Phosphatidic acid: Mono- and poly-unsaturated forms regulate distinct stages of neuroendocrine exocytosis

Emeline Tanguy, Alexander Wolf, Maite Montero-Hadjadje, Stéphane Gasman, Marie-France Bader, Nicolas Vitale

▶ **To cite this version:**

Emeline Tanguy, Alexander Wolf, Maite Montero-Hadjadje, Stéphane Gasman, Marie-France Bader, et al.. Phosphatidic acid: Mono- and poly-unsaturated forms regulate distinct stages of neuroendocrine exocytosis. Advances in Biological Regulation, 2021, 79, pp.100772. 10.1016/j.jbior.2020.100772 . hal-03184878

HAL Id: hal-03184878

https://normandie-univ.hal.science/hal-03184878

Submitted on 10 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Version of Record: https://www.sciencedirect.com/science/article/pii/S221249262030083X
Manuscript_2c83dac27bab9ac74ae92e87b52f5f4c

Phosphatidic acid: mono- and poly-unsaturated forms regulate distinct stages of neuroendocrine exocytosis

Emeline Tanguy[1], Alexander Wolf[1], Maité Montero-Hadjadje[2], Stéphane Gasman[1], Marie-France Bader[1] and Nicolas Vitale[1*]

[1]Centre National de la Recherche Scientifique, Université de Strasbourg, Institut des Neurosciences Cellulaires et Intégratives, F-67000 Strasbourg, France

[2]Normandie Univ, UNIROUEN, INSERM, U1239, Laboratoire de Différenciation et Communication Neuronale et Neuroendocrine, Institut de Recherche et d'Innovation Biomédicale de Normandie, 76000, Rouen, France

*Correspondence: vitalen@unistra.fr

Key words: Exocytosis, fatty acid, phosphatidic acid, phospholipase D, neuroendocrine secretion

© 2020 published by Elsevier. This manuscript is made available under the CC BY NC user license
https://creativecommons.org/licenses/by-nc/4.0/

Abstract

Lipids have emerged as important actors in an ever-growing number of key functions in cell biology over the last few years. Among them, glycerophospholipids are major constituents of cellular membranes. Because of their amphiphilic nature, phospholipids form lipid bilayers that are particularly useful to isolate cellular content from the extracellular medium, but also to define intracellular compartments. Interestingly, phospholipids come in different flavors based on their fatty acyl chain composition. Indeed, lipidomic analyses have revealed the presence in cellular membranes of up to 50 different species of an individual class of phospholipid, opening the possibility of multiple functions for a single class of phospholipid. In this review we will focus on phosphatidic acid (PA), the simplest phospholipid, that plays both structural and signaling functions. Among the numerous roles that have been attributed to PA, a key regulatory role in secretion has been proposed in different cell models. We review here the evidences that support the idea that mono- and poly-unsaturated PA control distinct steps in hormone secretion from neuroendocrine cells.

1. Introduction

Lipids found in eukaryotic membranes can be divided in three major classes including glycerophospholipids, sphingolipids, and sterols. Sophisticated techniques led to the identification of more than 30,000 different lipids suggesting that these molecules support many biological processes in addition to the well-known energy provider and compartment delimitation roles. Although their synthesis pathway, subcellular distribution as well as mechanisms by which they move from one compartment to another are getting better understood everyday (Yang et al., 2018), there is still a gap in the comprehension of the precise function of many of them.

The acyl chain diversity in glycerophospholipids has been studied for decades, but our understanding of its contribution to cell function has remained elusive for many years, largely because of technical and conceptual limitations. Phospholipids are divided in families based on the chemical nature of their headgroup. By order of quantities found in eucaryotic membranes, the main families of phospholipids are phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphoinositides (PI) and phosphatidic acid (PA). They can exist both as monoacyl- or diacyl-glycerophospholipid forms. Variety in acyl chain in position *sn-1* and *sn-2* further extends the diversity of phospholipids. These chains are divided in three classes: saturated, mono- and poly-unsaturated. Based on the location of the poly-unsaturation, the later class has been further divided in subclasses of which the omega-3 (ω3, e.g. docosahexaenoic acid, C22:6) and the omega-6 (ω6, e.g. arachidonic acid, C20:4) are the most popular.

The acyl chain profile of a biological membrane often presents predominant combination that can be specific to some organisms, cells, and even organelles. For instance, while the endoplasmic reticulum is particularly enriched in mono-unsaturated lipids, poly-unsaturated lipids are more abundant in secretory vesicles and saturated lipids are more present in the plasma membrane (Bigay and Antonny, 2012). In mammals, the acyl chain pairings of PI are frequently composed of stearic acid (C18:0) and arachidonic acid (C20:4), while other phospholipids present a wide variety of acyl chains of diverse length and saturation. Little is known regarding the mechanisms and functions behind the phospholipid fatty acyl chain composition (Barneda et al., 2019).

Among phospholipids, PA is an important intermediate metabolite in the synthesis of all membrane glycerophospholipids and is therefore playing an important structural role in living cells by contributing to membrane biogenesis. Chemically, PA consists of a glycerol backbone to which are attached, through esterification, two fatty acyl chains and a phosphate at positions *sn-1*, *sn-2*, and *sn-3*, respectively. The unique feature of PA compared to the other diacyl–glycerophospholipids is its phosphomonoester link to a small anionic phosphate headgroup (Jenkins and Frohman, 2005; Tanguy et al., 2018). The small headgroup of PA predicts that it adopts a cone-shaped structure in membranes and X-ray diffraction studies have contributed to postulate that local accumulation of PA in one leaflet of membranes modifies their topology by creating a negative curvature (Kooijman et al., 2005). This property of PA is often linked to its contribution in diverse membrane trafficking events (Ammar et al., 2013b; Tanguy et al., 2016). PA serves also as an essential signaling molecule in diverse cellular processes, through the recruitment of a broad range of cytosolic proteins to appropriate membrane locations (Kim and Wang, 2020; Tanguy et al., 2019b, 2018).

In membrane trafficking, pharmacological, molecular and genetic compelling evidences support an essential role for PA in a variety of cellular processes based on vesicle transport and fusion. For instance, PA is critical for various exocytotic events such as endocrine and neuroendocrine exocytosis (Bader and Vitale, 2009; Vitale et al., 2001; Waselle et al., 2005), mast cell degranulation (Choi et al., 2002), endothelial cell secretion (Disse et al., 2009), acrosomal exocytosis (Lopez et al., 2012), exosome biogenesis and secretion (Ghossoub et al., 2014) and even the fast synaptic neurotransmission (Humeau et al., 2001; Schwarz et al., 2011; Raben and Barber, 2017). PA is also implicated in autophagy (Holland et al., 2016) and phagocytosis (Corrotte et al., 2006; Tanguy et al., 2019a), intracellular pathways such as tubular endosome biogenesis (Giridharan et al., 2013) and Golgi vesicle formation (Ktistakis et al., 1996), membrane wound repair (Vaughan et al., 2014) and neurite outgrowth (Ammar et al., 2013a), However, despite or perhaps due to these large implications, precise functions of PA along the secretory pathway remain unclear. The diversity of the PA biosynthetic routes together with the possible occurrence of many different PA species based on their fatty acyl chain composition open the possibility for multiple roles in a given cellular function.

2. The different sources of phosphatidic acid

In mammals, cellular PA production involves several major routes (Pokotylo et al., 2018; Zegarlińska et al., 2018; Tanguy et al., 2019b) (Figure 1). Structural PA is synthesized through two acylation reactions: the glycerol 3-phosphate (G3P) pathway and the dihydroxyacetone phosphate (DHAP) pathway, both leading to lysoPA (LPA), a mono-acylated form of PA. LPA is subsequently transformed into PA during a second acylation step (Vance and Vance, 2004). PA generated from these *de novo* pathways is the main intermediate in the synthesis of all triacyglycerols and glycerophospholipids, as it is actively transformed by PA phosphohydrolase (PAP) into diacyglycerol (DAG) in the endoplasmic reticulum (Vance and Goldfine, 2002). PA can also be transformed by cytidyl phosphatidate (CDP)-DAG synthetase (CDS) into CDP-DAG, which fuels the synthesis of PI, phosphatidylglycerol (PG) and cardiolipin (CL). Different phospholipases A (PLA) can also deacylate PA into LPA.

Additionally, three alternative biosynthetic pathways use distinct lipid precursors to produce so-called “signaling” PA (Figure 1). The first pathway involves phosphorylation of DAG to produce PA using ATP as a phosphate source by one of the 10 different DAG kinase (DGK). The second pathway involves acylation of LPA by specific LPA-acyltransferases (LPAAT) to generate PA (Jenkins and Frohman, 2005). Six human LPAAT isoforms have been cloned and characterized (Leung, 2001), but more proteins displaying LPAAT activities have recently been identified. For instance, the RIBEYE protein in synaptic ribbon enabling ultra-fast synaptic release possesses an LPAAT activity providing PA (Schwarz et al., 2011). The third pathway involves phospholipase D (PLD), which catalyzes the hydrolysis of the distal phosphodiester bond of PC to form PA and choline. Six isoforms of PLDs have been identified based on sequence homology, among them PLD1 and PLD2 being the best characterized isoforms. It is of note that until now no direct PLD activity *per se* has been documented for the PLD3-5 isoforms (Frohman, 2015). The complexity and diversity of PLD, DGK and LPAAT families suggest that they are involved in various specific cellular functions which are, probably, not redundant. In general, signaling PA generated from these enzymes is implicated in multiple processes such as cytoskeleton organization, cell survival, proliferation, membrane and vesicle trafficking (Nelson and Frohman, 2015). Intriguingly PC to PA conversion by PLD transforms a cylindrical phospholipid into a conical one. Since

biophysical models have proposed that intermediates of the membrane fusion reaction require such cone- or inverted cone-shaped lipid, a direct role of PA as a fusogenic lipid has been proposed (Vitale, 2010). Interestingly, PLD localization to specific membranes compartments depends on key domains of the enzymes (Du et al., 2003) and probably involves deciphering the phosphoinositide landscape of intracellular membranes (Sztacho et al., 2019).

3. Role of phosphatidic acid in neuroendocrine exocytosis

The requirement for PA in calcium-regulated exocytosis in neuroendocrine and endocrine cells is probably one of the best documented role for PA in membrane trafficking (Bader and Vitale, 2009). Early studies in bovine chromaffin cells reported the inhibitory action of butanol on exocytosis through the inhibition of PA synthesis by PLD (Caumont et al., 1998; Galas et al., 1997). These observations were supported by experiments in different cell models (Choi et al., 2002; Hughes et al., 2004; O'Luanaigh et al., 2002), but the lack of specificity of butanol has later been questioned (Su et al., 2009). Overexpression of wild type and dominant-negative PLDs provided the first direct molecular evidence supporting the involvement of PA generated by the PLD1 isoform in hormone release in neuroendocrine chromaffin cells (Vitale et al., 2001). PLD1 silencing experiments further supported the notion that PLD1 is the major source for PA synthesis during exocytosis in chromaffin cells (Zeniou-Meyer et al., 2007). Furthermore, the use of a molecular sensor for PA revealed a predominant calcium-stimulated accumulation of PA at the plasma membrane in cells undergoing exocytosis (Zeniou-Meyer et al., 2007). Additional studies highlighted a tight regulation of PLD1 activity by several upstream signaling pathways involving small GTPases and the kinase RSK2 (Béglé et al., 2009; Momboisse et al., 2009; Vitale et al., 2005, 2010; Zeniou-Meyer et al., 2008), revealing a finely tuned production of PA at granule docking sites in stimulated chromaffin cells. Finally, in line with the selective production of PA near exocytotic sites at the plasma membrane, capacitance recordings of chromaffin cells silenced for PLD1 indicated that this enzyme controls the population of fusion competent secretory granules at the plasma membrane (Zeniou-Meyer et al., 2007).

More recently, electron microscopy and morphometric analysis on mouse adrenal gland slices revealed a reduced number of secretory granules in *Pld1* knockout mice adrenal chromaffin cells (Carmon et al., 2020) and a 50% reduction of the number of secretory granules visually docked at the plasma membrane (Tanguy et al., 2020). Supporting the idea that PA might play

a role in the biogenesis of dense-core secretory granules, the dual PLD1/PLD2 inhibitor 5-fluoro-2-indolyl des-chlorohalopemide (FIPI) reduced the number of secretory granules synthesized in a COS-7 cell line expressing chromogranin A (CgA), a member of the granin family playing an important role in granule biogenesis (Carmon et al., 2020). In line with these observations, a PA-binding domain (PABD) was found in the CgA sequence that effectively binds to PA in membrane models, and the overexpression of a CgA PABD-deleted form in endocrine cells significantly reduced the number of secretory granules indicating that the "granulogenic" activity of CgA might involve its ability to bind PA (Carmon et al., 2020).

Carbon fiber amperometry recordings of individual mouse chromaffin cells revealed that *Pld1* knockout decreased by nearly 60% the frequency of amperometric events corresponding to single granule fusion, in good agreement with the apparent reduction of the number of morphologically docked granules seen in the *Pld1*$^{-/-}$ adrenal gland (Tanguy et al., 2020). Shape analysis of individual amperometric spikes and pre-spikes, which provide information on kinetic parameters of single fusion events revealed that the kinetics of the pore dilation and the duration of the exocytotic event were affected by the absence of PLD1 (Tanguy et al., 2020). Supporting the physiological relevance of these *in vitro* observations, catecholamine levels in the blood of new-born mice were reduced by *Pld1* knockout (Tanguy et al., 2020). These observations were further substantiated by the use of FIPI and by the isoform-selective PLD1 inhibitor on bovine chromaffin cells that mimicked the effect observed by amperometry on *Pld1*$^{-/-}$ mouse chromaffin cells (Tanguy et al., 2020). Altogether, these findings indicate that the production of PA by PLD1 controls several stages of the neuroendocrine secretory pathway including biogenesis of secretory granules, the number of secretory granules undergoing exocytosis, and also at the single granule level, the duration of the initial fusion pore and its expansion required to release neuropeptides and peptide hormones (Figure 2). This composite role of PA led to the idea that the sequential stages underlying the secretory pathway could actually require distinct PA subspecies involved in specific functions.

4. Specific PA species contribute to different steps in neuroendocrine cell exocytosis

In neuroendocrine cells, subcellular fractionation experiments revealed that Golgi, secretory granule and plasma membranes contained detectable levels of up to 40 different PA species (Carmon et al., 2020; Tanguy et al., 2020). Secretory granules were particularly

enriched in poly-unsaturated forms of PA, especially PA 40:6 containing docosahexaenoic acid (DHA), a major ω3 fatty acid (Tanguy et al., 2020). Stimulation of exocytosis selectively increased the plasma membrane level of PA containing long chain mono- and di-unsaturated fatty acids (PA 36:1, 38:1 and 38:2), by more than 6-fold (Tanguy et al., 2020). These peculiar PA are largely decreased in adrenal glands from *Pld1* knockout mice, suggesting that PLD1 known to be activated in stimulated cells is most likely responsible for their synthesis at the plasma membrane (Tanguy et al., 2020).

Incubation of cultured chromaffin cells with PA micelles proved to be an easy and effective approach to identify the PA species able to compensate for the lack of PLD activity at the plasma membrane of PLD1-depleted cells. Indeed, exogenous PA rapidly reached the intracellular leaflet of the plasma membrane as seen using the PA sensor Spo20p-GFP which was recruited to the cell membrane within minutes when cells were incubated with PA micelles (Tanguy et al., 2020). Incubation of chromaffin cells with a mixture of various forms of PA did not significantly affect catecholamine secretion *per se*. However, it rescued secretion from cells treated with PLD1 inhibitors (Tanguy et al., 2020). Interestingly, mono- and di-unsaturated forms of PA rescued secretion in PLD1-depleted cells, whereas poly-unsaturated PA did not (Tanguy et al., 2020). Using a combination of amperometric recordings and ultrastructural observations on plasma membrane sheets, we observed that mono- and di-unsaturated forms of PA are apparently involved in granule recruitment and/or docking thereby controlling the number of exocytotic events (Tanguy et al., 2020). In contrast, poly-unsaturated ω3 PA seem to be specifically implicated in fusion pore dilation (Tanguy et al., 2020). At present, it is not known if secretory vesicle biogenesis requires a specific type of PA. Both PA (36:1) and PA (40:6) identified as predominant species in Golgi and secretory granule membranes interact with CgA and promote CgA-induced membrane deformation and remodeling (Carmon et al., 2020).

Based on these findings, we propose that various steps preceding dense-core granule exocytosis require distinct PA species, with mono- and di-unsaturated forms of PA being preferentially implicated in granule docking whereas poly-unsaturated ω3 PA favors subsequent fusion pore dilation (Figure 2).

5. Proteins interacting preferentially with specific species of PA

One possibility to explain the differential effects of mono- and poly-unsaturated PA species on exocytosis is that they recruit different effectors involved specifically in vesicle docking or fusion. Among the nearly 50 different proteins that have been shown to date to bind to PA (Pokotylo et al., 2018; Tanguy et al., 2019b; Zegarlińska et al., 2018), their ability to interact with specific forms of PA has not been extensively evaluated, but some information on these aspects are starting to emerge. Using an *in vitro* fluorescence liposome-based assay, we recently found that the PA-binding domain of Spo20p, Opi1p, and PDE4A1 proteins are partially sensitive to the fatty acyl length and saturation, displaying a clear preference for mono- and poly-unsaturated PA over saturated PA (Kassas et al., 2017). Alternatively, the catalytic domains of *Clostridium sordellii* lethal toxin and related large clostridial glucosylating toxins preferentially recognize saturated PA (Varela Chavez et al., 2015). Muscle creatine kinase specifically binds to PA containing saturated fatty acid- and mono-unsaturated fatty acid, but not to poly-unsaturated PA (Hoshino et al., 2019), whereas L-lactate dehydrogenase selectively interacts with poly-unsaturated PA (Hoshino and Sakane, 2020). Thus, PA-binding proteins seem to be able to discriminate between the diverse forms of PA, although the clues for fatty acid selectivity of the PA-binding domain remain obscure.

Another intriguing mode of interaction has been reported for the dynamin-related protein 1 as it interacts with the headgroup of PA and with the saturated fatty acids of nearby phospholipids (Adachi et al., 2016). Moreover, atomistic molecular dynamics simulations of artificial membranes made of PC and PA revealed that the fatty acyl chain composition of PA affected not only membrane fluidity, but also depth of the PA phosphate groups compared to the PC phosphate groups, which can also influence the presentation of PA for peripheral membrane proteins, affecting their accessibility for binding (Kulig et al., 2019). Finally, even though binding between PA and specific proteins are electrostatic at first, it has been predicted that these links are later solidified through hydrophobic interaction between the acyl chains of PA and apolar groups that can be found in the PA-binding domains (Kooijman and Burger, 2009; Tanguy et al., 2018). Therefore, variation in the hydrophobic amino acids composing these PA-binding domains could partially explain an interaction preference with specific PA species. It is important to note that these kinds of interactions could be exclusive to cone-shaped lipids, such as PA, because they induce lipid packing defects enabling a better and deeper penetration into cellular membranes.

The SNARE protein syntaxin-1 is one of the first target candidate that comes in mind as a target protein in the secretory machinery, as it has been shown to bind to several anionic lipids including PI and PA (Lam et al., 2008). Interestingly, mutations in the polybasic site, abolishing the ability of syntaxin-1 to bind PA, resulted in a reduction of the number of amperometric spikes and increased the pre-spike foot duration in PC12 cells (Lam et al., 2008), very similarly to the amperometric traces obtained in *Pld1*$^{-/-}$ mice chromaffin cells or in bovine chromaffin cells with PLD1 activity pharmacologically inhibited (Tanguy et al., 2020). A similar domain enriched in positive charges that could potentially bind to anionic PA has also been recently described in vesicular SNAREs, such as VAMP-2, and reported to be required for membrane destabilization and fusion (Rathore et al., 2019). It is also worth to recall that the yeast PA binding protein Spo20p is the orthologue of the SNARE protein SNAP25. *In vitro* experiments support the idea that PA directly affects SNARE complex assembly and/or zippering (Mima and Wickner, 2009), although the function of the different PA forms has not been yet tested in this system.

The actin cytoskeleton, originally viewed as a physical barrier preventing granule recruitment, is also emerging as an active network to stabilize granule docking sites, control fusion pore lifetime and/or directly expel granule secretory content (Li et al., 2018). Several actin-binding proteins have been shown to bind PA, including regulators of the Rho family of small GTPases, which play a key role in providing actin structures required for the late stages of exocytosis (Gasman et al., 2004; Malacombe et al., 2006; Houy et al., 2015). Spatial organization of the exocytotic sites at the plasma membrane also appears under the control of large actin structures bundled by annexin A2 (Gabel et al., 2015), a protein that binds anionic lipids including PA (Gabel et al., 2019). The next challenge will be to determine if the function of these regulators in the exocytotic machinery involves their ability to bind PA and whether they require a partnership with specific PA species.

6. Do different PA species differentially affect the fusion pore dynamics?

Fusion of the two membranes requires the formation of several non-bilayer intermediates resulting in hemifusion, which is ultimately followed by membrane breakdown and formation of a pore (Chernomordik et al., 1995). For most lipids found in biological membranes, the lamella bilayer is the most energetically favorable structure. However, cone-

shaped lipids, such as PA, promote negative curvature (Kooijman et al., 2005; Monck and Fernandez, 1994) and the formation of hemifusion intermediates (Chernomordik et al., 1995, 1993). Thus, the predicted effect of a local elevation of PA due to the activation of PLD at exocytotic sites would be to promote membrane bending, particularly in the presence of calcium (Cornell and Arnold, 1996), thereby facilitating hemifusion and subsequent formation of the exocytotic fusion pore and its dilation.

The ability of PA (40:6) to specifically restore kinetics of individual amperometric events in PLD1-depleted chromaffin cells suggests that poly-unsaturated PA regulate the speed of catecholamine release and thus the dynamics of the fusion pore. Molecular modeling of different species clearly indicates that the overall geometry of glycerophospholipids is affected by fatty acyl chain composition (Hodgkin et al., 1998). It is thus likely that the ability to affect membrane topology and more specifically to modulate the fusion pore stability may be variable among the different PA species. Clearly additional experimental evidence is now required to prove or refute this possibility, but it is of interest to note that a similar model, based on lipid-induced changes in membrane curvature, has been proposed for endophilin I-mediated recycling of synaptic vesicles at the plasma membrane (Schmidt et al., 1999).

Conclusion

Lipids are now viewed as essential partners for proteins in membrane trafficking and among them the negatively charged cone-shaped PA is recognized as a key mediator in vesicle exocytosis and membrane fusion events in a variety of cell types including neurons and neuroendocrine cells. The observation that ω3 forms of PA modulate fusion pore dynamics as seen by amperometry opens new routes of investigations. It is possible that the higher flexibility of the acyl chains of poly-unsaturated PA is particularly suited for membrane curvature, thereby reducing the energetic cost of various membrane deformation processes and facilitating fission and fusion events (Manni et al., 2018). It is tempting to propose that poly-unsaturated PA could regulate the fusion mode associated with either a large or a narrow fusion pore (Shin et al., 2020, 2018) or trigger the fusion pore dilation required for secretion of neuropeptides and peptide hormones (Ren et al., 2016). An additional challenge will be to determine if specific PA species contribute to diseases that have been associated to an alteration of PA production. These include intellectual disability

diseases such as Fragile-X disease (Tabet et al., 2016) and the Coffin Lowry Syndrome (Zeniou-Meyer et al., 2008), but also neurodegenerative diseases such as Alzheimer disease (Oliveira et al., 2010) and Parkinson where specific PA subtypes (18:1/18:1 PA) induce structural change and favor aggregation of α-synuclein, (Mizuno et al., 2017). Interestingly, a recent review also highlighted the important contribution of different DGKs isoforms and their product PA in neuronal pre- and post-synaptic functions and their potential contribution to pathologies (Barber and Raben, 2019). PA generated by PLD has also been proposed to facilitate multiple steps in cancer progression including growth, metabolism, angiogenesis, and mobility (Roth and Frohman, 2018). Although the functions of the individual PA species in these pathologies remain to be precisely established, the ability of exogenous PA to restore neuroendocrine exocytosis by provision of the adequate PA opens a route towards a better understanding of the importance of specific fatty acids in diet to improve human health.

Abbreviations

ADR: Acyldihydroxyacetone-phosphate reductase, CDP: Cytidyl phosphatidate; CDS: CDP-diacylglycerol synthase, CL: Cardiolipin, DAG: Diacyglycerol, DGK: Diacylglycerol kinase, DHAP: Dihydroxyacetone phosphate, DHAP AT: DHAP acyltransferase, FIPI: 5-Fluoro-2-indolyl des-chlorohalopemide, G3P: Glycerol 3-phosphate, GPAT: G3P acyltransferase, LPA: Lysophosphatidic acid, LPAAT: Lysophosphatidic acid-acyltransferases, PA: Phosphatidic acid, PABD: PA-binding domain, PAP: Phosphatidic acid phosphohydrolase, PC: Phosphatidylcholine, PE: Phosphatidylethanolamine, PG: Phosphatidylglycerol, PI: Phosphoinositides, PLA: Phospholipase A, PLD: Phospholipase D, PS: Phosphatidylserine, TG: triglycerides.

Figure Legends

Figure 1. The biosynthetic routes of PA, a central phospholipid

Enzymatic pathways highlighted in orange are involved in structural PA synthesis, whereas enzymes in blue lead to the formation of signaling pools of PA. Among this last category, PLD activity transforms the cylindrical phospholipid PC into PA, a conical one, potentially

inducing local negative membrane curvature. Enzymes triggering PA catabolism are shown in green.

Figure 2. Model depicting the different PA species involved in neuroendocrine secretion. Secretory granules are formed by budding from the Golgi apparatus and then actively transported toward the plasma membrane, where they undergo regulated exocytosis to release their cargo into the extracellular medium. Among lipids involved in this journey, specific PA species can modulate different steps of the regulated secretory pathway. PLD1 contributes to the synthesis of mono- and poly unsaturated PA species found in the *trans*-Golgi network membrane, where they may promote granule biogenesis through their interaction with chromogranin A (CgA). Stimulation of exocytosis increases the synthesis by plasma-membrane bound PLD1 of mono-unsaturated PA that favors docking of granules to the plasma membrane and poly-unsaturated PA promoting membrane curvature and fusion pore formation. Note that PA can rapidly flip across membrane leaflets when it is not in tight interaction with specific proteins, suggesting that PA can probably distribute between the two leaflets of granule membranes.

Additional Information

The authors declare no competing financial interests.

This work was supported by grants from the Fondation pour la Recherche Médicale (DEI20151234424) and the Agence Nationale pour la Recherche (ANR-19-CE44-0019) to N.V.

References

Adachi, Y., Itoh, K., Yamada, T., Cerveny, K.L., Suzuki, T.L., Macdonald, P., Frohman, M.A., Ramachandran, R., Iijima, M., Sesaki, H., 2016. Coincident Phosphatidic Acid Interaction Restrains Drp1 in Mitochondrial Division. Molecular Cell 63, 1034–1043. https://doi.org/10.1016/j.molcel.2016.08.013

Ammar, M.-R., Humeau, Y., Hanauer, A., Nieswandt, B., Bader, M.-F., Vitale, N., 2013a. The Coffin-Lowry syndrome-associated protein RSK2 regulates neurite outgrowth through phosphorylation of phospholipase D1 (PLD1) and synthesis of phosphatidic acid. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 33, 19470–19479. https://doi.org/10.1523/JNEUROSCI.2283-13.2013

Ammar, M.R., Kassas, N., Chasserot-Golaz, S., Bader, M.-F., Vitale, N., 2013b. Lipids in Regulated Exocytosis: What are They Doing? Frontiers in Endocrinology 4, 125. https://doi.org/10.3389/fendo.2013.00125

Bader, M.-F., Vitale, N., 2009. Phospholipase D in calcium-regulated exocytosis: lessons from chromaffin cells. Biochimica Et Biophysica Acta 1791, 936–941. https://doi.org/10.1016/j.bbalip.2009.02.016

Barber, C.N., Raben, D.M. 2020. Roles of DGKs in neurons: Postsynaptic functions? Adv Biol Regul. 75:100688. https://doi.org/10.1016/j.jbior.2019.100688

Barneda, D., Cosulich, S., Stephens, L., Hawkins, P., 2019. How is the acyl chain composition of phosphoinositides created and does it matter? Biochemical Society Transactions 47, 1291–1305. https://doi.org/10.1042/BST20190205

Béglé, A., Tryoen-Tóth, P., de Barry, J., Bader, M.-F., Vitale, N., 2009. ARF6 regulates the synthesis of fusogenic lipids for calcium-regulated exocytosis in neuroendocrine cells. Journal of Biological Chemistry 284, 4836–4845. https://doi.org/10.1074/jbc.M806894200

Bigay, J., Antonny, B., 2012. Curvature, lipid packing, and electrostatics of membrane organelles: defining cellular territories in determining specificity. Developmental Cell 23, 886–895. https://doi.org/10.1016/j.devcel.2012.10.009

Carmon, O., Laguerre, F., Riachy, L., Delestre-Delacour, C., Wang, Q., Tanguy, E., Jeandel, L., Cartier, D., Thahouly, T., Haeberlé, A.-M., Fouillen, L., Rezazgui, O., Schapman, D., Haefelé, A., Goumon, Y., Galas, L., Renard, P.-Y., Alexandre, S., Vitale, N., Anouar, Y., Montero-Hadjadje, M., 2020. Chromogranin A preferential interaction with Golgi phosphatidic acid induces membrane deformation and contributes to secretory granule biogenesis. The FASEB Journal: official publication of the Federation of American Societies for Experimental Biology 34, 6769–6790. https://doi.org/10.1096/fj.202000074R

Caumont, A.S., Galas, M.C., Vitale, N., Aunis, D., Bader, M.F., 1998. Regulated exocytosis in chromaffin cells. Translocation of ARF6 stimulates a plasma membrane-associated phospholipase D. The Journal of Biological Chemistry 273, 1373–1379. https://doi.org/10.1074/jbc.273.3.1373

Chernomordik, L., Chanturiya, A., Green, J., Zimmerberg, J., 1995. The hemifusion intermediate and its conversion to complete fusion: regulation by membrane composition. Biophysical Journal 69, 922–929. https://doi.org/10.1016/S0006-3495(95)79966-0

Chernomordik, L.V., Vogel, S.S., Sokoloff, A., Onaran, H.O., Leikina, E.A., Zimmerberg, J., 1993. Lysolipids reversibly inhibit Ca(2+)-, GTP- and pH-dependent fusion of biological membranes. FEBS letters 318, 71–76. https://doi.org/10.1016/0014-5793(93)81330-3

Choi, W.S., Kim, Y.M., Combs, C., Frohman, M.A., Beaven, M.A., 2002. Phospholipases D1 and D2 regulate different phases of exocytosis in mast cells. Journal of Immunology 168, 5682–5689. https://doi.org/10.4049/jimmunol.168.11.5682

Cornell, R.B., Arnold, R.S., 1996. Modulation of the activities of enzymes of membrane lipid metabolism by non-bilayer-forming lipids. Chemistry and Physics of Lipids, The Properties and Biological Roles of Non-Lamellar Forming Lipids 81, 215–227. https://doi.org/10.1016/0009-3084(96)02584-4

Corrotte, M., Chasserot-Golaz, S., Huang, P., Du, G., Ktistakis, N.T., Frohman, M.A., Vitale, N., Bader, M.-F., Grant, N.J., 2006. Dynamics and function of phospholipase D and phosphatidic acid during phagocytosis. Traffic 7, 365–377. https://doi.org/10.1111/j.1600-0854.2006.00389.x

Disse, J., Vitale, N., Bader, M.-F., Gerke, V., 2009. Phospholipase D1 is specifically required for regulated secretion of von Willebrand factor from endothelial cells. Blood 113, 973–980. https://doi.org/10.1182/blood-2008-06-165282

Du, G., Altshuller, Y.M., Vitale, N., Huang, P., Chasserot-Golaz, S., Morris, A.J., Bader, M-F., Frohman, M.A. 2003. Regulation of phospholipase D1 subcellular cycling through coordination of multiple membrane association motifs. J. Cell Biol. 162, 305-315. https://doi.org/10.1083/jcb.200302033.

Frohman, M.A., 2015. The phospholipase D superfamily as therapeutic targets. Trends in Pharmacological Sciences 36, 137–144. https://doi.org/10.1016/j.tips.2015.01.001

Gabel, M., Delavoie, F., Demais, V., Royer, C., Bailly, Y., Vitale, N., Bader, M.-F., Chasserot-Golaz, S., 2015. Annexin A2-dependent actin bundling promotes secretory granule docking to the plasma membrane and exocytosis. Journal of Cellular Biology 210, 785–800. https://doi.org/10.1083/jcb.201412030

Gabel, M., Delavoie, F., Royer, C., Tahouly, T., Gasman, S., Bader, M.-F., Vitale, N., Chasserot-Golaz, S., 2019. Phosphorylation cycling of Annexin A2 Tyr23 is critical for calcium-regulated exocytosis in neuroendocrine cells. Biochimica et Biophysica Acta Molecular Cell Research 1866, 1207–1217. https://doi.org/10.1016/j.bbamcr.2018.12.013

Galas, M.-C., Helms, J.B., Vitale, N., Thiersé, D., Aunis, D., Bader, M.-F., 1997. Regulated Exocytosis in Chromaffin Cells A potential role for a secretory granule-associated ARF6 protein. Journal of Biological Chemistry 272, 2788–2793. https://doi.org/10.1074/jbc.272.5.2788

Gasman, S., Chasserot-Golaz, S., Malacombe, M., Way, M., Bader, M.-F., 2004. Regulated exocytosis in neuroendocrine cells: a role for subplasmalemmal Cdc42/N-WASP-induced actin filaments. Molecular Biology of the Cell 15, 520–531. https://doi.org/10.1091/mbc.e03-06-0402

Ghossoub, R., Lembo, F., Rubio, A., Gaillard, C.B., Bouchet, J., Vitale, N., Slavík, J., Machala, M., Zimmermann, P., 2014. Syntenin-ALIX exosome biogenesis and budding into multivesicular bodies are controlled by ARF6 and PLD2. Nature Communications 5, 3477. https://doi.org/10.1038/ncomms4477

Giridharan, S.S.P., Cai, B., Vitale, N., Naslavsky, N., Caplan, S., 2013. Cooperation of MICAL-L1, syndapin2, and phosphatidic acid in tubular recycling endosome biogenesis. Molecular Biology of the Cell 24, 1776–1790, S1-15. https://doi.org/10.1091/mbc.E13-01-0026

Hodgkin, M.N., Pettitt, T.R., Martin, A., Michell, R.H., Pemberton, A.J., Wakelam, M.J., 1998. Diacylglycerols and phosphatidates: which molecular species are intracellular messengers? Trends in Biochemical Sciences 23, 200–204. https://doi.org/10.1016/s0968-0004(98)01200-6

Holland, P., Knævelsrud, H., Søreng, K., Mathai, B.J., Lystad, A.H., Pankiv, S., Bjørndal, G.T., Schultz, S.W., Lobert, V.H., Chan, R.B., Zhou, B., Liestøl, K., Carlsson, S.R., Melia, T.J., Di Paolo, G., Simonsen, A., 2016. HS1BP3 negatively regulates autophagy by modulation of phosphatidic acid levels. Nature Communications 7, 13889. https://doi.org/10.1038/ncomms13889

Hoshino, F., Murakami, C., Sakai, H., Satoh, M., Sakane, F., 2019. Creatine kinase muscle type specifically interacts with saturated fatty acid- and/or monounsaturated fatty acid-containing phosphatidic acids. Biochemical and Biophysical Research Communications 513, 1035–1040. https://doi.org/10.1016/j.bbrc.2019.04.097

Hoshino, F., Sakane, F., 2020. Polyunsaturated fatty acid-containing phosphatidic acids selectively interact with L-lactate dehydrogenase A and induce its secondary structural change and inactivation. Biochimica et Biophysica Acta. Molecular and Cell Biology of Lipids 1865, 158768. https://doi.org/10.1016/j.bbalip.2020.158768

Houy, S., Estay-Ahumada, C., Croisé, P., Calco, V., Haeberlé, A.-M., Bailly, Y., Billuart, P., Vitale, N., Bader, M.-F., Ory, S., Gasman, S., 2015. Oligophrenin-1 Connects Exocytotic Fusion to Compensatory Endocytosis in Neuroendocrine Cells. The Journal of Neuroscience. 35, 11045–11055. https://doi.org/10.1523/JNEUROSCI.4048-14.2015

Hughes, W.E., Elgundi, Z., Huang, P., Frohman, M.A., Biden, T.J., 2004. Phospholipase D1 regulates secretagogue-stimulated insulin release in pancreatic beta-cells. The Journal of Biological Chemistry 279, 27534–27541. https://doi.org/10.1074/jbc.M403012200

Humeau, Y., Vitale, N., Chasserot-Golaz, S., Dupont, J.L., Du, G., Frohman, M.A., Bader, M.F., Poulain, B., 2001. A role for phospholipase D1 in neurotransmitter release. Proceedings of the National Academy of Sciences of the United States of America 98, 15300–15305. https://doi.org/10.1073/pnas.261358698

Jenkins, G.M., Frohman, M.A., 2005. Phospholipase D: a lipid centric review. Cellular and Molecular Life Sciences 62, 2305–2316. https://doi.org/10.1007/s00018-005-5195-z

Kassas, N., Tanguy, E., Thahouly, T., Fouillen, L., Heintz, D., Chasserot-Golaz, S., Bader, M.-F., Grant, N.J., Vitale, N., 2017. Comparative Characterization of Phosphatidic Acid Sensors and Their Localization during Frustrated Phagocytosis. The Journal of Biological Chemistry 292, 4266–4279. https://doi.org/10.1074/jbc.M116.742346

Kim, S.-C., Wang, X., 2020. Phosphatidic acid: an emerging versatile class of cellular mediators. Essays in Biochemistry 64, 533–546. https://doi.org/10.1042/EBC20190089

Kooijman, E.E., Burger, K.N.J., 2009. Biophysics and function of phosphatidic acid: a molecular perspective. Biochimica et Biophysica Acta 1791, 881–888. https://doi.org/10.1016/j.bbalip.2009.04.001

Kooijman, E.E., Chupin, V., Fuller, N.L., Kozlov, M.M., de Kruijff, B., Burger, K.N.J., Rand, P.R., 2005. Spontaneous curvature of phosphatidic acid and lysophosphatidic acid. Biochemistry 44, 2097–2102. https://doi.org/10.1021/bi0478502

Ktistakis, N.T., Brown, H.A., Waters, M.G., Sternweis, P.C., Roth, M.G., 1996. Evidence that phospholipase D mediates ADP ribosylation factor-dependent formation of Golgi coated vesicles. Journal of Cell Biology 134, 295–306. https://doi.org/10.1083/jcb.134.2.295

Kulig, W., Korolainen, H., Zatorska, M., Kwolek, U., Wydro, P., Kepczynski, M., Róg, T., 2019. Complex Behavior of Phosphatidylcholine-Phosphatidic Acid Bilayers and Monolayers: Effect of Acyl Chain Unsaturation. Langmuir: the ACS journal of surfaces and colloids 35, 5944–5956. https://doi.org/10.1021/acs.langmuir.9b00381

Lam, A.D., Tryoen-Toth, P., Tsai, B., Vitale, N., Stuenkel, E.L., 2008. SNARE-catalyzed fusion events are regulated by Syntaxin1A-lipid interactions. Molecular Biology of the Cell 19, 485–497. https://doi.org/10.1091/mbc.e07-02-0148

Leung, D.W., 2001. The structure and functions of human lysophosphatidic acid acyltransferases. Frontiers in Bioscience: A Journal and Virtual Library 6, D944-953. https://doi.org/10.2741/leung

Li, P., Bademosi, A.T., Luo, J., Meunier, F.A., 2018. Actin Remodeling in Regulated Exocytosis: Toward a Mesoscopic View. Trends in Cell Biology 28, 685–697. https://doi.org/10.1016/j.tcb.2018.04.004

Lopez, C.I., Pelletán, L.E., Suhaiman, L., De Blas, G.A., Vitale, N., Mayorga, L.S., Belmonte, S.A., 2012. Diacylglycerol stimulates acrosomal exocytosis by feeding into a PKC- and PLD1-dependent positive loop that continuously supplies phosphatidylinositol 4,5-bisphosphate. Biochimica et Biophysica Acta 1821, 1186–1199. https://doi.org/10.1016/j.bbalip.2012.05.001

Malacombe, M., Ceridono, M., Calco, V., Chasserot-Golaz, S., McPherson, P.S., Bader, M.-F., Gasman, S., 2006. Intersectin-1L nucleotide exchange factor regulates secretory granule exocytosis by activating Cdc42. EMBO Journal 25, 3494–3503. https://doi.org/10.1038/sj.emboj.7601247

Manni, M.M., Tiberti, M.L., Pagnotta, S., Barelli, H., Gautier, R., Antonny, B., 2018. Acyl chain asymmetry and polyunsaturation of brain phospholipids facilitate membrane vesiculation without leakage. Elife 7. https://doi.org/10.7554/eLife.34394

Mima, J., Wickner, W., 2009. Complex lipid requirements for SNARE- and SNARE chaperone-dependent membrane fusion. Journal of Biological Chemistry 284, 27114–27122. https://doi.org/10.1074/jbc.M109.010223

Mizuno, S., Sasai, H., Kume, A., Takahashi, D., Satoh, M., Kado, S., Sakane, F., 2017. Dioleoyl-phosphatidic acid selectively binds to α-synuclein and strongly induces its aggregation. FEBS letters 591, 784–791. https://doi.org/10.1002/1873-3468.12592

Momboisse, F., Lonchamp, E., Calco, V., Ceridono, M., Vitale, N., Bader, M.-F., Gasman, S., 2009. betaPIX-activated Rac1 stimulates the activation of phospholipase D, which is associated with exocytosis in neuroendocrine cells. Journal of Cellular Science 122, 798–806. https://doi.org/10.1242/jcs.038109

Monck, J.R., Fernandez, J.M., 1994. The exocytotic fusion pore and neurotransmitter release. Neuron 12, 707–716. https://doi.org/10.1016/0896-6273(94)90325-5

Nelson, R.K., Frohman, M.A., 2015. Physiological and pathophysiological roles for phospholipase D. Journal of Lipid Research 56, 2229–2237. https://doi.org/10.1194/jlr.R059220

Oliveira, T.G., Chan, R.B., Tian, H., Laredo, M., Shui, G., Staniszewski, A., Zhang, H., Wang, L., Kim, T.-W., Duff, K.E., Wenk, M.R., Arancio, O., Di Paolo, G., 2010. Phospholipase d2 ablation ameliorates Alzheimer's disease-linked synaptic dysfunction and cognitive deficits. The Journal of Neuroscience 30, 16419–16428. https://doi.org/10.1523/JNEUROSCI.3317-10.2010

O'Luanaigh, N., Pardo, R., Fensome, A., Allen-Baume, V., Jones, D., Holt, M.R., Cockcroft, S., 2002. Continual Production of Phosphatidic Acid by Phospholipase D Is Essential for Antigen-stimulated Membrane Ruffling in Cultured Mast Cells. Molecular Biology of the Cell 13, 3730–3746. https://doi.org/10.1091/mbc.e02-04-0213

Pinot, M., Vanni, S., Pagnotta, S., Lacas-Gervais, S., Payet, L.-A., Ferreira, T., Gautier, R., Goud, B., Antonny, B., Barelli, H., 2014. Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins. Science 345, 693–697. https://doi.org/10.1126/science.1255288

Pokotylo, I., Kravets, V., Martinec, J., Ruelland, E., 2018. The phosphatidic acid paradox: Too many actions for one molecule class? Lessons from plants. Progress in Lipid Research 71, 43–53. https://doi.org/10.1016/j.plipres.2018.05.003

Raben, D.M., Barber, C.N., 2017. Phosphatidic acid and neurotransmission. Advances in Biological Regulation 63, 15–21. https://doi.org/10.1016/j.jbior.2016.09.004

Rathore, S.S., Liu, Y., Yu, H., Wan, C., Lee, M., Yin, Q., Stowell, M.H.B., Shen, J., 2019. Intracellular Vesicle Fusion Requires a Membrane-Destabilizing Peptide Located at the Juxtamembrane Region of the v-SNARE. Cell Reports 29, 4583-4592.e3. https://doi.org/10.1016/j.celrep.2019.11.107

Ren, L., Mellander, L.J., Keighron, J., Cans, A.-S., Kurczy, M.E., Svir, I., Oleinick, A., Amatore, C., Ewing, A.G., 2016. The evidence for open and closed exocytosis as the primary release mechanism. Quaterly Review of Biophysics 49, e12. https://doi.org/10.1017/S0033583516000081

Roth, E., Frohman, M.A., 2018. Proliferative and metastatic roles for Phospholipase D in mouse models of cancer. Adv Biol Regul. Jan;67:134-140. https://doi.org/10.1016/j.jbior.2017.11.004

Schmidt, A., Wolde, M., Thiele, C., Fest, W., Kratzin, H., Podtelejnikov, A.V., Witke, W., Huttner, W.B., Söling, H.D., 1999. Endophilin I mediates synaptic vesicle formation by transfer of arachidonate to lysophosphatidic acid. Nature 401, 133–141. https://doi.org/10.1038/43613

Schwarz, K., Natarajan, S., Kassas, N., Vitale, N., Schmitz, F., 2011. The synaptic ribbon is a site of phosphatidic acid generation in ribbon synapses. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 31, 15996–16011. https://doi.org/10.1523/JNEUROSCI.2965-11.2011

Shin, W., Arpino, G., Thiyagarajan, S., Su, R., Ge, L., McDargh, Z., Guo, X., Wei, L., Shupliakov, O., Jin, A., O'Shaughnessy, B., Wu, L.-G., 2020. Vesicle Shrinking and Enlargement Play Opposing Roles in the Release of Exocytotic Contents. Cell Reports 30, 421-431.e7. https://doi.org/10.1016/j.celrep.2019.12.044

Shin, W., Ge, L., Arpino, G., Villarreal, S.A., Hamid, E., Liu, H., Zhao, W.-D., Wen, P.J., Chiang, H.-C., Wu, L.-G., 2018. Visualization of Membrane Pore in Live Cells Reveals a Dynamic-Pore Theory Governing Fusion and Endocytosis. Cell 173, 934-945.e12. https://doi.org/10.1016/j.cell.2018.02.062

Su, W., Yeku, O., Olepu, S., Genna, A., Park, J.-S., Ren, H., Du, G., Gelb, M.H., Morris, A.J., Frohman, M.A., 2009. 5-Fluoro-2-indolyl des-chlorohalopemide (FIPI), a phospholipase D pharmacological inhibitor that alters cell spreading and inhibits chemotaxis. Molecular Pharmacology 75, 437–446. https://doi.org/10.1124/mol.108.053298

Sztacho, M., Sobol, M., Balaban, C., Escudeiro Lopes, S.E., Hozák, P. 2019. Nuclear phosphoinositides and phase separation: Important players in nuclear compartmentalization. Adv Biol Regul. 71:111-117. https://doi.org/0.1016/j.jbior.2018.09.009

Tabet, R., Moutin, E., Becker, J.A.J., Heintz, D., Fouillen, L., Flatter, E., Krężel, W., Alunni, V., Koebel, P., Dembélé, D., Tassone, F., Bardoni, B., Mandel, J.-L., Vitale, N., Muller, D., Le Merrer, J., Moine, H., 2016. Fragile X Mental Retardation Protein (FMRP) controls diacylglycerol kinase activity in neurons. Proceedings of the National Academy of Sciences of the United States of America 113, E3619-3628. https://doi.org/10.1073/pnas.1522631113

Tanguy, E., Carmon, O., Wang, Q., Jeandel, L., Chasserot-Golaz, S., Montero-Hadjadje, M., Vitale, N., 2016. Lipids implicated in the journey of a secretory granule: from

biogenesis to fusion. Journal of Neurochemistry 137, 904–912. https://doi.org/10.1111/jnc.13577

Tanguy, E., Costé de Bagneaux, P., Kassas, N., Ammar, M.-R., Wang, Q., Haeberlé, A.-M., Raherindratsara, J., Fouillen, L., Renard, P.-Y., Montero-Hadjadje, M., Chasserot-Golaz, S., Ory, S., Gasman, S., Bader, M.-F., Vitale, N., 2020. Mono- and Poly-unsaturated Phosphatidic Acid Regulate Distinct Steps of Regulated Exocytosis in Neuroendocrine Cells. Cell Reports 32, 108026. https://doi.org/10.1016/j.celrep.2020.108026

Tanguy, E., Kassas, N., Vitale, N., 2018. Protein–Phospholipid Interaction Motifs: A Focus on Phosphatidic Acid. Biomolecules 8. https://doi.org/10.3390/biom8020020

Tanguy, E., Tran Nguyen, A.P., Kassas, N., Bader, M.-F., Grant, N.J., Vitale, N., 2019a. Regulation of Phospholipase D by Arf6 during FcγR-Mediated Phagocytosis. Journal of Immunology 202, 2971–2981. https://doi.org/10.4049/jimmunol.1801019

Tanguy, E., Wang, Q., Moine, H., Vitale, N., 2019b. Phosphatidic Acid: From Pleiotropic Functions to Neuronal Pathology. Frontiers in Cellular Neuroscience 13, 2. https://doi.org/10.3389/fncel.2019.00002

Vance, D.E., Goldfine, H., 2002. Konrad Bloch--a pioneer in cholesterol and fatty acid biosynthesis. Biochemical and Biophysical Research Communications 292, 1117–1127. https://doi.org/10.1006/bbrc.2002.2031

Vance, J.E., Vance, D.E., 2004. Phospholipid biosynthesis in mammalian cells. Biochemistry and Cell Biology 82, 113–128. https://doi.org/10.1139/o03-073

Varela Chavez, C., Hoos, S., Haustant, G.M., Chenal, A., England, P., Blondel, A., Pauillac, S., Lacy, D.B., Popoff, M.R., 2015. The catalytic domains of *C lostridium sordellii* lethal toxin and related large clostridial glucosylating toxins specifically recognize the negatively charged phospholipids phosphatidylserine and phosphatidic acid: LCGT catalytic domains specifically bind to anionic phospholipids. Cellular Microbiology 17, 1477–1493. https://doi.org/10.1111/cmi.12449

Vaughan, E.M., You, J.-S., Elsie Yu, H.-Y., Lasek, A., Vitale, N., Hornberger, T.A., Bement, W.M., 2014. Lipid domain-dependent regulation of single-cell wound repair. Molecular Biology of the Cell 25, 1867–1876. https://doi.org/10.1091/mbc.E14-03-0839

Vitale, N., 2010. Synthesis of fusogenic lipids through activation of phospholipase D1 by GTPases and the kinase RSK2 is required for calcium-regulated exocytosis in neuroendocrine cells. Biochemical Society Transactions 38, 167–171. https://doi.org/10.1042/BST0380167

Vitale, N., Caumont, A.S., Chasserot-Golaz, S., Du, G., Wu, S., Sciorra, V.A., Morris, A.J., Frohman, M.A., Bader, M.F., 2001. Phospholipase D1: a key factor for the exocytotic machinery in neuroendocrine cells. EMBO Journal 20, 2424–2434. https://doi.org/10.1093/emboj/20.10.2424

Vitale, N., Mawet, J., Camonis, J., Regazzi, R., Bader, M.-F., Chasserot-Golaz, S., 2005. The Small GTPase RalA controls exocytosis of large dense core secretory granules by interacting with ARF6-dependent phospholipase D1. Journal of Biological Chemistry 280, 29921–29928. https://doi.org/10.1074/jbc.M413748200

Waselle, L., Gerona, R.R.L., Vitale, N., Martin, T.F.J., Bader, M.-F., Regazzi, R., 2005. Role of phosphoinositide signaling in the control of insulin exocytosis. Molecular Endocrinology 19, 3097–3106. https://doi.org/10.1210/me.2004-0530

Yang, Y., Lee, M., Fairn, G.D., 2018. Phospholipid subcellular localization and dynamics. The Journal of Biological Chemistry 293, 6230–6240. https://doi.org/10.1074/jbc.R117.000582

Zegarlińska, J., Piaścik, M., Sikorski, A.F., Czogalla, A., 2018. Phosphatidic acid - a simple phospholipid with multiple faces. Acta Biochimica Polonica 65, 163–171. https://doi.org/10.18388/abp.2018_2592

Zeniou-Meyer, M., Liu, Y., Béglé, A., Olanich, M.E., Olanish, M., Hanauer, A., Becherer, U., Rettig, J., Bader, M.-F., Vitale, N., 2008. The Coffin-Lowry syndrome-associated protein RSK2 is implicated in calcium-regulated exocytosis through the regulation of PLD1. Proceedings of the National Academy of Sciences of the United States of America 105, 8434–8439. https://doi.org/10.1073/pnas.0710676105

Zeniou-Meyer, M., Zabari, N., Ashery, U., Chasserot-Golaz, S., Haeberlé, A.-M., Demais, V., Bailly, Y., Gottfried, I., Nakanishi, H., Neiman, A.M., Du, G., Frohman, M.A., Bader, M.-F., Vitale, N., 2007. Phospholipase D1 production of phosphatidic acid at the plasma membrane promotes exocytosis of large dense-core granules at a late stage. Journal of Biological Chemistry 282, 21746–21757. https://doi.org/10.1074/jbc.M702968200

Figure 1

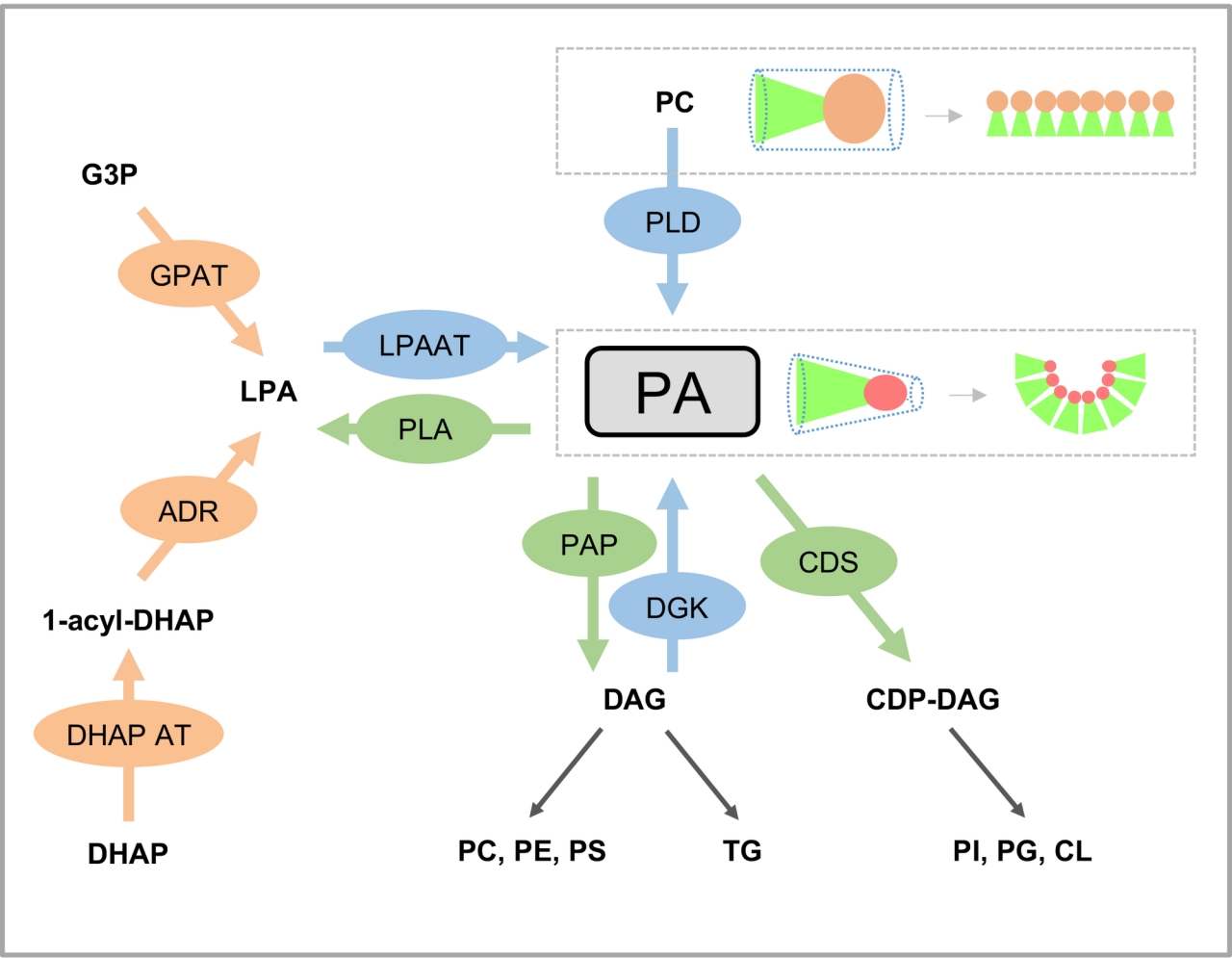
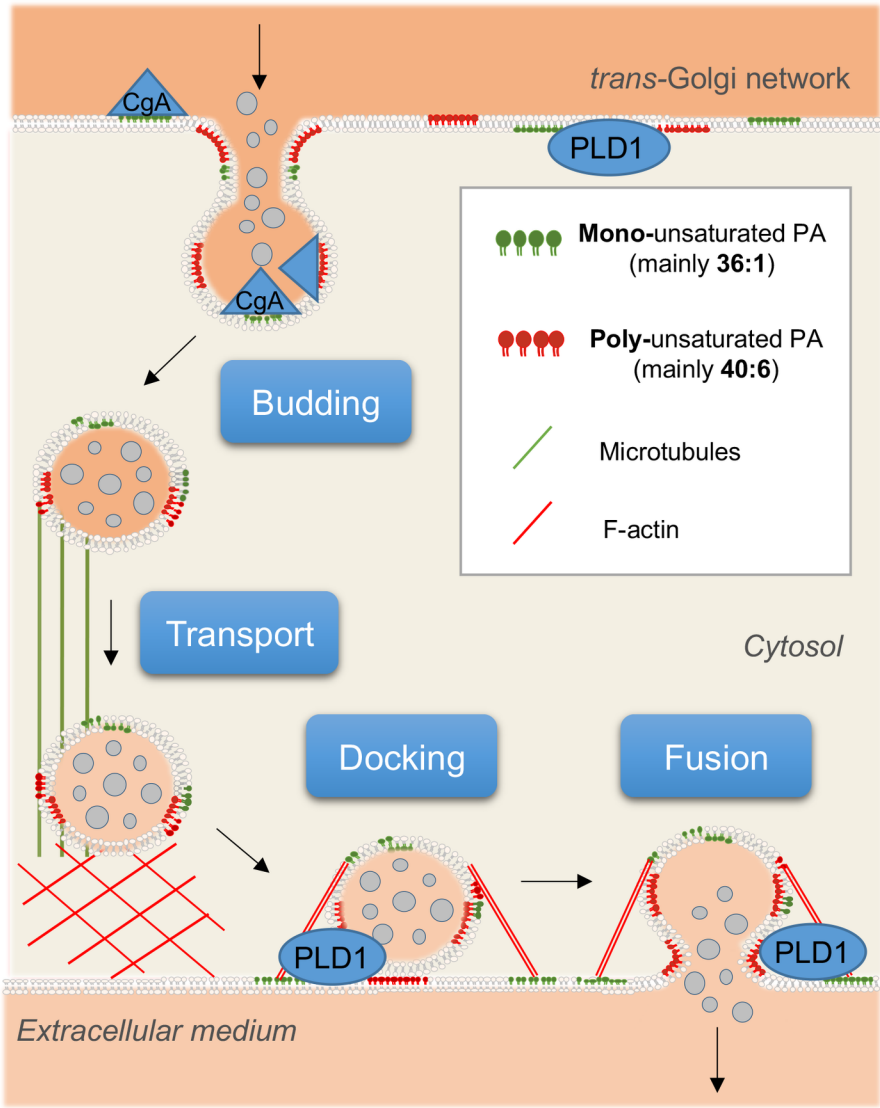

PC
PLD
G3P
GPAT
LPAAT
LPA
PA
PLA
ADR
PAP
DGK
CDS
1-acyl-DHAP
DAG
CDP-DAG
DHAP AT
DHAP
PC, PE, PS
TG
PI, PG, CL

Figure 2


trans-Golgi network
CgA
CgA
PLD1
Budding
Transport
Docking
Fusion
Cytosol
Extracellular medium
PLD1
PLD1
Mono-unsaturated PA
(mainly 36:1)
Poly-unsaturated PA
(mainly 40:6)
Microtubules
F-actin