible that there are other components on the Golgi that bind to this site. Alternatively mutating this site may result in subtle conformational alteration of the CBLs and affect Ca<sup>2+</sup>-dependent translocation in cells. Clearly more studies are required to resolve the discrepancies regarding the role of CERK and C-1-P in regulating cPLA<sub>2</sub>α.

### 4. Regulation of cPLA<sub>2</sub>α activity at the Golgi

Calcium-dependent translocation of cPLA<sub>2</sub>α positions the catalytic domain on the membrane for interaction with phospholipid substrate (Fig. 1). A comparison of the calcium-induced translocation of full-length cPLA<sub>2</sub>α and the C2 domain to Golgi revealed that the C2 domain acts as a calcium sensor and translocates in direct proportion to [Ca<sup>2+</sup>]<sub>i</sub> [74]. Translocation of full-length cPLA<sub>2</sub>α is slightly slower but it remains associated with the Golgi in a calcium-independent fashion unlike the C2 domain, which rapidly dissociates as [Ca<sup>2+</sup>]<sub>i</sub> decreases. There is little experimental data that well defines the basis of catalytic domain binding to the interface of the Golgi membrane in cells. The only clear result is that a tryptophan residue on the membrane-exposed face of the catalytic domain contributes to the prolonged calcium-independent residence of cPLA<sub>2</sub>α at the Golgi [74].

Translocation to membrane is necessary but not sufficient for cPLA<sub>2</sub>α to release arachidonic acid. The hydrolytic activity of cPLA<sub>2</sub>α is independently regulated by phosphorylation and by interaction

of an anionic membrane component with basic residues in the catalytic domain (Fig. 1). Phosphorylation on Ser-505 has been studied most extensively since it is the site for MAPKs, a common signaling pathway that regulates the release of arachidonic acid [4,75]. cPLA<sub>2</sub>α that is completely dephosphorylated is active when assayed *in vitro* and phosphorylation on Ser-505 modestly enhances activity by about 2-fold [57,75–77]. Ser-505 phosphorylation is not sufficient for cPLA<sub>2</sub>α to release arachidonic acid but acts synergistically with increases in calcium [75,78–80]. Phosphorylation of cPLA<sub>2</sub>α on Ser-505 has been reported to increase the membrane binding affinity at low calcium [81]. However, wild type cPLA<sub>2</sub>α and cPLA<sub>2</sub>α Ser-505A mutant exhibit similar rates of translocation to Golgi in response to physiological agonists, but the mutant releases less arachidonic acid [57,82]. These results suggest that phosphorylation at Ser-505 enhances release of arachidonic acid but not calcium-dependent translocation, which is consistent with *in vitro* data showing that Ser-505 phosphorylation increases catalytic activity but not calcium-dependent interfacial binding [57].

The phosphorylation sites of cPLA<sub>2</sub>α, Ser-505 and Ser-727, are located in a flexible region that is not near either the membrane binding face or the active site (Fig. 1). This raises questions as to how phosphorylation regulates cPLA<sub>2</sub>α function. It has been suggested that phosphorylation of Ser-505, which is located near the interdomain flexible linker region, may promote optimal orientation of the C2 and catalytic domains for efficient catalysis [83]. Phosphorylation at Ser-505 by MAPKs is generally accompanied by phosphorylation at Ser-727, which occurs by kinases downstream of MAPK such as MAPK-interacting kinase [80,84,85]. Ser-727 phosphorylation plays a novel role in regulating the interaction of cPLA<sub>2</sub>α with p11 (S100A10)/annexin A2 complexes (Fig. 1) [86]. This bulky complex binds to the hydroxy group of Ser-727, which maintains cPLA<sub>2</sub>α in an inactive state by interfering with membrane binding *in vitro* and blocking translocation of cPLA<sub>2</sub>α to perinuclear membranes in cells (Fig. 1) [86–88]. The interaction of p11/annexin A2 complex with cPLA<sub>2</sub>α is prevented by phosphorylation of Ser-727 thus allowing calcium-dependent interaction of cPLA<sub>2</sub>α with membrane and release arachidonic acid [86].

The activity cPLA<sub>2</sub>α is enhanced by several types of polyphosphoinositides through interaction with a patch of basic residues (K488/K541/K543/K544) in the catalytic domain [68,89–92]. It was shown that PI(4,5)P<sub>2</sub> facilitates binding of cPLA<sub>2</sub>α to PC/Triton X-100 mixed micelles and promotes activation of cPLA<sub>2</sub>α in a calcium-independent manner [68,91]. However, other studies have shown that PI(4,5)P<sub>2</sub> in PC liposomes enhances cPLA<sub>2</sub>α activity in the presence but not in the absence of calcium [57,89]. This is reminiscent of studies with C-1-P described above showing that cPLA<sub>2</sub>α activation by the anionic additive is much more pronounced with mixed micelles versus liposomes. *In vitro* data showing that PI(4,5)P<sub>2</sub> primarily acts to enhance catalytic activity and not interfacial binding is consistent with cellular data showing that wild type cPLA<sub>2</sub>α and the K488N/K543N/K544N mutant exhibit similar rates of translocation to Golgi in response to serum but the mutant releases less arachidonic acid (Fig. 1) [57]. In addition, it has been shown that translocation of cPLA<sub>2</sub>α to internal membranes induced by loading cells with PI(4,5)P<sub>2</sub> occurs in the presence but not in the absence of intracellular calcium [92]. Although polyphosphoinositides activate cPLA<sub>2</sub>α *in vitro* and when loaded into cells, further studies are needed to definitively identify the endogenous anionic component on the Golgi that activates cPLA<sub>2</sub>α through the basic residues in the catalytic domain.

## 5. Function of cPLA<sub>2</sub>α at the Golgi

Several studies have provided evidence that cPLA<sub>2</sub>α regulates the structure and function of the Golgi. The expression of cPLA<sub>2</sub>α in

LLC-PK1 kidney epithelial cells, which are deficient in cPLA<sub>2</sub>α, causes disruption of the Golgi cisternae and blocks the constitutive transport of aquaporin-2 to the plasma membrane [93]. The effect on Golgi structure is suggested to be due to the ability of cPLA<sub>2</sub>α to generate free fatty acids and lysophospholipids that modify the properties of the Golgi membrane.

cPLA<sub>2</sub>α also plays a role in regulating the structure and function of the Golgi that occurs by modifying cellular cholesterol content (Fig. 1). Increasing cellular cholesterol induces vesiculation of the Golgi apparatus that occurs by a cPLA<sub>2</sub>α- and dynamin-dependent mechanism [94]. Loading cells with cholesterol induces the translocation of cPLA<sub>2</sub>α to the Golgi and an increase in arachidonic acid release. Vesiculation of the Golgi by cholesterol is blocked by a cPLA<sub>2</sub>α inhibitor suggesting that enzymatic production of free fatty acids and lysophospholipids are involved. Conversely it has been shown that lowering Golgi cholesterol inhibits the association of cPLA<sub>2</sub>α with the Golgi resulting in a decrease in the release of arachidonic acid and accumulation of caveolin-1 in the Golgi [95]. The ability of a cPLA<sub>2</sub>α inhibitor to block transport of caveolin-1 from the Golgi supports a role for cPLA<sub>2</sub>α activity in regulating trafficking. These studies highlight the important role of membrane lipid composition in regulating the association of cPLA<sub>2</sub>α with the Golgi and a role for cPLA<sub>2</sub>α in regulating Golgi structure and transport.

There is considerable evidence that modification of Golgi lipid composition by phospholipases and acyltransferases influences membrane curvature and regulates tubulation and vesicle formation (Fig. 1) [22,23,96,97]. cPLA<sub>2</sub>α has recently been shown to be required for formation of tubules that connect the Golgi stacks and for regulation of intra-Golgi transport [22]. When transport through the Golgi is activated, cPLA<sub>2</sub>α is recruited to the rims of the Golgi cisternae and to rim-associated tubules. Similar translocation to Golgi of the cPLA<sub>2</sub>α C2 domain during transport suggests a role for local calcium increases perhaps from the high intraluminal levels in the Golgi. cPLA<sub>2</sub>α is also associated with the Golgi during steady state trafficking. The function of cPLA<sub>2</sub>α is specific for regulating tubule formation and intra-Golgi trafficking but not formation of Golgi vesicles or other trafficking pathways [22]. cPLA<sub>2</sub>α is proposed to act by generating lysophospholipids at the rims of the Golgi cisternae to promote positive membrane curvature for formation of tubules (Fig. 1).

Several studies have identified a role for cPLA<sub>2</sub>α and its association with Golgi in regulating the function of endothelial cells. Endothelial cells line the luminal surfaces of blood vessels and serve a barrier function due to tight junctions and endothelial adherens [98]. Endothelial cells proliferate during wound healing for formation of new blood vessels and during tumor formation. The proliferation of sub-confluent human umbilical vein endothelial cells, and cell cycle entry, is blocked by the cPLA<sub>2</sub>α inhibitor pyrrolidine-1 and siRNA knockdown of cPLA<sub>2</sub>α [99]. It is known that sub-confluent endothelial cells release greater amounts of arachidonic acid than confluent cells [100]. The decrease in proliferation and arachidonic acid release in confluent endothelial cells correlates with a redistribution of cPLA<sub>2</sub>α from the cytosol and nucleus to the Golgi [99]. Localization to Golgi does not require an increase in [Ca<sup>2+</sup>]<sub>i</sub> and correlates with inhibition of cPLA<sub>2</sub>α due to interaction with annexin A1 [101]. It is suggested that annexin-1 at the Golgi sequesters cPLA<sub>2</sub>α and prevents direct interaction with Golgi membrane and association with substrate.

However, recent findings have demonstrated that localization of cPLA<sub>2</sub>α at the Golgi in confluent endothelial plays a functional role in regulating cell–cell junction formation [102]. The translocation of cPLA<sub>2</sub>α to the Golgi correlates with the initial maturation of adherens junctions, and disruption of junctions using a VE-cadherin blocking antibody causes cPLA<sub>2</sub>α to dissociate from the Golgi.

Results suggest that VE-cadherin clustering may provide signals for promoting translocation of cPLA<sub>2</sub>α to the Golgi. Once on the Golgi cPLA<sub>2</sub>α regulates the transport of junction proteins through and/or from the Golgi to cell–cell contacts, which is blocked by siRNA knockdown of cPLA<sub>2</sub>α. Treating confluent endothelial cell with cPLA<sub>2</sub>α inhibitors causes accumulation of proteins that form adherens junction (VE-cadherin) and tight junctions (occludin, claudin-5) in the Golgi suggesting that cPLA<sub>2</sub>α enzymatic activity is required for transport of junction proteins [102]. Therefore although cPLA<sub>2</sub>α localization to Golgi in confluent endothelial cells may dampen the agonist-induced release of arachidonic acid, cPLA<sub>2</sub>α maintains a level of activity that is required for regulating Golgi trafficking. The regulation of junction formation in endothelial cells by cPLA<sub>2</sub>α is not due to the production of prostaglandins [102]. These studies implicate a role for formation of the immediate products of cPLA<sub>2</sub>α action, free fatty acids and lysophospholipids, or a non-prostanoid lipid mediator, in regulating Golgi function although the actual mechanism remains to be defined.

## 6. The need for specific PLA<sub>2</sub> inhibitors

Studies presented above provide strong evidence that cPLA<sub>2</sub>α plays a role in regulating fundamental cellular processes such as Golgi trafficking. This raises the question of why the cPLA<sub>2</sub>α knockout mouse does not exhibit more profound phenotypic alterations. It is certainly possible that there is adaptation in response to the genetic ablation of cPLA<sub>2</sub>α in mice and compensatory pathways come into play. It is unlikely that cells would rely on a single pathway for regulating important physiological cell functions. This is illustrated in studies that clearly show a role for cPLA<sub>2</sub>α in regulating tubulation and intra-Golgi transport, yet these processes function normally in fibroblasts from the cPLA<sub>2</sub>α knockout mouse [22]. An siRNA screen to determine the role of other PLA<sub>2</sub>s of the Group IV, VI, VII and VIII families, found that silencing of only the GVIII PLA<sub>2</sub> inhibited Golgi transport in cPLA<sub>2</sub>α-deficient fibroblasts [22]. The cPLA<sub>2</sub>α knockout mouse has been very useful in elucidating the role of cPLA<sub>2</sub>α in disease, but compensatory pathways may obscure our understanding of the full functional role of cPLA<sub>2</sub>α. This argues for the importance of developing specific inhibitors for mammalian PLA<sub>2</sub>s to more accurately evaluate the role of these important enzymes in regulating normal cellular processes and in disease pathogenesis.

Probably the most reliable cPLA<sub>2</sub>α inhibitors for use in cellular studies are the pyrrolidines developed at Shionogi and the indoles developed at Wyeth [103,104]. These compounds inhibit cPLA<sub>2</sub>α in the sub-micromolar range using a number of different *in vitro* lipolysis assays and they block cPLA<sub>2</sub>α-dependent arachidonate release in mammalian cells in the 0.01–1 μM range [103–107]. The pyrrolidine and indole inhibitors have recently been shown to be efficacious in mouse models showing a role for cPLA<sub>2</sub>α in collagen-induced arthritis and in experimental autoimmune encephalomyelitis, respectively [108,109]. They do not inhibit the human cPLA<sub>2</sub>β and cPLA<sub>2</sub>γ isoforms but data on the mouse isoforms are not yet available [105,110]. However, recent data show that the pyrrolidines and indoles work about as well on mouse cPLA<sub>2</sub>ζ as on cPLA<sub>2</sub>α *in vitro* [111]. In fact, these compounds block arachidonate release in mouse lung fibroblasts prepared from cPLA<sub>2</sub>α deficient mice, suggesting that cPLA<sub>2</sub>ζ can contribute at least some of the arachidonate in these cells. There is no data on the effect of these inhibitors on purified human cPLA<sub>2</sub>ζ or on mouse and human cPLA<sub>2</sub>δ and cPLA<sub>2</sub>ε. The groups of Lehr and Kokotos have reported on a series of ketone-containing compounds that are also potent inhibitors of cPLA<sub>2</sub>α [112,113]. Studies in the author's laboratories are underway to fully explore the specificity of cPLA<sub>2</sub>α inhibitors on the complete set of human and mouse Group IV PLA<sub>2</sub> isoforms.

In summary, we have available sub-micromolar cPLA<sub>2</sub>α inhibitors that work in cell models, but the full reliability of these compounds must await further analysis in terms of cross-specificity with other cPLA<sub>2</sub> isoforms. Such inhibitors, when combined with siRNA and gene deletion, will lead to a comprehensive understanding of the function of individual members of the Group IV cPLA<sub>2</sub> family.

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