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Phospholipase D in the Golgi Apparatus

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Summary

Phospholipase D has long been implicated in vesicle formation and vesicular transport through the secretory pathway. The Golgi apparatus has been shown to exhibit a plethora of mechanisms of vesicle formation at different stages to accommodate a wide variety of cargo. Phospholipase D has been found on the Golgi apparatus and is regulated by ADP-ribosylation factors which are themselves regulators of vesicle trafficking. Moreover, the product of phospholipase D activity, phosphatidic acid, as well as its degradation product diacylglycerol, have been implicated in vesicle fission and fusion events. Here we summarize recent advances in the understanding of the role of phospholipase D at the Golgi apparatus.

Keywords

Phospholipase D; Phosphatidic acid; Golgi apparatus; vesicular trafficking

Introduction

Studies into the role of phospholipase D (PLD) in the regulation of vesicle transport and protein trafficking were provoked by the observation that ADP-ribosylation factor (ARF) proteins are efficacious activators of PLD [1–3]. ARF proteins have been previously implicated as factors for regulation of intracellular vesicle trafficking and are found on the Golgi apparatus and the plasma membrane [4]. The PLD discussed in this review hydrolyzes phosphatidylcholine (PC) to yield phosphatidic acid (PA) and choline. PA has been demonstrated to be a signaling molecule as well as a crucial lipid during vesicle fusion and fission [5–8]. Furthermore, PA can be hydrolyzed to generate diacylglycerol (DG) which also acts as signaling molecule as well as a functional lipid in membrane modulation [9,10]. This review, which focuses on recent advances in the localization and function of PLD in the Golgi apparatus is dedicated to the memory of Dennis Shields, who unexpectedly passed away December 1st, 2008.

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Structure and Regulation of mammalian PLD enzymes

Two mammalian PC-PLD genes have been associated with intracellular vesicle trafficking: PLD1 [11] and PLD2 [12]. Both share the same basic domain organization of a phox (PX) and a pleckstrin homology (PH) domain in tandem in their amino terminus, the conserved dual HKD motif which forms the active site, and a central phosphoinositide binding domain. This domain structure is also shared by the single PLD genes of Yeast, Nematodes and Drosophila [13], as well as by one of the plant PC-PLD families, PLDζ[6]. PLD1 exhibits low intrinsic basal activity and is strongly and synergistically activated by members of the ARF and Rho families of monomeric G proteins as well as by PKCα and PKCβ. Conversely, heterologous expression of PLD2 results in high basal activity. However, endogenous PLD2 cannot be measured without prior purification and therefore may be regulated by inhibitory proteins such as synucleins [14]. In addition, the activity of PLD1 and PLD2 is highly dependent on the presence of phosphatidylinositol 4,5-bisphosphate (PIP_2) or phosphatidylinositol 3,4,5-trisphosphate (PIP_3).

Subcellular localization and membrane association of mammalian PLDs

Overexpression of epitope tagged variants has been widely used to examine the subcellular localization of PLD1 and PLD2. GFP- or HA-tagged PLD1 and PLD2 localize mainly to the plasma membrane and several other organelles, including endosomes, lysosomes, secretory granules and the Golgi apparatus [12,15,16]. Cryo-immunoelectron microscopy using selective antibodies, and subcellular fractionation, demonstrated that 25–30% of endogenous PLD1 was localized to the Golgi apparatus. Although a fraction of endogenous PLD2 was evident on the plasma membrane, much of the enzyme localized to the region of the Golgi apparatus and cytosolic puncta [17]. Most significantly, cryoimmunoelectron microscopy demonstrated that PLD2 was present almost exclusively on Golgi cisternal rims in pituitary GH3 cells; it was enriched 80-fold in Golgi rims relative to cisternae [17]. The differential distribution of PLD1 and PLD2 suggests that these enzymes have separate functions in the Golgi apparatus.

The mechanisms by which PLD1 and PLD2 associate with cellular membranes have been studied extensively. PLD1 and PLD2 exhibit three lipid-binding domains in addition to the active site, a PX domain, a PH domain and a polybasic domain that is responsible for PIP_2 stimulated enzyme activity and is sufficient for the membrane recruitment of PLD2 [18]. This multi-domain organization reflects the complex cellular distribution of these proteins. The PX domain preferentially binds to PIP_3 but also mono-phosphorylated inositides, and its membrane association is enhanced by binding of acidic lipids such as PA or PS at a secondary site [19, 20]. This is thought to be responsible for internalization of PLD after recruitment to the plasma membrane [19]. PIP_2 binds to the PLD2 PH domain albeit with low affinity, suggesting that this domain functions in conjunction with other membrane association domains to target the protein to different intracellular sites [21]. In addition, the PH domain of PLD1 is palmitoylated [22,23]. Protein palmitoyl transferases are found along the secretory pathway [24] and it has not been established where PLD is acylated. In fact, the effect of palmitoylation on the subcellular localization of PLD1 is controversial [25,26]. Both, PX and PH domains have been shown to bind to proteins in addition to lipids [27–30], and a model of combinatorial binding of such domains has been suggested [30]. Interestingly, the four-phosphate-adaptor protein PH-domain binds phosphatidylinositol 4-phosphate and ARF simultaneously to recruit these proteins to the trans-Golgi network [31]. Investigations into the role of phosphoinositides in membrane localization of PLD1 and PLD2 are complicated by the finding that certain isoforms of the enzyme responsible for PIP_2 synthesis, phosphatidylinositol 4-phosphate 5-kinase (PI4P5K), are themselves stimulated by the PLD product PA [32,33]. PIP_2 is synthesized on isolated Golgi apparatus membranes incubated with cytosol in a PLD dependent manner [34]. In vivo, synthesis of PIP_2 on the Golgi apparatus is required for its structural integrity

and treatment of cells with primary alcohols causes rapid fragmentation of the Golgi apparatus, in part by PIP_2 dependent relocalization of βIII-spectrin [35]. Stimulation of PI4P5K activity by PLD-produced PA provides an attractive explanation for these observations. This process forms a feed forward loop with PLD. PIP_2 is rapidly degraded on the Golgi apparatus [35] presumably by PIP_2 5-phosphatases such as Ocrl1 [36]. This suggests a tightly regulated local and temporal spike of PA and PIP_2 production upon recruitment of PLD and PI4P5Ks by ARF on the Golgi apparatus, contrasting with the comparatively large and static PIP_2 pool of the plasma membrane.

ARF as a PLD1 effector in membrane trafficking

PLD1 can be activated by all members of the ARF family in their GTP-bound states, and the potency of this effect is enhanced by myristoylation of ARF [37]. However, although the effects are likely to be direct because they can be readily observed using purified proteins, the mechanism whereby ARF stimulates enzyme activity and a site of direct interaction on PLD1 remain to be identified. ARF1-5 localize to the perinuclear region and the Golgi apparatus, and combinatorial RNAi mediated knock-down revealed that when ARF1 was depleted together with ARF3, ARF4 or ARF5, the distribution of the COPI coat protein β-cop was disrupted [38]. ARF1 regulated PLD activity at the Golgi apparatus has been implicated in vesicle budding [39,40], whereas the plasma membrane localized ARF6 regulates PLD activity at the plasma membrane [41,42]. It has been demonstrated that ARF1 is a more potent activator of PLD1 than ARF6 [43]. However, a study using effector domain mutants of ARF3 suggested that ARF activation of PLD1 and coatomer recruitment are separable processes [44]. Whereas ARF proteins are strong stimulators of PLD1 activity, their effect on PLD2 activity is low. However, paradoxically, N-terminally truncated PLD2 mutants are more sensitive to ARF stimulation [45]. Effects of ARF on PLD2 activity in cell-based assays might in fact be explained by ARF stimulated PI4P5K activity [43].

Insights from the transphosphatidylation reaction

A large body of work implicating PLD activity in an extensive variety of cellular functions, including Golgi membrane transport, is based on the long-established preference of PLD for primary alcohols over H_2O to hydrolyze PC, yielding a phosphatidylalcohol instead of PA [46,47]. This reaction, termed transphosphatidylation, has been used to infer the involvement of PLD-generated PA in cellular processes based on the following presumptions: diversion of PA to the corresponding phosphatidylalcohol would be complete, and phosphatidylalcohols, which are metabolically stable and accumulate to significant levels in primary alcohol treated cells, would be themselves inert and do not exert inhibitory effects on the cellular processes under study. In support of the suggested involvement of PLD in intracellular trafficking, primary alcohols were shown to inhibit both protein transport from the endoplasmic reticulum to the Golgi apparatus and release of secretory vesicles from the trans-Golgi network [40,48, 49]. Moreover, the Golgi apparatus reversibly fragments in the presence of primary alcohols [34,50].

Initiation of assembly of the COPI coat on Golgi membranes was demonstrated to occur independently of ARF in cell lines exhibiting high constitutive PLD activity [39,48]. COPI coated vesicles mediate intra-Golgi apparatus trafficking as well as retrograde transport to the endoplasmic reticulum [51]. The formation of coated vesicles was sensitive to ethanol at concentrations that inhibit PLD catalyzed PA production. Furthermore, exogenous bacterial PLD was able to induce the binding of coatomers to Golgi membranes. Additionally, ARF1, reconstituted purified COPI coatomer proteins and chemically defined synthetic liposomes containing PA can form coated vesicles in vitro in the absence of PLD [52]. This led to the idea that PLD catalyzed production of PA is a key event in the formation of coatomer-coated

vesicles, which is supported by the finding that the coatomer protein β-cop and ARF directly bind to PA in vitro [53]. Recently, PLD2 has been described as a crucial component of COPI vesicle formation [54]. The BARS protein, a structural protein acting at the fission step of COPI vesicle formation [55] requires PA in liposomes to induce tubulation. Depletion of PLD2 from Golgi membranes by either RNAi or using antibodies trapped β-cop on membranes [54]. This indicates that in absence of PA generated by PLD2 fission is not completed.

In Drosophila, absence of PLD was lethal by preventing embryonic cellularization and caused abnormal Golgi apparatus structure and vesicle trafficking [13]. The observation of abnormal Golgi apparatus structure in Pld^{null} Drosophila embryos suggested a role for PLD in facilitating the fission of vesicles from the trans Golgi network which are targeted to the embryonic cortex. In addition, fusion of vesicles into the plasma membrane was inhibited [13], demonstrating that Drosophila PLD is required for both events.

Interestingly, it has been demonstrated that SNARE mediated fusion of vesicles is enhanced by the presence of PA and PIP_2 [56,57], where PA has to be present on the tSNARE side whereas PIP_2 is stimulatory only on the vSNARE side. Since PLD1 and PLD2 require PIP_2 for their activity, it can be speculated that PLD binds to PIP_2 on the vesicle while generating PA on the target membrane. The structure of PLD1 and PLD2 is unknown, however, their ability to form homo- as well as heterodimers [58] would support this scenario. However, these studies have not yet been extended to Golgi apparatus SNAREs and it is unclear if those exhibit a similar lipid requirement. Finally, the budding yeast spo14p PLD plays a conditional role in maintaining normal secretory function during the “sec14 bypass” and an obligatory role in formation of the prospore membrane during meiosis [59]. The latter process involves a redirection of the secretory pathway to sites of membrane fusion at the spindle pole bodies [60]. The identification of a PA binding function for the putative PLD effector, the SNARE protein spo20p, provides a potential mechanism for this process [56].

Role of PLD in production of diacylglycerol

In addition to its role in production of PA, PLD may also be important in the production of other lipid messenger molecules. In many systems hormone-induced production of DG is biphasic by nature [61,62]. A small transient increase is followed by a larger sustained increase. Polyunsaturated DG predominates during the initial phase of stimulation and is thought to be derived from PIP_2 by the action of phospholipase C [63]. During the sustained phase, the concentration of mono-unsaturated and saturated DG rises. PLD has some preference for mono-unsaturated and saturated PC molecular species [64]. Therefore, it is proposed that PLD contributes to the production of DG by promoting the formation of PA, which is subsequently converted into DG by PA phosphohydrolase (Fig. 1). Since phosphatidylalcohols are not a substrate for PA phosphohydrolase, as described above, the transphosphatidylation reaction can be exploited to demonstrate that the second peak of DG accumulation results from the actions of PLD [65]. Additionally, the PA phosphohydrolase inhibitor propranolol can attenuate DG production in some systems [65]. The most prominent function of DG is activation of PKC; and PKCμ (PKD1), PKD2 and PKCν (PKD3) regulate vesicle trafficking at the Golgi apparatus [66]. Intriguingly, brief treatment with propranolol causes reversible relocalization of PKD from the Golgi apparatus into the cytoplasm [67]. Moreover, ilimaquinone, a drug that causes fragmentation of the Golgi apparatus, requires PLD-mediated PA generation and its subsequent conversion to DG to cause PKD stimulation [68]. It is important to note that DG on the Golgi apparatus can also be derived from sphingomyelin synthesis. Phosphocholine is transferred from PC to ceramide, producing sphingomyelin and DG [69]. Depletion of ceramide using fumonisin B1 therefore reduces DG production through this pathway, and it has been shown that extended treatment with fumonisin B1 causes cytosolic distribution of PKD [67]. Moreover, ten mammalian DG kinases have been described [70]

which can utilize DG to create a pool of PA independent of PLD. Indeed, activation of PLD may not be an essential part of the basic ARF-mediated vesicle budding machinery in the Golgi apparatus of eukaryotic cells, because in yeast such a functionality does not exist [71], and any PA required in these processes might be produced by DG kinase [72]. The only known PLD gene in the yeast Saccharomyces cerevisiae, SPO14, is not essential for secretion, but does play a conditional role in this process during the “sec14 bypass” [73]. Spo14p is mainly involved in prospore formation which depends on vesicle transport from the Golgi apparatus to the prospore membrane [74]. The contributions of the PLD and the DG kinase pathways likely differ between cell types and for the transported cargo by way of adaptor and coat proteins.

Role of PLD in Golgi fragmentation during apoptosis

Recently, it has been demonstrated that fragmentation of the Golgi apparatus is an early event in apoptosis coinciding with cytochrome c release from the mitochondria [75]. Several Golgi apparatus proteins are cleaved during apoptosis by caspases, and some, such as golgin-160, or p115 are targets of upstream caspases such as caspase-2 and caspase-8 [76,77]. Since treatment of cells with n-butanol but not t-butanol causes reversible fragmentation of the Golgi apparatus [34], the fate of PLD1 and PLD2 during apoptosis is clearly of interest. In vitro, PLD1 and PLD2 were substrates for caspases, including the upstream caspase-8 but not caspase-2 [78]. Interestingly, both isoforms retained their activity after cleavage and PLD1 showed an altered response to regulatory stimuli. Cleavage of PLD1 resulted in an enzyme not responsive to phorbol ester and increased response to GTPγS. The caspase-mediated removal of the amino terminus that is responsible for the loss of response to phorbol ester would also prevent PLD1 from being recruited by PKC to the plasma membrane. It is therefore tempting to speculate that this cleaved form of PLD1 accumulates on the Golgi apparatus and its activity is in part responsible for Golgi fragmentation during apoptosis.

Conclusion

Recent advances have provided insight as to how PLD is involved in regulating structure and function of the Golgi apparatus (Fig. 2). However, PA and DG, the immediate and secondary lipid products of PLD activity respectively, can also be derived via other pathways (Fig. 1). Mammalian cells exhibit a plethora of mechanisms of vesicle formation on the Golgi apparatus [79,80], and alternative pathways of lipid modification add to the specific regulation of each. This complexity demands further characterization of the role of PLD in particular pathways of vesicular transport at the Golgi apparatus. Although considerable progress has been made in this area, the range of experimental tools available for manipulations of PLD activity in intact cells limits the types of experiments that can be attempted. In particular, concerns about the specificity and persistence of the effects of primary alcohols to manipulate PLD activity may limit their usefulness while more specific “epigenetic” approaches such as overexpression or RNA interference are slow in onset. The very recent identification of small molecule inhibitors of PLD1 and PLD2 activity may provide an invaluable new tool to re-examine these issues with greater precision [81,82]. If these probes can be rapidly accumulated by, and washed out of cells, then their use in conjunction with live cell imaging techniques will provide a way to directly evaluate the role of PLD in intracellular transport and organelle dynamics.

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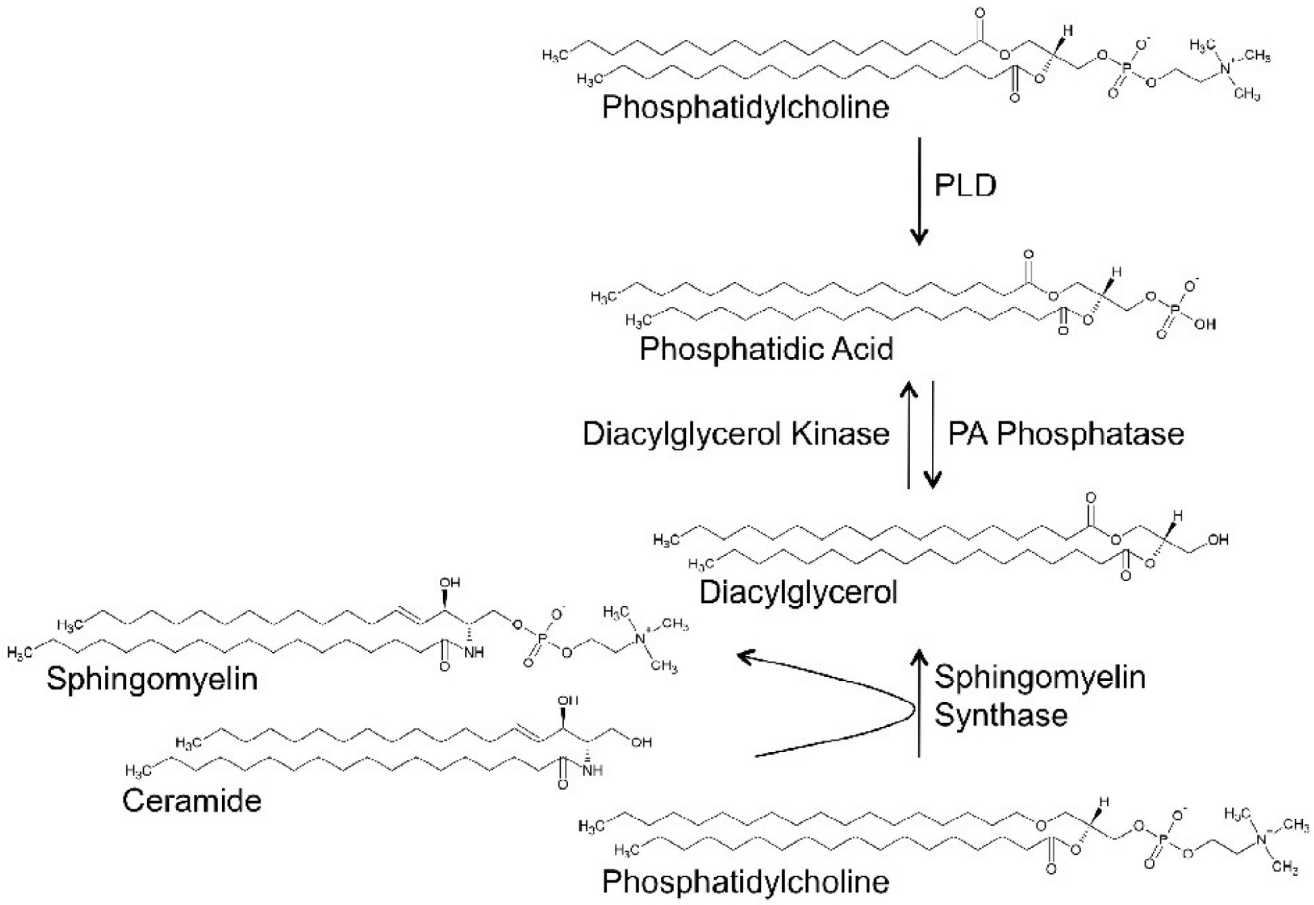

Fig. 1.

Phosphatidic acid and diacylglycerol can be synthesized through separate routes on the Golgi apparatus. In mammalian cells all pathways are active, allowing for complexity of regulation. Note that diacylglycerol might be derived by additional pathways. PA, phosphatidic acid; PLD, phospholipase D.

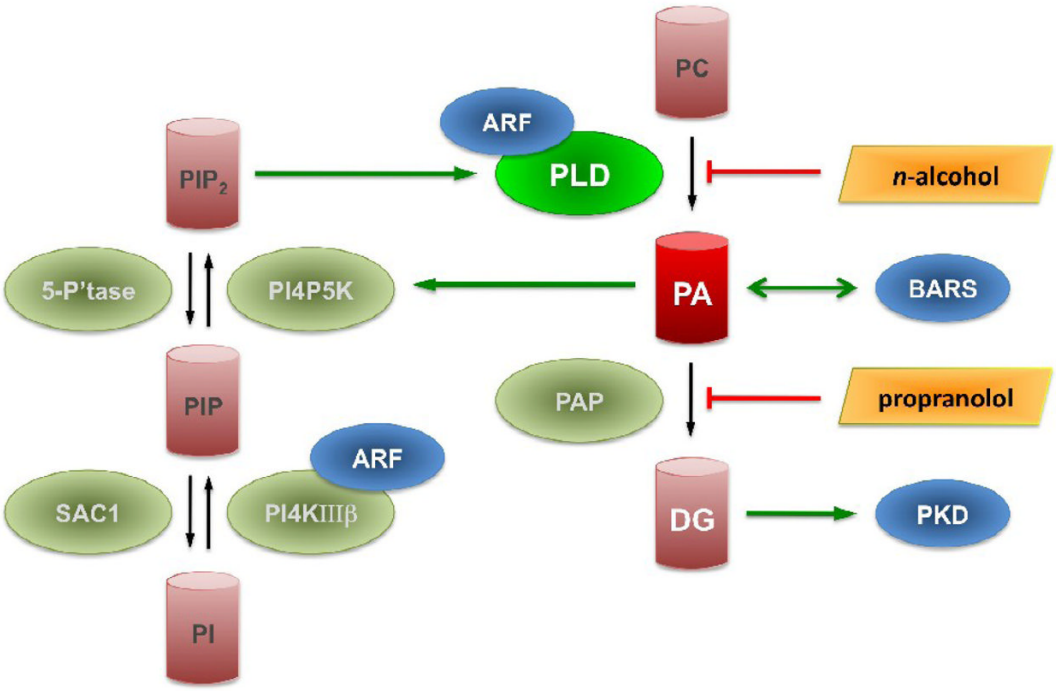

Fig. 2.

Regulation and effectors of phospholipase D and phosphatidic acid on the Golgi apparatus. See text for details. ARF, ADP-ribosylation factor; BARS, Brefeldin-A ADP-ribosylated substrate; DG, diacylglycerol; PA, phosphatidic acid; PAP, PA phosphatase; PC, phosphatidylcholine; PI, phosphatidylinositol; PIP, PI 4-phosphate; PIP_2, PI 4,5-bisphosphate; PI4KIIIβ, type III PI 4-kinase β; PI4P5K, type I PIP 5-kinase; PKD, protein kinase D; PLD, phospholipase D; SAC1, PIP 4-phosphatase; 5-P'tase, PIP_2 5-phosphatase.