‘Slip, sliding away’: phospholipase D and the Golgi apparatus

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Phospholipase D enzymes (PLDs) constitute a family of phosphodiesterases that catalyze the hydrolysis of phosphatidylcholine (PtdCho) to generate choline and phosphatidic acid (PtdOH), a potent lipid signaling molecule implicated in numerous physiological processes. Mammalian PLDs have been localized to multiple organelles, including the nucleus, Golgi apparatus, lysosomes, secretory granules and plasma membrane. However, the detailed mechanisms that govern targeting of PLDs to different organelles, how their local activity is controlled or indeed the nature of PA effectors are not well understood. Here, we discuss recent observations on PLD localization to the Golgi apparatus and how members of this enzyme family might play a role in regulating the structure of this organelle.

Phospholipids play a key role in mediating signal-transduction events and intracellular membrane trafficking. In the past decade, phospholipid-modifying enzymes and, in particular, phospholipase D (PLD) have become the subjects of intense study [1–4]. Phospholipase D is involved in numerous physiological processes, including vesicle coat recruitment, budding from the Golgi apparatus, exocytosis, endocytosis, organization of actin filaments in membrane ruffles and meiosis [3,4]. Its enzymatic activity, which hydrolyzes phosphatidylcholine (PtdCho) to produce phosphatidic acid (PtdOH) and choline, was originally discovered in cabbage leaves from which it was purified and cloned; these sequences were instrumental in cloning PLD from the budding yeast *Saccharomyces cerevisiae* [5]. Both the plant and yeast sequences were used subsequently to identify the two major mammalian PLD enzymes – PLD1 (1074 amino acid residues) and PLD2 (933 amino acid residues) [6–8] (Figure 1). Although both isoforms share many similarities, increasing evidence [9] indicates significant differences. For example, PLD1 and PLD2 are differentially regulated by small GTP-binding proteins, including members of the ADP-ribosylation factor (ARF) family [10]; RhoA has been demonstrated to stimulate PLD1, while having no effect on PLD2 [11]. Additionally, the enzymes have different intracellular distributions, although there is overlap in some organelles, and only PLD2 activity appears to be enhanced by coexpression with phosphatidylinositol 4-phosphate 5-kinases [PtdIns(4)*P* 5-kinases] [12]. Here, we discuss recent observations on PLD localization to the Golgi apparatus and how members of this enzyme family might play a role in regulating the structure of this organelle.

Since their initial characterization, PLDs from many species have been cloned and functionally important motifs have been identified (Figure 1); these studies have been the subject of many excellent reviews and will not be discussed in detail [3,4,8,13]. PLD1 and PLD2 show ~50% homology in the conserved catalytic core, with more variable N- and C-termini [4,8] (Figure 1). The catalytic core, subdivided into four conserved subdomains (I–IV), contains two unique motifs – $HxK(x)_4D(x)_6GSxN$ – termed HKD motifs, which are responsible for enzymatic activity [14,15]. PLDs are members of a large enzyme family, including phosphodiesterases and transphosphatidyl transferases that catalyze transphosphatidylation reactions. In the presence of primary alcohols only, the phosphatidyl group is transferred to the alcohol to generate phosphatidyl-alcohol, rather than PtdOH. Although PtdOH can be generated from a variety of sources, including DAG kinase and glycerol 3-phosphate acyltransferase, the PLD enzymes appear unique in their ability to utilize primary alcohols – this reaction has been used extensively to assay PLD activity.

Yeast PLD

During a screen for genes involved in meiosis, a *Saccharomyces cerevisiae* PLD ortholog, the product of the *SPO14* gene, was identified as a mutant that is defective in meiotic divisions required to package nuclei into spores [5]. The gene encodes a 1683-residue protein, whose N-terminus is ~350 residues longer than mammalian PLD1. Similar to mammalian PLDs, Spo14p activity is stimulated by phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], and the enzyme possesses transphosphatidylation activity. During meiosis, Spo14p relocates from the cytoplasm to spindle pole bodies, adjacent to sites of membrane formation; enzyme relocation is dependent on the N-terminus, which becomes hyperphosphorylated [16]. N-terminal-truncated Spo14p has diminished phosphorylation activity, resulting in its failure to relocate to spindle pole bodies or complement sporulation defects in *spo14* mutants, even though it maintains catalytic activity *in vitro*. Similarly, catalytically dead Spo14p was unable to support spore membrane formation even though meiotic relocalization was unaffected [16].

In otherwise wild-type yeast cells, *spo14* mutants have little, if any, effect on secretion. However, in *sec14* ‘bypass’ mutants, Spo14p activity is absolutely necessary for

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 0962-8924/$ - see front matter doi:10.1016/j.tcb.2003.08.004

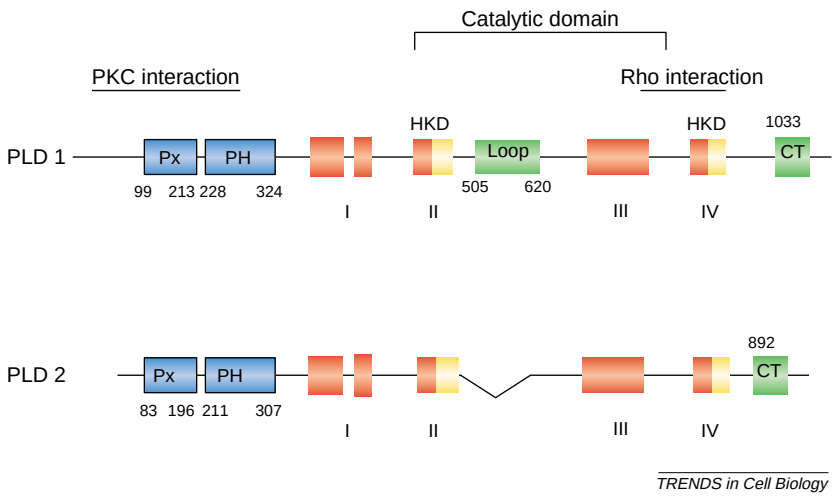

Fig. 1. Structure of mammalian phospholipase D (PLD) enzymes. Comparison of domains in PLD1 and 2 [6,7,8,11,22]. The four conserved regions are indicated by roman numerals, and the 'HKD' motifs are in yellow. Evidence suggests that HKD motifs mediate intra- and inter-molecular interactions between PLDs necessary for their catalytic activity [8]. PLD1 possesses a 116-residue loop, located after the first HKD motif (absent in PLD2) and termed the 'activation loop', which might be involved in the regulation of enzyme activity. Both enzymes undergo alternative splicing; in some cells, a 33-residue insert within the 116-residue loop is spliced out of the full-length PLD1, termed PLD1a, to generate the smaller PLD1b. Similarly, the full-length PLD2 (PLD2a) is spliced to generate two truncated splice variants, PLD2b and PLD2c [4,8]. Abbreviations: CT, carboxyl-terminal region essential for enzyme activity; PH, pleckstrin-homology domain; Px, phox domain.

survival [17]. The *SEC14* gene encodes a PtdIns–PtdCho transfer protein (PITP) that is essential for exit from the Golgi apparatus; mutations in this gene are lethal [18]. Seven loss-of-function mutants have been identified that can 'bypass' the lethality of *sec14* mutations, and several of these mutants are defective in enzymes of the PtdCho biosynthetic pathway. Most significantly, in the absence of Spo14p enzyme activity, secretion is inhibited in *sec14* mutant cells [19] and the bypass mutants are lethal [19,20]. These observations demonstrate that PtdOH synthesis through PLD can restore Golgi function when Sec14p activity is lacking. More recently, the two functions of Spo14p – relocalization to spore-forming sites and *SEC14* bypass – have been uncoupled [21]. The data demonstrate that, although phosphorylation and relocation to spore-forming sites are necessary for meiosis, only PLD activity is required for Sec14p-independent secretion. At present, the role of PtdOH in either sporulation or Golgi function is unclear. In both situations, a local production of PtdOH could alter the composition of membrane microdomains, leading to changes in their physical properties and shape. In this respect, the function of Spo14p might be analogous to that of PLD2 at the rims of Golgi cisternae, where a locally high production of PtdOH could play a role in regulating membrane curvature (see below).

Mammalian PLDs

It is not surprising that, given their involvement in disparate functions, mammalian PLD activities are subject to complex regulation by a variety of molecules, including small GTP-binding proteins and protein kinase C (PKC) isoforms [8]. PKC binds to the N-terminus of PLD (Figure 1) and renders the enzyme responsive to agonists, such as phorbol esters, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) [8]. The stimulation of PLD1 and PLD2 activities by PKCα, a Ca^{2+}-dependent isoform of PKC, has been well documented [22,23], and, more recently, PKCδ has been demonstrated to stimulate PLD activity [24]. Additionally, members of the Rac- and Rho-GTPase family regulate PLD1 and PLD2 activities [4,9]. However, one of the best-characterized regulators of PLD1 is the small GTP-binding protein ARF [3,25,26]. All members of the ARF family, ARF1–ARF6, can activate PLD1 activity in their GTP-bound states, particularly when myristoylated [3]. However, the specific site(s) on the PLD1 molecule that govern this interaction and the mechanism whereby ARF stimulates enzyme activity remain to be identified. Whereas ARF1 regulates PLD1 activity at the Golgi apparatus and has been implicated in vesicle budding [27,28], ARF6 regulates PLD activity at the plasma membrane [29]. Although these earlier studies suggested PLD activation by ARFs occurred in the Golgi, where it might regulate coat recruitment/vesicle production, subsequent studies, using point mutants of ARF3, have demonstrated that the situation is more complex than originally envisioned. Kuai *et al.* [30] generated ARF3 point mutants in which coatomer recruitment was inhibited but their ability to stimulate PLD was almost normal, as well as mutants exhibiting the opposite phenotype. These results suggest that ARF activation of PLD and coatomer recruitment are separable processes and that PLD activation alone is insufficient for coatomer protein I (COP-I) recruitment to Golgi membranes [30]. Although PLD1 and PLD2 are activated by ARFs, the two isoforms vary in their sensitivities to individual ARFs. The GTP-bound form of ARF1 stimulates PLD1 catalytic activity more than 13-fold, whereas PLD2 is only stimulated 1.5-fold [10,11]. Interestingly, deletion of the N-terminal 308 amino acid residues renders PLD2 as ARF responsive as PLD1 [11]. This observation suggests that full-length PLD2 might interact with proteins at its N-terminus that protect it from ARF activation [4]; alternatively, the N-terminus of PLD2 itself could act as an inhibitory domain.

PLD activity requires PtdIns(4,5)P_2

Classic *in vitro* studies, using purified cell membranes, demonstrated that PLD activity was dependent on PtdIns(4,5)P_2 [4,25]; how PtdIns(4,5)P_2 regulates PLD activity is still unknown. PLDs possess N-terminal phoX (PX) and pleckstrin-homology (PH) domains that bind to phosphoinositide phospholipids and mediate PLD–lipid interactions (Figure 1). It was reported that the PH domain of PLD1 is required for catalytic activity and localization to membranes *in vitro* [31]. This was supported by the recent observation that mutagenesis of the PH domain alters PLD1 distribution from membranes to the cytosol [32]. Previous work had shown that PLD1 is palmitoylated on two adjacent cysteines (C240 and C241) in a flexible loop within the PH domain [33]. Mutation of these residues significantly limited PLD1 acylation, affecting its membrane localization and diminishing its activity [32]. However, acylation alone is unlikely to play a role in the membrane targeting of other PLDs, such as Spo14p or mouse PLD2, because neither enzyme possesses the tandem cysteine residues. Other studies reported that deletion or mutagenesis of the PH domain had little effect on PLD1 or PLD2 activity [34]. Instead, a small region consisting of basic residues (amino acids 691–712 for PLD1 and 554–575 for PLD2), known as a 'KR' motif, interacted with phosphoinositides and was responsible for PtdIns(4,5)P_2-stimulated PLD activity [34]. More recent observations have demonstrated that the PH domain of PLD2 is required for efficient enzyme targeting *in vivo* [35]. Mutations in this domain resulted in catalytically active enzymes, which maintained a requirement for PtdIns(4,5)P_2; however, these enzymes relocated from the plasma membrane to endosomes and were rendered unresponsive to activation by agonists [35]. These authors suggest a model in which the PH domain of PLD2 binds to PtdIns(4,5)P_2 selectively, but with low affinity. However, in concert with the KR motif, which functions as a high-affinity but less-selective anchor, these two domains cooperate to localize PLD2 to the plasma membrane [35].

The role of the PX domain in PLD enzymology is unclear. Previous reports have implicated binding of PX domains to a variety of phosphoinositides, including PtdIns(3)*P*, PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3, in addition to PtdIns(4,5)P_2 [36]. It is possible that this diverse range of potential interacting lipid molecules could contribute to PLD localization; however, this seems less probable in the light of a recent study demonstrating that deletion of the PX domain of PLD1 affected neither the intracellular localization nor the degree of acylation of PDL1 [32]. It is possible that the PH domain and polybasic motifs, in combination with acylation, might create a tighter, more selective and less transient membrane association that facilitates stimulation of PLD catalytic activity [32]. Therefore, multiple protein–protein interactions with these modules, mediated by ARFs, Rac, Rho or PKC isoforms (the latter being membrane associated), might confer organelle-binding specificity on PLDs. Thus, a current challenge for the field will be to identify putative binding proteins and/or motifs that confer membrane specificity.

Localization of PLD to Golgi membranes

Several studies have provided biochemical evidence for the presence of an ARF-stimulated PLD on Golgi membranes [27,37]. Additionally, ARF-activated PLD1, and the resultant PtdOH, stimulated release of nascent secretory vesicles from the trans-Golgi network (TGN) [28,38]. Using cell fractionation, Jones *et al.* [39] found only minor levels of ARF-activated PLD in Golgi membranes, where most of the activity was associated with a dense membrane fraction. A complication with all these studies [28,37,39] is that different methods of Golgi isolation were used by the various laboratories, making it difficult to compare results directly. Furthermore, overexpressed GFP- or HA-tagged PLD1 localized to several organelles, including endosomes, lysosomes, secretory granules and the plasma membrane; little was detected on the Golgi apparatus [40,41]. To resolve these discrepancies, morphological techniques were used to localize endogenous PLD1 and PLD2. PLD1 exhibited a Golgi-like localization, as well as diffuse cytoplasmic staining [42]. Cryo-immunoelectron microscopy and subcellular fractionation demonstrated that ~25–30% of endogenous PLD1 was localized to the Golgi apparatus. However, overexpression of an HA-tagged PLD1 led to loss of Golgi staining, through displacement of endogenous PLD1 into peripheral vesicular structures [42].

Overexpressed PLD2 was primarily found at the plasma membrane [7,43], in agreement with studies that demonstrated an association between PLD2, ARF6 and EGF receptor at sites of membrane ruffling [44]. Although a fraction of endogenous PLD2 was evident on the plasma membrane, much of the enzyme localized to the Golgi region and cytosolic puncta [45]. Most significantly, cryo-immunoelectron microscopy demonstrated that PLD2 was present almost exclusively on Golgi cisternal rims; it was enriched 80-fold in Golgi rims relative to cisternae [45]. These findings suggest that some caution might be necessary when interpreting data from experiments that utilize overexpression protocols to define the subcellular localization of enzymes normally present only in catalytic amounts.

The differential distribution of PLD1 and PLD2 implies that these enzymes have separate functions in the Golgi apparatus. Because PLD2 has a relatively high basal activity, it might function as a 'housekeeping' enzyme and maintain a localized pool of PtdOH, whereas ARF1-activated PLD1 might play a role in modulating PtdOH levels. Furthermore, the rim localization of PLD2 implies a regulatory role in mediating vesicular trafficking, analogous to the recent observations that protein components of the retrograde transport machinery are localized to rims of the Golgi apparatus [46]. By catalyzing formation of PtdOH, PLDs might alter the membrane composition at specific sites to facilitate release of vesicles. It has been suggested that increasing local concentrations of PtdOH, as mediated by endophilin, alters membrane curvature sufficiently to facilitate vesicular budding [47]; PLD2 might have an analogous function at the Golgi rims. Consistent with this idea, it is noteworthy that caveoli, which manifest a high degree of membrane curvature, are enriched in PLD2 [4,45].

A role for PLD and PtdOH in the Golgi apparatus?

There are several possibilities as to how PtdOH might function in the Golgi apparatus. First, it could act as a 'structural' lipid – not only to promote membrane curvature at cisternal rims but also to recruit coats and other components required for vesicle formation and budding [3]. In this context, Manifava *et al.* [48] demonstrated that several proteins involved in vesicle trafficking (COP-I complex, ARF, NSF and kinesin) bound to a PtdOH-agarose affinity resin incubated with brain cytosol; interestingly, a PtdIns(4,5)P_2 affinity column bound these proteins poorly. Second, PtdOH could be dephosphorylated by a Golgi-associated phosphatidic-acid phosphatase to generate DAG and activate a signaling pathway through PKC. In this context, recent observations by Baron and Malhotra [49] suggest that protein kinase D (PKD) stimulates vesicle budding from the TGN; of particular interest is their observation that PKD recruitment was dependent on DAG levels. Treatment of cells with propanolol, an inhibitor of phosphatidic acid phosphatase, caused reversible relocation of PKD from the TGN to the cytoplasm [49], suggesting that PtdOH was the source of Golgi DAG. Consistent with this idea, PtdOH synthesis has been shown to promote Golgi vesicle release *in vitro* [38]. Third, during viral infection, PtdOH could promote the release of post-Golgi vesicles containing viral proteins or virions [15]. Studies on vaccinia virus assembly from the Golgi apparatus have suggested a role for PLD activity. Interestingly, a vaccinia viral protein F13L induces the formation of post-Golgi vesicles, and this protein has a phospholipase D motif. Significantly, primary butanol inhibited F13L-induced vesicle formation and decreased formation of extracellular virus, without affecting the yield of intracellular mature virus. These data support a role for F13L in inducing the formation of vesicle precursors of the vaccinia virus membrane through phospholipase activity [15].

A role for PLD in phosphoinositide synthesis in the Golgi apparatus?

It is well documented that PtdOH stimulates PtdIns(4,5)P_2 synthesis *in vitro*, and, in a feedback loop, PtdIns(4,5)P_2 stimulates PLD activity [25,50]. It is possible that these pathways could intersect in the Golgi apparatus. At least two enzymes in the PtdIns(4,5)P_2 biosynthetic pathway (PtdIns 4-kinase types IIα and β) are Golgi associated [51,52] and a third (PtdIns 4-kinase type IIIβ) is recruited to Golgi membranes by ARF1 [53]. Thus, *in-vitro*-isolated Golgi membranes are capable of robust PtdIns(4)*P* synthesis [39,53–56]. By contrast, the type I PtdIns(4)*P* 5-kinases [the final enzymes in the PtdIns(4,5)P_2 biosynthetic pathway] are soluble and recruited to their target membrane by interaction with lipids [54] and, presumably, other proteins. Incubation of isolated Golgi membranes with cytosolic extracts or purified enzymes results in synthesis of PtdIns(4,5)P_2 [39,53,55] in a reaction that can be stimulated by ARF1 [39,53]. Our laboratory demonstrated that *in vitro* synthesis of PtdIns(4,5)P_2 was decreased by phosphatidyl alcohols, suggesting a role for PLD in regulating these reactions [55] (Figure 2). By contrast, other investigators found no effect of primary alcohols on PtdIns(4,5)P_2 synthesis *in vitro* [39,53]; the reason for this discrepancy is unclear at present. Interestingly, brief treatment of cells (~15 min) with primary butanol, but not with secondary or tertiary butanol (neither of which participate in the transphosphatidylation reaction), leads to rapid Golgi fragmentation and blebbing of the plasma membrane; upon alcohol removal, the Golgi apparatus reassembles [55,56].

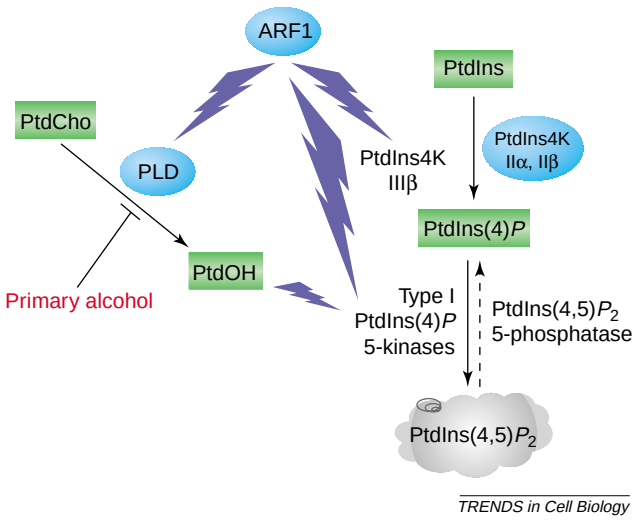

Fig. 2. Schematic representation of phosphatidic acid (PtdOH) and phosphoinositide synthesis in the Golgi apparatus. Phospholipase D (PLD)-mediated hydrolysis of phosphatidylcholine (PtdCho) leads to generation of PtdOH. In the case of PLD1, the small GTP-binding protein ARF1 stimulates enzyme activity significantly, whereas basal activity of PLD2 is high. The type II phosphatidylinositol 4-kinases IIα and IIβ (PtdIns4K IIα and IIβ), which associate with the Golgi apparatus as well as other organelles [51,52], phosphorylate phosphatidylinositol (PtdIns) at the D4 position to synthesize phosphatidylinositol (4)-phosphate [PtdIns(4)*P*], the major inositol phospholipid in the Golgi [62,63]. ARF1 also recruits phosphatidylinositol 4-kinase IIIβ to Golgi membranes [53] and stimulates phosphatidylinositol (4)-phosphate 5-kinase activity *in vitro* to generate PtdIns(4,5)P_2 [39,53]. *In vitro*, type I phosphatidylinositol (4) phosphate 5-kinases are stimulated by PtdOH to generate phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] [50]. In the presence of primary alcohols, the transphosphatidylation reaction of PLD synthesizes a phosphatidyl alcohol (instead of PtdOH); this metabolite cannot stimulate type I PtdIns(4)*P* 5-kinase activities and PtdIns(4,5)P_2 synthesis is inhibited [55,56]. Although significant levels of PtdIns(4,5)P_2 have not been detected in the Golgi apparatus [61–63], isolated Golgi membranes possess potent phosphatidylinositol 5-phosphatase activity, the function of which remains to be determined [56].

On the basis of the correlation between diminished PtdIns(4,5)P_2 synthesis *in vitro* and Golgi fragmentation *in vivo*, PtdOH production in Golgi membranes might regulate the local synthesis of PtdIns(4,5)P_2. The resulting increase in its concentration could function in maintaining Golgi architecture by recruitment of specific cytoskeletal proteins, such as βIII-spectrin or ankyrin, to the membrane [57,58]; increased ARF1 activity could also promote actin binding [59]. PtdIns(4,5)P_2 levels could be maintained by a balance between synthesis and turnover because numerous reports [1,2] have demonstrated the existence of a large family of inositol polyphosphate 5-phosphatases. This family includes several synaptojanin-like enzymes, members of the type II phosphatase family that can hydrolyze PtdIns(4,5)P_2 and have been localized to the Golgi apparatus – for example, the Ocrl1 protein [60]. It was recently shown that isolated Golgi membranes possess a potent PtdIns(4,5)P_2 5-phosphatase activity, the nature of which remains to be determined; interestingly, these membranes have little if any PtdIns(4)*P* 4-phosphatase activity [56] (Figure 2).

Although this model is consistent with a PtdIns(4,5)P_2 requirement for PLD activity, several reports have suggested that the major site of PtdIns(4,5)P_2 synthesis is the plasma membrane. By exploiting a PH domain of GST–PLCδ that is specific for PtdIns(4,5)P_2, Watt *et al.* [61] used cryo-immunoelectron microscopy to demonstrate PtdIns(4,5)P_2-immunoreactive gold particles on the plasma membrane and nuclei, with only minor localization to the Golgi apparatus. Similarly, Levine and Munro [62] found that virtually all PtdIns(4,5)P_2 was localized to the plasma membrane in yeast cells; identical results were obtained by Stefan *et al.* [63]. Indeed, in cells expressing a single temperature-sensitive synaptojanin-like mutant, PtdIns(4,5)P_2 was only present on intracellular compartments at the non-permissive temperature, suggesting that, in yeast, this enzyme and other phosphatases control the plasma membrane localization of PtdIns(4,5)P_2 [63]. Although these are compelling results and the PH domain of PLCδ has been used extensively to detect PtdIns(4,5)P_2, some caution in the use of such reagents might be necessary. Given that these reporter molecules are frequently overexpressed in cells, it is possible that lipid binding might be a function of mass-action effects rather than resulting from specific high-affinity interactions; such a scenario could provide a misleading picture of the level and localization of PtdIns(4,5)P_2. Also, not all PH domains are equal, and it is possible that the affinity of the PH domain of PLCδ for a putative PtdIns(4,5)P_2 pool in the Golgi apparatus (if indeed it exists) could be much lower than at the plasma membrane. Consequently, additional tools are required to determine the relative levels and identity of phospholipids in all organelles including the Golgi apparatus.

Limitations and prospects

Two shortcomings in this area of research are the lack of specific, high-affinity reagents, analogous to PH domains, that could be used to measure or localize PtdOH in organelles, and the absence of specific inhibitors of PLD activity. In the first case, an exciting prospect is the recent identification of an 11-residue N-terminal module from the cAMP-specific phosphodiesterase PDE4A1 [64]. This novel phospholipid-binding domain interacts with PtdOH selectively and has been designated 'TAPAS' (tryptophan-anchoring phosphatidic-acid-selective binding domain) [64], and it might turn out to be a powerful reagent for identifying PtdOH interactions. Additionally, the absence of specific PLD inhibitors has limited ability to dissect the function of the enzyme and that of PtdOH in cells and in *in vitro* assays. Although primary alcohols have been used to inhibit PtdOH synthesis, there is concern that such a relatively nonspecific reagent could be toxic even when suitable controls, employing secondary and tertiary alcohols, are included.

The most powerful tool to analyze PtdOH, PtdIns(4,5)P_2 or indeed other lipids in organelles is mass spectrometry, combined with 'lipidomics'. Not only can this technology identify different lipids individually, but it can also separate all the different fatty-acyl-chain derivatives of each phospholipid species [65]. Mass spectrometry, in combination with measuring changes in different lipid species and isoforms in organelles from control and agonist/antagonist-treated cells can probably provide novel and important insights into how the levels of different lipids are regulated. Furthermore, it will generate definitive answers as to the relative levels of PtdOH and PtdIns(4,5)P_2 in the Golgi apparatus. Recently, this technology has been exploited to characterize changes in phosphoinositide profiles in human fibroblasts from patients with the Lowe Syndrome, a disorder affecting phosphoinositide turnover [49].

Concluding remarks

A major challenge in the next few years will be to determine how PLD localization and the local production of PtdOH are controlled, and the mechanisms by which the relatively simple PtdOH molecule can affect a multitude of cellular processes. Of particular importance will be to dissect the relationship between the synthesis of PtdOH and DAG, to understand how these lipids interact in regulating Golgi structure and function. Finally, it is apparent that ARFs play a central role in regulating the activities and recruitment of several key lipid-modifying enzymes on the Golgi apparatus (Figure 2); how this multitude of functions is integrated remains to be determined. Clearly, much work remains ahead to elucidate the roles of PLD and inositol phospholipids in Golgi biology; the next several years will bear the fruits of such endeavors as our understanding 'slip slides ahead'.

Acknowledgments

We apologize to the many authors whose important work could not be cited owing to space limitations. We thank all the members of our laboratory for helpful discussions. Work in our laboratory is supported by a grant from the National Institutes of Health (DK 21860).

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