Phospholipase D signaling: orchestration by PIP2 and small GTPases

Paschal A. Oude Weernink · Maider López de Jesús · Martina Schmidt

Received: 12 October 2006 / Accepted: 20 December 2006 / Published online: 24 January 2007
© Springer-Verlag 2007

Abstract Hydrolysis of phosphatidylcholine by phospholipase D (PLD) leads to the generation of the versatile lipid second messenger, phosphatidic acid (PA), which is involved in fundamental cellular processes, including membrane trafficking, actin cytoskeleton remodeling, cell proliferation and cell survival. PLD activity can be dramatically stimulated by a large number of cell surface receptors and is elaborately regulated by intracellular factors, including protein kinase C isoforms, small GTPases of the ARF, Rho and Ras families and, particularly, by the phosphoinositide, phosphatidylinositol 4,5-bisphosphate (PIP2). PIP2 is well known as substrate for the generation of second messengers by phospholipase C, but is now also understood to recruit and/or activate a variety of actin regulatory proteins, ion channels and other signaling proteins, including PLD, by direct interaction. The synthesis of PIP2 by phosphoinositide 5-kinase (PIP5K) isoforms is tightly regulated by small GTPases and, interestingly, by PA as well, and the concerted formation of PIP2 and PA has been shown to mediate receptor-regulated cellular events. This review highlights the regulation of PLD by membrane receptors, and describes how the close encounter of PLD and PIP5K isoforms with small GTPases permits the execution of specific cellular functions.

Keywords Phospholipase D · Phosphatidic acid · PIP2 · Phosphoinositide 5-kinase · ARF · Rho · Ras

Introduction

The activation of membrane receptors by hormones and growth factors results in the localized generation of intracellular second messengers. The hydrolysis of membrane phospholipids and the generation of biologically active products play important roles in the regulation of cell function and cell fate. Well known is the activation of phosphoinositide-specific phospholipase C (PLC) isoforms, which hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2), a membrane phospholipid found in all eukaryotic cells (Schmidt et al. 2004). Stimulation of PLC isoforms plays a major role in many early and late cellular responses to receptor activation, including smooth muscle contraction, secretion and neuronal signaling as well as fertilization, cell growth and differentiation (Berridge 2005; Nishizuka 2003). Phospholipase D (PLD) was first described 60 years ago as a distinct, phospholipid-specific phosphodiesterase activity in cabbage leaves (Hanahan and Chaikoff 1948). This pioneering research indicated that PLD hydrolyzes phosphatidylcholine to yield phosphatidic acid (PA) and choline. The recognition that PLD is rapidly and dramatically activated in response to extracellular stimuli in cultured animal cells, now 20 years ago (Bocckino et al. 1987; Cockcroft 1984), has brought PLD signaling to the very forefront of current biological and biomedical research. Meanwhile, phosphatidylcholine-hydrolyzing PLD has been identified in bacteria, protozoa, fungi, plants and animals, and, due to this widespread distribution, is assumed to be involved in the regulation of fundamental cellular functions. Indeed, it has now been established that activation
of PLD and the generation of PA by a vast number of membrane receptors modulate such a wide array of cellular responses as calcium mobilization, secretion, superoxide production, endocytosis, exocytosis, vesicle trafficking, glucose transport, rearrangements of the actin cytoskeleton, mitogenesis and survival (Cockcroft 2001; Exton 2002b; Jenkins and Frohman 2005; Liscovitch et al. 2000).

PIP2 is a critical cofactor for PLD, and profoundly affects the activity, membrane localization and receptor activation of both PLD isoforms, PLD1 and PLD2 (Brown et al. 1993; Hodgkin et al. 2000; Liscovitch et al. 1994; Pertile et al. 1995; Schmidt et al. 1996d). Thus, reduction of cellular PIP2 levels, for instance via scavenging of PIP2 by the actin-binding protein fodrin (Lukowski et al. 1998) or via forced PIP2 hydrolysis by the phosphatase synaptojanin (Chung et al. 1997), has been shown to inhibit PLD activity. Vice versa, the synthesis of PIP2 by phosphoinositide 5-kinase (PIP5K) isoforms can be directly stimulated by the PLD product PA (Jenkins et al. 1994; Moritz et al. 1992), and this regulation has also been confirmed to occur at the whole cell level (Divecha et al. 2000; Jones et al. 2000b; Skippen et al. 2002). It is now hypothesized that the reciprocal stimulation of PLD and PIP5K enzymes enables rapid feed-forward stimulation loops for a localized and explosive generation of PA and PIP2, which may then govern the recruitment and activation of proteins to execute specific cellular tasks, especially membrane trafficking, and changes in the organization of the actin cytoskeleton. The activity and localization of both PLD and PIP5K are under control of GTPases of the Arf and Rho families, which are well-defined regulators of membrane transport and actin-reorganization processes. The reciprocal stimulation of PIP5K and PLD, and the regulation of these enzymes by ARF and Rho GTPases, point to concerted mechanisms in cellular actions, involving acute, localized PIP2 and PA synthesis (Fig. 1). This review will focus on the regulation of PLD enzymes by membrane receptors and monomeric GTPases, and on how PLD signaling is organized and connected by PIP2 metabolism.

**Phosphatidic acid and PLD isoforms**

Most cellular responses following PLD activation are probably mediated by the immediate reaction product PA. PA is a multifunctional lipid that can be further metabolized to the bioactive lipids, lysophosphatidic acid (LPA) and diacylglycerol (DAG), can by itself alter membrane curvature, and can serve as a protein attachment site and affect both cellular localization and activity of various proteins, including Raf-1 kinase, protein phosphatase 1, sphingosine kinase 1, and mTOR (mammalian target of rapamycin), a key regulator of cell growth and proliferation (Jenkins and Frohman 2005). PLD enzymes can catalyze a transphosphatidylation reaction in which the phosphatidyl moiety of phosphatidylcholine is accepted by primary...
alcohols, thereby producing stable phosphatidylalcohol instead of PA. This transphosphatidylation reaction is widely applied to measure PLD activity in biological samples, and quenching of PA synthesis by primary alcohols has proven extremely helpful to identify the involvement of PLD enzymes in cell physiology. In this way, a role for PLD has been demonstrated in a variety of signaling processes, such as activation of phosphoinositide (PI3K, PIP5K) and protein (Akt, ERK1/2) kinases, calcium mobilization, cytoskeleton remodeling, endocytosis, exocytosis, membrane trafficking, superoxide production, glucose transport, cell migration, cell proliferation, and survival signaling (Exton 2002a; Foster and Xu 2003).

There are two mammalian PLD genes, PLD1 and PLD2. PLD1 has a low basal activity and is extensively regulated by conventional protein kinase C (PKC, -β, -γ) isozymes and small GTPases of the ARF (ARF1 - ARF6) and Rho (RhoA, Rac1, Cdc42) families (Henage et al. 2006). PLD2 has a higher basal activity than PLD1, but has been shown to respond to ARF and PKC as well (Chen and Exton 1998). PLD2 has a low basal activity and is extensively regulated on PIP2 for activity. Experiments utilizing inactive PLD mutants and RNA interference have discriminated isoform-specific PLD functions, and showed that PLD1 is involved in agonist-induced secretion, actin organization, and cell adhesion and migration (Exton 2002a; Iyer et al. 2006; Kim et al. 2006; Vitale et al. 2001), and PLD2 in endocytosis and recycling of membrane receptors (Du et al. 2004; Koch et al. 2006; Padrón et al. 2006).

The PLD isoforms, both with two splice variants, share an ~50% amino-acid sequence identity (Colley et al. 1997; Hammond et al. 1995, 1997; Steed et al. 1998). The catalytic core of both PLD enzymes are composed of four conserved domains (domain I-IV), and the HKD motifs in the domains II and IV probably associate together to form a catalytic centre (Xie et al. 2000). PLD1 is characterized by a 116-amino acid loop region following domain II, which has been proposed to function as a negative regulatory element (Sung et al. 1999). PLD1 and PLD2 further possess N-terminal PH (pleckstrin homology) and PX (phox homology) domains. PLP2 binds to the PH domain (Hodgkin et al. 2000), but also to a polybasic PIP2 binding motif within the catalytic core (Sciorra et al. 1999), and interaction of PIP2 with both domains has been suggested to be involved in membrane targeting of PLD as well as stimulation of PLD catalytic activity (Du et al. 2003; Hodgkin et al. 2000; Sciorra et al. 2002). The PX domain of PLD1 has been reported to preferentially bind to phosphatidylinositol-3,4,5-trisphosphate (PIP3) (Lee et al. 2005; Stahelin et al. 2004), but interaction with PISP has been observed as well (Du et al. 2003). Recently, it was shown that the PX domain of PLD has GTPase-activating protein (GAP) activity towards dynamin, and that PLD supports EGF receptor endocytosis (Lee et al. 2006). The PH and PX domains probably contribute to the proper localization of the PLD enzymes within cells. In line with a role for PLD enzymes in different cellular tasks, PLD1 and PLD2 show a diverse subcellular distribution. PLD1 is found throughout the cell, but primarily localizes to perinuclear endosomes and the Golgi apparatus (Brown et al. 1998; Freyberg et al. 2001; Hughes and Parker 2001). PLD2 is almost exclusively present at the plasma membrane in lipid raft fractions (Czarny et al. 1999). The localization of PLD1 does not seem to be static, and regulated translocation and recycling of the enzyme between cellular compartments may be crucial to its proper functioning. In an elegant study, coordinated subcellular targeting of the lipid binding motifs has been demonstrated to drive this subcellular cycling of PLD1 (Du et al. 2003). Upon stimulation, PLD1 was found to translocate from the intracellular compartments to the plasma membrane, and this process was probably dependent on the polybasic PIP2 binding site. The PH domain then facilitated entry of PLD1 into lipid rafts, a step critical for internalization of the enzyme, whereafter interaction of the PX domain with PISP may control the efficient return of PLD1 to the endosomes.

**PIP2 and PIP5K isoforms**

PIP2 is an essential and versatile factor in cellular signaling. Hydrolysis of PIP2 by PLC into the second messengers, inositol-1,4,5-trisphosphate (IP3) and DAG, is a general and well-defined answer of cells in response to stimulation of many membrane receptors (Schmidt et al. 2004). Phosphorylation of PIP2 by PI3K results in the rapid accumulation of PIP3, which recruits and activates mediators involved in actin remodeling, mitogenesis and survival (Vanhaesebroeck et al. 2001). But it is now recognized that PIP2, as well as other phosphoinositides, are signaling molecules by themselves and can, by binding to unique phosphoinositide-binding sequences, such as the PH and PX domains, affect the activity and subcellular localization of many proteins, including many actin regulatory proteins, a wide range of ion channels, and PLD (Niggli 2005; Suh and Hille 2005; Yin and Janmey 2003). In this way, PIP2 can modulate a remarkable variety of cellular processes, including cortical actin organization, membrane ruffling, vesicle trafficking, gene expression, cell migration and cell survival (Ling et al. 2006; Oude Weernink et al. 2004b; Toker 2002). Subsequent dephosphorylation of PIP2 by inositol polyphosphate 5-phosphatases, such as synaptojanin, is believed to terminate local PIP2 signaling, for instance in the process of vesicle trafficking (Majerus et al. 1999).
To execute this variety of functions, PIP2 may be organized in discrete functional pools within cells, but the existence of PIP2 clusters in the plasma membrane is currently under debate. Using green fluorescent protein-tagged PH domains or antibodies to visualize PIP2, the lipid was found to concentrate in highly dynamic, actin-rich regions (Tall et al. 2000) and lipid rafts (Laux et al. 2000; Parmyr et al. 2003) in the plasma membrane, feeding the idea that spatially organized PIP2 synthesis regulates actin polymerization and other cellular processes. The localization of PIP2 in rafts is supported by biochemical data (Pike and Casey 1996); however, specific PIP2 clustering has been disputed (van Rheezen et al. 2005).

PIP2 is generated after phosphorylation of phosphatidylinositol-4-phosphate by PIP5K. In mammals, cDNAs encoding three isoforms of PIP5K (designated Iα, Iβ and Iγ) with alternative splice variants have been cloned and characterized (Ishihara et al. 1996, 1998; Loijens and Anderson 1996). Sequence analysis has shown that PIP5K enzymes are related to PIP4K enzymes, but that they share no identity with most other lipid (PI3K and PI4K) or protein kinases. The sequence similarity between the PIP4Ks and PIP5Ks is clustered in the catalytic core of the kinases (Anderson et al. 1999; Hinchliffe et al. 1998). An activation loop spanning the catalytic domain has been shown to determine both substrate specificity and subcellular targeting of PIP5Ks, which can be swapped by substitution of a single amino acid within this loop (Kunz et al. 2002). In murine PIP5K-Iβ, two dimerization domains were identified, which may contribute to the proper subcellular localization and functioning of the enzyme (Galiano et al. 2002).

The identification of three PIP5K isoforms raised the expectation of a differential regulation of the enzymes by cellular signal transduction components, but up to now the regulatory properties of PIP5K-Iα, Iβ and Iγ appear to be remarkably similar. All PIP5K isoforms are stimulated by PA, are extensively regulated by ARF and Rho GTPases, remarkably similar. All PIP5K isoforms are stimulated by membrane ruffles (Doughman et al. 2003) and may selectively control functional PIP2 pools, which may contribute to the proper subcellular localization and functioning of the enzyme (Galiano et al. 2002).

The execution of specific PIP2-modulated processes is very probably achieved by an orchestration of appropriate signaling partners within discrete subcellular microdomains, and PLD-derived PA as well as the PLD enzymes by themselves can contribute to this organization. Indeed, both PLD1 and PLD2 interact with PIP5K-Iα, and PLD2 recruits PIP5K-Iα to a submembranous vesicular compartment (Divecha et al. 2000). PLD2-derived PA was shown to stimulate PIP5K-Iγ splice variants, and the subsequent formation of PIP2 to drive the initial stages of integrin-mediated cellular adhesion (Powne et al. 2005). In many processes, the temporal activation and correct localization of PLD and PIP5K isoforms by monomeric GTPases appears crucial to achieve the spatially organized production of PIP2 and PA (Santarius et al. 2006).

**ARF GTPases and membrane traffic**

Although the direct interaction site on PLD for ARF has not yet been unequivocally defined, it is well established that ARF proteins, particularly ARF1 and ARF6, activate both PLD enzymes, but especially PLD1 (Hammond et al. 1995, 1997). ARF GTPases regulate intracellular vesicle trafficking and actin remodeling. ARF1 is localized to the Golgi complex, and is required for proper Golgi structure and function. The use of primary alcohols has also pointed to a role for PLD in vesicle transport to Golgi (Bi et al. 1997; Kitstakis et al. 1996). PLD activity has been shown to stimulate the release of nascent secretory vesicles from the trans-Golgi network (Chen et al. 1997), and to be required for maintaining the structural integrity and function of the Golgi apparatus, but the precise role for PLD in vesicle formation is still controversial. PIP5K is also a direct effector of ARF1, and an ARF1 mutant that selectively activates PIP5K, but not PLD activity, demonstrated that both PLD-derived PA and direct activation of PIP5K by ARF1 contribute to increased PIP2 synthesis (Skippen et al. 2002). In permeabilized cells, ARF1 has been shown to restore secretion by promoting PIP2 synthesis (Fensome et al. 1996), and ARF1-mediated PIP5K activation (Jones et al. 2000a) and recruitment to the Golgi complex (Godi et al. 1999) appears to be critical in Golgi functioning.

ARF6 regulates vesicular transport, secretion, and cortical actin reorganization. ARF6 activates PLD, and PA has been implicated in the mediation of the effects of ARF6 in vesicular trafficking events. A critical role for PLD1 in exocytosis has been established in different cell types.
including neurons (Humeau et al. 2001), neuroendocrine cells (Vitale et al. 2001) and pancreatic β cells (Hughes et al. 2004). PLD2 has recently emerged as a mediator of ARF-dependent internalization of the µ-opioid receptor (Koch et al. 2003), and both PLD isoforms have been implicated in macrophage phagocytosis (Corrotte et al. 2006; Iyer et al. 2004). In addition, PIP5K colocalizes and interacts with, and is directly activated by ARF6 at the plasma membrane (Honda et al. 1999), and ARF6 and PIP2 colocalize on the plasma membrane and on endosomal structures (Brown et al. 2001). ARF6-organized PIP2 turnover at the plasma membrane is apparently involved in regulated secretion (Aikawa and Martin 2003; Brown et al. 2001; Lawrence and Birnbaum 2003). Focal and transient accumulation of PIP2 by PIP5K is required for phagocytosis as well (Botelho et al. 2000; Coppolino et al. 2002; Wong and Isberg 2003), and PIP2 hydrolysis probably dictates the remodeling of actin (Paris et al. 2002). PLD2 has recently emerged as a mediator of ARF-membranes (Sweeney et al. 2002), blockade of clathrin-coat fusogenic lipid, PA. But PA also takes a function as an additional protein — PLD cleaves the non-fusogenic lipid, PC, to form the directly contribute to vesicle fusion in a biophysical manner, vesicle recycling (Krauss et al. 2003). PLD-derived PA may coat recruitment to synaptic membranes to allow synaptic PIP5K-Iγ initiate dense-core vesicle exocytosis. Direct activation of PIP5K-1γ by ARF6 has been shown to stimulate clathrin-coat recruitment to synaptic membranes to allow synaptic vesicle targeting and activation of both PIP5K and PLD (Galandrini et al. 2005). PIP2 also recruits additional proteins—for instance the endocytic proteins AP-2, epsin and AP180—to initiate clathrin-coat formation preceding endocytosis (Ford et al. 2001; Itoh et al. 2001; Padrón et al. 2003), and CAPS (Grishanin et al. 2004) to initiate dense-core vesicle exocytosis. Direct activation of PIP5K-1γ by ARF6 has been shown to stimulate clathrin-coat recruitment to synaptic membranes to allow synaptic vesicle recycling (Krauss et al. 2003). PLD-derived PA may directly contribute to vesicle fusion in a biophysical manner, as PLD cleaves the non-fusogenic lipid, PC, to form the fusogenic lipid, PA. But PA also takes a function as an essential cofactor for PIP5K, and disruption of Golgi membranes (Sweeney et al. 2002), blockade of clathrin-coat assembly (Arneson et al. 1999) and inhibition of ARF1-reconstituted secretion (Way et al. 2000) after quenching of PA production could be attributed to inhibited PIP2 synthesis. Thus, both PLD and PIP2 synthesis seem necessary for membrane trafficking aspects in the endo- and exocytotic machinery. But PLD and PIP5K also mediate other processes down-stream of ARF6. Epidermal growth factor (EGF)-induced membrane ruffling requires ARF6-induced PIP5K-1γ translocation to the ruffles and local PIP2 production. This leads to the recruitment of PLD2, and PLD-derived PA and ARF6 may then synergistically activate PIP5K (Honda et al. 1999).

The relationship between ARF and PIP2 is also bidirectional, as phosphoinositides can regulate ARF activity by binding and activating both ARF-specific guanine nucleotide exchange factors (ARF-GEFs) (Klarlund et al. 1998; Paris et al. 1997) and ARF-GTPase-activating proteins (ARF-GAPs) (Kam et al. 2000; Nie et al. 2002) via their PH domains. The fact that ARF-GAPs bind PIP2 with high affinity and specificity offers an attractive feed-back mechanism for terminating ARF activation after a cycle of ARF-induced PIP2 synthesis.

### Rho GTPases and actin dynamics

PA formation, especially by PLD1, has been reported to induce stress fibre formation in specific cell types (Cross et al. 1996; Ha and Exton 1993; Kam and Exton 2001; Porcelli et al. 2002). Rho proteins, in particular RhoA, Rac1 and Cdc42, which control actin cytoskeleton reorganization, exclusively activate PLD1 by direct interaction with its C-terminus (Exton 2002b; Powner and Wakelam 2002). Thus, PLD stimulation by RhoA may happen by direct interaction, but may involve indirect, Rho-dependent mechanisms as well. Inactivation of Rho GTPases, with *Clostridium difficile* toxin B or *Clostridium botulinum* C3 exoenzyme, reduced cellular PIP2 levels, resulting in inhibition of receptor-mediated PIP2 hydrolysis by PLC (Schmidt et al. 1996a) as well as diminished PLD stimulation (Schmidt et al. 1996d). As the inhibition of PLD signaling after Rho inactivation could be largely rescued by the addition of PIP2, Rho proteins do seem to affect PLD via PIP5K regulation (Schmidt et al. 1996c,d). PIP2 is well-known to associate with and regulate the activity of a plethora of actin-binding proteins that organize actin dynamics (Hilpela et al. 2004; Yin and Janmey 2003), and PA and PIP2 may act in concert to mediate Rho-dependent actin cytoskeleton remodeling. PIP5K isoforms are, like PLD, under direct control of Rho GTPases. PIP5K isoforms are markedly stimulated by RhoA, Rac1, and Cdc42 (Chong et al. 1994; Hartwig et al. 1995; Oude Weernink et al. 2004a), and physically associate with both RhoA (Ren et al. 1996) and Rac1 (Tolias et al. 2000), but not with Cdc42 (Oude Weernink et al. 2004a; van Hennik et al. 2003). PIP5K isoforms are now seen as critical mediators of RhoA- and Rac1-induced actin organization and remodeling (Doughman et al. 2003; Shibasaki et al. 1997; Tolias et al. 2000). The established Rho effector Rho-kinase, a serine/threonine kinase, is apparently involved in Rho-dependent regulation of both PLD (Schmidt et al. 1999) and PIP5K activities (Oude Weernink et al. 2000), and PIP5K was found to play an essential role as downstream effector of Rho and Rho-kinase in neurite remodeling (van Horck et al. 2002; Yamazaki et al. 2002) and platelet cytoskeleton assembly (Gratacap et al. 2001; Yang et al. 2004). But Rho may also directly signal to PIP5K
independently of Rho-kinase, as RhoA-induced activation of ERM (ezrin, radixin, moesin) proteins, that cross-link actin filaments to plasma membranes, was found to be mediated by PIP5K, but not by Rho-kinase (Matsui et al. 1999). PLD and PIP5K were also demonstrated to collectively mediate Rho-induced changes in the actin cytoskeleton. Thus, myogenic differentiation induced by arginine-vasopressin, which involves actin fiber formation, is mediated by Rho proteins and PLD1, and involves PLD-induced PIP2 synthesis along the actin fibers (Komati et al. 2005). These findings suggest that PLD and PIP5K enzymes may co-operate down-stream of Rho in processes that depend on actin organization.

Another Rho effector, PKC-related protein kinase N (PKN), also directly interacts with PLD (Oishi et al. 2001) and mediates PLD activation by the α1-adrenergic receptor (Parmentier et al. 2002). Interestingly, components of the actin regulatory machinery, β-actin and α-actinin, have been found to directly associate with and inhibit the activity of PLD isoforms (Lee et al. 2001; Park et al. 2000). PLD also binds to and is stimulated by filamentous F-actin, and PLD1 in particular may act as a signal transduction component responsive to dynamic changes of the actin cytoskeleton (Kusner et al. 2002). PKN interacts with α-actinin, and PKN may modulate PLD signaling by reversing the inhibitory effect of α-actinin on PLD1, and by direct interaction with PLD1.

**Regulation of PLD and PIP5K by membrane receptors**

In line with the critical role of PA in cellular processes, the enzymatic activity of PLD is tightly regulated by a variety of hormones, neurotransmitters, and growth factors. Regulation of PLD enzymes by membrane receptors, including G protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs), is complex and mediated by several cytosolic factors, including PKC as well as ARF, Rho and Ras GTPases (Exton 2002b; Liscovitch et al. 2000; López De Jesús et al. 2006; Powner and Wakeland 2002). Most receptors that stimulate PLD also increase PLC activity, leading to activation of the PLD regulator PKC, and it was assumed that PLD activation might be secondary to PLC activation. A physical association between PLD with PKC isoforms has been reported, resulting in strong activation of in vitro PLD1 activity, and the major interaction site was identified within the N-terminus of PLD1 (Park et al. 1998). Indeed, inhibition of PKC was shown to reduce receptor-induced PLD responses, and PLD1 mutants unresponsive to PKC did respond poorly to activation of GPCRs (Zhang et al. 1999) or to active Gαq proteins (Xie et al. 2002). However, stimulation of PLD in several receptor systems, including M3 muscarinic and α1-adrenergic receptors, was actually PKC-independent (Balboa and Insel 1998; Muthalif et al. 2000; Rümenapp et al. 1997; Schmidt et al. 1994), suggesting that PLD stimulation must not necessarily be secondary to PLC stimulation.

Brefeldin A, an inhibitor of certain ARF-GEFs, reduced receptor signaling to PLD in several cell types, indicating that ARF proteins participate in receptor-mediated PLD stimulation (Fensome et al. 1998; Mitchell et al. 1998; Rümenapp et al. 1995; Shome et al. 2000). Likewise, sequestration of ARF-GEFs by the ARF-related protein ARP inhibited M3 muscarinic receptor signaling to PLD (Schäffirnann et al. 1999). Clostridial toxins and enzymes that specifically inactivate Rho proteins and expression of inactive Rho mutants have been used to identify the role of Rho in signaling to PLD. Thus, Rho proteins were found to be involved in PLD stimulation by GPCRs (M3 muscarinic, bradykinin, sphingosine-1-phosphate and LPA), RTKs (PDGF, EGF), and immunoglobulin (FcεRI) receptors (Hess et al. 1997; Ojio et al. 1996; Schmidt et al. 1996c).

Stimulation of PLD by GPCRs was shown to be mediated by both pertussis toxin (PTX)-insensitive (Gosau et al. 2002; Schmidt et al. 1994) and PTX-sensitive (Cummings et al. 2002; Fensome et al. 1998) heterotrimeric G proteins. G12 family proteins can stimulate PLD (Plonk et al. 1998), and RGS (regulators of G protein signaling) proteins, that act as α subunit-specific GAPs, have been used to position G12 in PLD activation by the M3 muscarinic (Rümenapp et al. 2001), the PAR1 (Fahimi-Vahid et al. 2002), and the Ca2+-sensing receptor (Huang et al. 2004), as well as mechanical force (Ziembicki et al. 2005). As forskolin and cAMP were shown to cause activation of PLD via PKA and ERK1/2 (Ginsberg et al. 1997; Yoon et al. 2005) or, alternatively, via the cAMP-activated GEF for Ras-like GTTPases, Epac and R-Ras (López De Jesús et al. 2006), G proteins also mediate stimulation of PLD. PLD activation is also controlled by βγ-subunits, possibly via Src and/or ARF6 (Le Stunff et al. 2000; Usbho-Fukai et al. 1999), but Gβγ can also directly interact with and inhibit PLD (Preiminger et al. 2006).

As the precise mechanism of PLD stimulation in intact cells was only poorly understood, during the last 10 years our laboratory in Essen has focused on the regulation of PLD activity by membrane receptors. In HEK-293 cells, signaling to PLD by a typical GPCR, the M3 muscarinic receptor, and an RTK, the EGF receptor, was studied and shown to be executed by several distinct pathways (Fig. 2). In addition, by expressing inactive PLD mutants, the M3 muscarinic and the EGF receptors were found to signal to individual PLD isoforms and to selectively stimulate PLD1 and PLD2 respectively (Han et al. 2001). The M3 muscarinic receptor stimulates both PLC and PLD via PTX-insensitive mechanisms (Offermanns et al. 1994; Peralta et al. 1988; Schmidt et al. 1994). Interestingly, stimulation of PLD by the agonist carbachol was not affected by PKC inhibitors, suggesting
that activation of PLD by the M₃ muscarinic receptor was rather independent of PLC (Rümenapp et al. 1997; Schmidt et al. 1994). Expression of α-subunits of G proteins and of specific RGS proteins was used to identify the G proteins involved in these pathways, and demonstrated that whereas the M₃ receptor signals to PLC via Gq proteins, activation of PLD is mediated by G₁₂ family proteins (Rümenapp et al. 2001). PLD activation by the M₃ receptor, but not by the EGF receptor, was further found to be under control of ARF (Rümenapp et al. 1995, 1997) as well as Rho proteins, particularly RhoA (Schmidt et al. 1996c,d). Likewise, regulation of mTOR by LPA, but not PDGF, involved PLD₁ activation by Rho GTPases (Kam and Exton 2004). Both ARF₁ and RhoA were found to become activated after M₃ receptor activation (Keller et al. 1997; Rümenapp et al. 1995), and a role for Rho-kinase in RhoA-controlled PLD stimulation could be demonstrated (Schmidt et al. 1999). In further studies, it was shown that activation of PLD by RhoA and Rho-kinase is mediated by G₁₂ and the tyrosine kinase Pyk₂, whereas activation by ARF₁ is mediated by G₁₃, PI3K and the Arf-GEF ARNO (Han et al. 2003). In cardiomyocytes, Rho proteins were shown to affect signaling to PLD by both endothelin-1 and thrombin, apparently by controlling PI₃K synthesis, whereas ARF selectively affects signaling by the PAR₁ receptor (Fahimi-Vahid et al. 2002).

PLD can directly interact with RaLA, and a Ras/Ral signaling cascade was shown to regulate PLD responses. In HEK-293 cells, Ras and RaLA— but not Rho proteins— were located in RTK signaling to PLD, and this Ras/Ral-dependent signaling cascade was found to be dependent on PKC-α and a Ral-specific GEF (Fig. 2) (Schmidt et al. 1998; Voss et al. 1999). RaLA apparently co-operates with ARF (Kim et al. 1998; Xu et al. 2003) and Rho proteins (Frankel et al. 1999; Wilde et al. 2002) to achieve full PLD activation. Likewise, Ras proteins were found to modulate PLD responses by PDGF (Lucas et al. 2000), and RaLA to affect EGF receptor signaling to PLD (Lu et al. 2000). It was recently shown that direct activation of Ras-related R-Ras by Epac is involved in PLD stimulation by the M₃ muscarinic receptor, apparently by coupling to Gₛ proteins (López de Jesús et al. 2006), but a contribution of Ral proteins to GPCR-induced PLD activation has not been found (Meacci et al. 2002). Collectively, these data demonstrate that heterotrimeric G proteins as well as small GTPases co-ordinate PLD activation by specific membrane receptors in particular cell types, and these mechanisms probably contribute to the organization of agonist-induced PA production for the execution of diverse cellular signaling tasks.

In addition, the synthesis of PI₃K can be directly stimulated by GPCRs (thrombin, LPA, M₃ muscarinic) as well as RTKs (Cochet et al. 1991; Nolan and Lapetina 1990; Pike and Eakes 1987). Receptor activation leads to increased association of PI₃K with the actin cytoskeleton (Grondin et al. 1991; Payrastre et al. 1991), and receptor-induced stimulation and cytoskeletal association of PI₃K may be directly involved in actin cytoskeletal regulation and initialize the assembly of enzymes into signaling complexes. GPCR-induced stimulation of PI₃K synthesis was found to be mediated by pertussis toxin-sensitive Gₛ proteins (Schmidt et al. 1996b; Stephens et al. 1993), but also by G₁₂ and Gₛ proteins (Oude Weernink et al. 2003).
Enhanced PIP2 synthesis is also caused by conventional PKC isoforms, which may increase PIP5K activity by stimulating PIP5K dephosphorylation by the okadaic acid-sensitive protein phosphatase 1 (Park et al. 2001).

Concluding remarks

In the last decade, PLD has taken a firm position as all-round player in cellular signaling events. It is now appreciated that PLD and PIP5K act together to execute several important cellular functions, including vesicle transport, cytoskeleton dynamics and cell adhesion. Because of the reciprocal stimulation of their activities it seems inappropriate to generally assign a conventional “upstairs-downstairs” relationship to PLD and PIP5K isozymes. The localized generation of the lipid messengers by PLD and PIP5K sozymes. The localized stimulation of their activities it seems inappropriate to cellu

References

Aikawa Y, Martin TJF (2003) ARF6 regulates a plasma membrane pool of phosphatidylinositol(4,5)bisphosphate required for regulated exocytosis. J Cell Biol 162:647–659
Anderson RA, Boronenkov IV, Doughman SD, Kunz J, Loijens JC (1999) Phosphatidylinositol phosphate kinases, a multifaceted family of signaling enzymes. J Biol Chem 274:9907–9910
Ameslon LS, Kunz J, Anderson RA, Traub LM (1999) Coupled inositol phosphorylation and phospholipase D activation initiates clathrin-coat assembly on lysosomes. J Biol Chem 274:17794–17805
Balboa MA, Insel PA (1998) Stimulation of phospholipase D via α-adrenergic receptors in Madin-Darby canine kidney cells is independent of PKCα and -ε activation. Mol Pharmacol 53:221–227
Berridge MJ (2005) Unlocking the secrets of cell signaling. Annu Rev Physiol 67:1–21
Bi K, Roth MG, Kistakas NT (1997) Phosphatidic acid formation by phospholipase D is required for transport from the endoplasmic reticulum to the Golgi complex. Curr Biol 7:301–307
Bocckino SB, Blackmore PF, Wilson PB, Exton JH (1987) Phosphatidate accumulation in hormone-treated hepatocytes via a phospholipase D mechanism. J Biol Chem 262:15309–15315
Botelho RJ, Tenem M, Dierickx M, Anderson R, Wells A, York JD, Meyer T, Grinstein S (2000) Localized biphasic changes in phosphatidylinositol-4,5-bisphosphate at sites of phagocytosis. J Cell Biol 151:1353–1367
Brown HA, Gutowksi S, Moomaw CR, Slaughter C, Stemweis PC (1993) ADP-riboseylation factor, a small GTP-dependent regulatory protein, stimulates phospholipase D activity. Cell 75:1137–1144
Brown FD, Thompson N, Saqib KM, Clark JM, Pownar D, Thompson NT, Solari R, Wakelam MJ (1998) Phospholipase D1 localises to secretory granules and lysosomes and is plasma-membrane translocated on cellular stimulation. Curr Biol 8:835–838
Brown FD, Rozelle AL, Yin HL, Balla T, Donaldson JG (2001) Phosphatidylinositol 4,5-bisphosphate and Arf6-regulated membrane traffic. J Cell Biol 154:1007–1017
Chen JS, Exton JH (2004) Regulation of phospholipase D2 activity by protein kinase Ca. J Biol Chem 279:22076–22083
Chen YG, Siddhanta A, Austin CD, Hammond SM, Sung TC, Frohman MA, Morris AJ, Shields D (1997) Phospholipase D stimulates release of nascent secretory vesicles from the trans-Golgi network. J Cell Biol 138:495–504
Chong LD, Traynor-Kaplan A, Bokoch GM, Schwartz MA (1994) The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate 5-kinase in mammalian cells. Cell 79:507–513
Chung JK, Sekiya F, Kang HS, Lee C, Han JS, Kim SR, Bae YS, Morris AJ, Rhee SG (1997) Synaptotagmin inhibition of phospholipase D activity by hydrolysis of phosphatidylinositol 4,5-bisphosphate. J Biol Chem 272:15980–15985
Cochet C, Filhol O, Payrastre B, Hunter T, Gill GN (1991) Interaction between the epidermal growth factor receptor and phosphoinositide kinases. J Biol Chem 266:637–644
Cockcroft S (2001) Signalling roles of mammalian phospholipase D1 and D2. Cell Mol Life Sci 58:1674–1687
Colley WC, Sung TC, Roll R, Jenco J, Hammond SM, Altschuler Y, Bar-Sagi D, Morris AJ, Frohman MA (1997) Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization. Curr Biol 7:191–201
Coppolino MG, Dierckman R, Loijens J, Collins RF, Pouladi M, Cross MJ, Roberts S, Ridley AJ, Hodgkin MN, Stewart A, Claesson-Welsh L, Wakelam MJ (1996) Stimulation of actin stress fibre formation mediated by activation of phospholipase D. Curr Biol 6:588–597
Fahimi-Vahid M, Gosau N, Michalek C, Han L, Jakobs KH, Schmidt Exton JH (2002a) Phospholipase D-structure, regulation and function.

Ginsberg J, Gupta S, Matowie WC, Kline L, Brindley DN (1997) Activation of phospholipase D in FRTL-5 thyroid cells by forskolin and dibutyl-cyclic adenosine monophosphate. Endocrinology 138:3645–3651

Godi A, Pertile P, Meyers R, Marra P, Di Tullio G, Iurisci C, Luni A, Corda D, De Matteis MA (1999) ARF mediates recruitment of PtdIns-4-OH kinase-β and stimulates synthesis of PtdIns(4,5)P2 on the Golgi complex. Nat Cell Biol 1:280–287

Gosau N, Fahimi-Vahid M, Michalek C, Schmidt M, Wieland T (2002) Signalling components involved in the coupling of αi-adrenoceptors to phospholipase D in neonatal rat cardiac myocytes. Naunyn Schmiedebergs Arch Pharmacol 365:468–476

Gratacoup M-P, Payrastre B, Nieswandt B, Offermanns S (2001) Differential regulation of Rho and Rac through heterotrimeric G-proteins and cyclic nucleotides. J Biol Chem 276:47906–47913

Grishanin RN, Kovalchyk JA, Klenchin VA, Ann K, Earles CA, Chapman ER, Gerona RR, Martin TF (2004) CAPS act at a prestin position in dense-core vesicle exocytosis as a C2P binding protein. Neuron 43:551–562

Groom J, Plantavid M, Sultan C, Breton M, Maeco G, Chap H (1991) Interaction of pp60c-src, phosphatidylinositol, inositol, and diacylglycerol kinases with the cytoskeletons of thrombin-stimulated platelets. J Biol Chem 266:15705–15709

Ha KS, Exton JH (1993) Activation of actin polymerization by phosphatidic acid derived from phosphatidylcholine in IIE fibroblasts. J Cell Biol 123:1789–1796

Hammond SM, Altshuller YM, Sung TC, Rudge SA, Rose K, Engebrecht J, Morris AJ, Frohman MA (1995) Human ADP-ribosylation factor-activated phosphatidylinositol-specific phospholipase D defines a new and highly conserved gene family. J Biol Chem 270:29640–29643

Hammond SM, Jenco JM, Nakashima S, Cadwallader K, Gu Q, Cook S, Nozawa Y, Prestwich GD, Frohman MA, Morris AJ (1997) Characterization of two alternately spliced forms of phospholipase D1. Activation of the purified enzymes by phosphatidylinositol 4,5-bisphosphate, ADP-ribosylation factor, and Rac. J Biol Chem 272:3860–3868

Han L, Woznicki M, Limper B, Caracciola P, Oude Weernink PA, Jakobs KH, Muzino K, Schmidt M (2001) Involvement of Lim-kinase in stimulation of phospholipase D (PLD) by Rho and Rho-kinase. Naunyn Schmiedebergs Arch Pharmacol 363:R61

Han L, Rother T, Eicken S, Kindhäuser F, Oude Weernink PA, Schmidt M, Jakobs KH (2003) Role of Gα12 and Gα13 in RhoA and ARF1-mediated signalling to phospholipase D by the M3 muscarinic receptor. Naunyn Schmiedebergs Arch Pharmacol 367:R52

Hanahan DI, Chaikoff IL (1948) On the nature of the phosphorus-containing lipides of cabbage leaves and their relation to a phospholipide-splitting enzyme contained in these leaves. J Biol Chem 272:191–198

Hartwig JH, Bokoch GM, Carpenter CL, Janmey PA, Taylor LA, Toker A, Stossel TP (1995) Thrombin receptor ligation and activated Rac uncap actin filament barbed ends through phosphoinositide synthesis in permeabilized human platelets. Cell 82:643–653

Henage LG, Exton JH, Brown HA (2006) Kinetic analysis of a mammalian phospholipase D. Allosteric modulation by monomeric GTPases, protein kinase C, and polyphosphoinositides. J Biol Chem 281:3408–3417

Hess JA, Ross AH, Qiu RG, Symons M, Exton JH (1997) Role of Rho family proteins in phospholipase D activation by growth factors. J Biol Chem 272:1615–1620

Hilpela P, Vartiainen MK, Lappalainen P (2004) Regulation of the actin cytoskeleton by PtdIns(4,5)P2 and PtdIns3,4,5P3. Curr Top Microbiol Immunol 282:117–163

Hinchcliffe KA, Ciruela A, Irvine RF (1998) PIPkins, their substrates and their products: new functions for old enzymes. Biochim Biophys Acta 1436:87–100

Hodgkin MN, Masson MR, Powner D, Sajib KM, Ponting CP, Wakelam MJO (2000) Phospholipase D regulation and localisation is...
dependent upon a phosphatidylinositol 4,5-bisphosphate-specific PH domain. Curr Biol 10:43–46

Honda A, Nogami M, Yokozeki T, Yamazaki M, Nakamura H, Watanabe H, Kawamoto K, Nakayama K, Morris AJ, Frohman MA, Kanaho Y (1999) Phosphatidylinositol 4-phosphate 5-kinase α is a downstream effector of the small G protein ARF6 in membrane ruffle formation. Cell 99:521–532

Huang C, Hujer KM, Wu Z, Miller RT (2004) The Ca2+-sensing receptor couples to Gα12/13 to activate phospholipase D1 in Madin-Darby canine kidney cells. Am J Physiol Cell Physiol 286:C22–C30

Hughes WE, Parker PJ (2001) Endosomal localization of phospholipase D1 and 2 is independent of activity. Biochem J 356:727–736

Hughes WE, Elgundi Z, Huang P, Frohman MA, Biden TJ (2004) Phospholipase D1 regulates secretagogue-stimulated insulin release in pancreatic beta-cells. J Biol Chem 279:27534–27541

Humeau Y, Vitale N, Chasserot-Golaz S, Du Dupont JLG, Frohman MA, Bader MF, Poulain B (2001) A role for phospholipase D1 in neurotransmitter release. Proc Natl Acad Sci USA 98:15300–15305

Ishihara H, Shibasaki Y, Kizuki N, Katagiri H, Yazaki Y, Asano T, Oka Y (1996) Cloning of cDNAs encoding two isoforms of 68-kDa type I phosphatidylinositol-4-phosphate 5-kinase. J Biol Chem 271:23611–23614

Ishihara H, Shibasaki Y, Kizuki N, Wada T, Yazaki Y, Asano T, Oka Y (1998) Type I phosphatidylinositol-4-phosphate 5-kinases. J Biol Chem 273:8747–8748

Itoh T, Koshiba S, Kigawa T, Kikuchi A, Yokoyama S, Takenawa T (2001) Role of the ENTH domain in phosphatidylinositol-4,5-bisphosphate binding and endocytosis. Science 291:1047–1051

Iyer SS, Barton JA, Bourgoin S, Kunser DJ (2004) Phospholipases D1 and D2 coordinately regulate macrophage phagocytosis. J Immunol 173:2615–2623

Iyer SS, Agrawal RS, Thompson CR, Thompson S, Barton JA, Kunser DJ (2006) Phospholipase D1 regulates phagocyte adhesion. J Immunol 176:3686–3696

Jenkins GM, Frohman MA (2005) Phospholipase D: a lipid centric review. Cell Mol Life Sci 62:2305–2316

Jenkins GH, Fisette PL, Anderson RA (1994) Type I phosphatidylinositol 4-phosphate 5-kinase isoforms are specifically stimulated by phosphatidic acid. J Biol Chem 269:11547–11554

Jones DH, Morris JB, Morgan CP, Kondo H, Irvine RF, Cockcroft S (2000a) Type I phosphatidylinositol 4-phosphate 5-kinase directly interacts with ARF-ribosylation factor I and is responsible for phosphatidylinositol 4,5-bisphosphate synthesis in the golgi compartment. J Biol Chem 275:13962–13966

Jones DR, Sanjuan MA, Mérida I (2000b) Type Ix phosphatidylinositol 4-phosphate 5-kinase is a putative target for increased intracellular phosphatidic acid. FEBS Lett 476:160–165

Kam Y, Epton JH (2001) Phospholipase D activity is required for actin stress fiber formation in fibroblasts. Mol Cell Biol 21:4055–4066

Kam Y, Epton JH (2004) Role of phospholipase D1 in the regulation of mTOR activity by lysophosphatidic acid. FASEB J 18:311–319

Kam JL, Miura K, Jackson TR, Gruschus J, Roller P, Stauffer S, Clark J, Aneja R, Randazzo PA (2000) Phosphoinositide-dependent activation of the ARF-ribosylation factor GTase-activating protein ASAP1. J Biol Chem 275:9326–9332

Keller J, Schmidt M, Hussein B, Rümenapp U, Jakobs KH (1997) Mucarinic receptor-stimulated cytosol-membrane translocation of RhoA. FEBS Lett 403:299–302

Kim JH, Lee SD, Han JM, Lee TG, Kim Y, Park JB, Lambeth JD, Suh PG, Ryu SH (1998) Activation of phospholipase D1 by direct interaction with ARF-ribosylation factor I and RalA. FEBS Lett 430:231–235

Kim JH, Kim HW, Jeon H, Suh PG, Ryu SH (2000) Phospholipase D1 regulates cell migration in a lipase activity-independent manner. J Biol Chem 275:15747–15756

Kisseleva M, Feng Y, Ward M, Song C, Anderson RA, Longmore GD (2005) The LIM protein Aujaba regulates phosphatidylinositol 4,5-bisphosphate levels in migrating cells through an interaction with and activation of PIPKlα. Mol Cell Biol 25:3956–3966

Klärner JD, Rameh LE, Cantley LC, Buxton JM, Holik JJ, Sakelis C, Patki V, Corvera S, Czech MP (1998) Regulation of GRP1-catalyzed ADP ribosylation factor guanine nucleotide exchange by phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 273:1859–1862

Koch T, Brandenburg LO, Schulz S, Liang Y, Klein J, Höllt V (2003) ADP-ribosylation factor-dependent phospholipase D2 activation is required for agonist-induced μ-opioid receptor endocytosis. J Biol Chem 278:9979–9985

Koch T, Wu DF, Yang LQ, Brandenburg LO, Höllt V (2006) Role of phospholipase D2 in the agonist-induced and constitutive endocytosis of G-protein coupled receptors. J Neurochem 97:365–372

Komati H, Naro F, Mebarek S, De Angelis V, Adamo S, Lagarde M, Prigent AF, Némoz G (2005) Phospholipase D is involved in myogenic differentiation through remodeling of actin cytoskeleton. Mol Biol Cell 16:1232–1244

Krauss M, Kinuta M, Wenk MR, De Camilli P, Takei K, Haucke V (2003) ARF6 stimulates clathrin/AP-2 recruitment to synaptic membranes by activating phosphatidylinositol phosphate kinase type 1y. J Cell Biol 162:113–124

Kristakis NT, Brown HA, Waters MG, Sternweis PC, Roth MG (1996) Evidence that phospholipase D mediates ADP ribosylation factor-dependent formation of Golgi coated vesicles. J Cell Biol 134:295–306

Kunz J, Fuelling A, Kolbe L, Anderson RA (2002) Stereo-specific substrate recognition by phosphatidylinositol phosphate kinases is swapped by changing a single amino acid residue. J Biol Chem 277:5611–5619

Kunser DJ, Barton JA, Wen KK, Wang X, Rubenstein PA, Iyer SS (2002) Regulation of phospholipase D activity by actin. Actin exerts bidirectional modulation of mammalian phospholipase D activity in a polymerization-dependent, isoform-specific manner. J Biol Chem 277:50683–50692

Laux T, Fukami K, Thelen M, Golub T, Frey D, Caroni P (2000) GAP43, MARCKS, and CAP23 mediate PI(4,5)P2 at plasma membrane rafts, and regulate cell cortex actin dynamics through a common mechanism. J Cell Biol 149:1455–1471

Lawrence JTR, Birnbaum MJ (2003) ADP-ribosylation factor 6 regulates insulin secretion through plasma membrane phosphatidylinositol 4,5-bisphosphate. Proc Natl Acad Sci USA 100:13320–13325

Le Stunff H, Dokhcac L, Bourgoin S, Bader MF, Harbon S (2000) Phospholipase D in rat myometrium: occurrence of a membrane-bound ARF6 (ADP-ribosylation factor 6)-regulated activity controlled by βγ subunits of heterotrimeric G-proteins. Biochem J 352:491–499

Lee S, Park JB, Kim JH, Kim Y, Kim JH, Shin KJ, Lee JS, Ha SH, Suh PG, Ryu SH (2001) Actin directly interacts with phospholipase D, inhibiting its activity. J Biol Chem 276:28252–28260

Lee JS, Kim JH, Jang IH, Kim HS, Han JM, Kazlauskas A, Yagisawa H, Suh PG, Ryu SH (2005) Phosphatidylinositol (3,4,5)-trisphosphate specifically interacts with the phox homology domain of phospholipase D1 and stimulates its activity. J Cell Sci 118:4405–4413

Lee CS, Kim IS, Park JB, Lee MN, Lee YH, Suh PG, Ryu SH (2006) The phox homology domain of phospholipase D activates dynamin GTPase activity and accelerates EGF receptor endocytosis. Nat Cell Biol 8:477–484

Ling K, Doughman RL, Firestone AJ, Bunce MW, Anderson RA (2002) Type Iy phosphatidylinositol phosphate kinase targets and regulates focal adhesions. Nature 420:89–93

Ling K, Schill NJ, Wagoner MP, Yun S, Anderson RA (2006) Movin’ on up: the role of PtdIns(4,5)P2 in cell migration. Trends Cell Biol 16:276–284
Liscovitch M, Chalifa V, Pertile P, Chen CS, Cantley LC (1994) Novel function of phosphatidylinositol 4,5-bisphosphate as a cofactor for brain membrane phospholipase D. J Biol Chem 269:21403–21406

Liscovitch M, Czarny M, Fiucci G, Tang X (2000) Phospholipase D: molecular and cellular biology of a novel gene family. Biochem J 345:401–415

Loijens JC, Anderson RA (1996) Type I phosphatidylinositol-4-phosphate 5-kinases are distinct members of this novel lipid kinase family. J Biol Chem 271:32937–32943

López de Jesús M, Stoque MB, Ouwe Weernink PA, Mahlke Y, Börgemann A, Anana VA, Rimmbach C, Rosskopf D, Michel MC, Jakobs KH, Schmidt M (2006) Cyclic AMP-dependent and Epac-mediated activation of R-Ras by G protein-coupled receptors leads to phospholipase D stimulation. J Biol Chem 281:21837–21847

Lu ZM, Hornia A, Joseph T, Sukezane T, Frankel P, Zhong M, Bychenok S, Xu LZ, Feig LA, Foster DA (2000) Phospholipase D and RaLαA cooperate with the epidermal growth factor receptor to transform 3Y1 rat fibroblasts. Mol Cell Biol 20:462–467

Lucas L, del Peso L, Rodriguez P, Penavla L, Lalc JC (2000) Ras protein is involved in the physiological regulation of phospholipase D by platelet-derived growth factor. Oncogene 19:431–437

Lukowski S, Mira JP, Zachowski A, Geny B (1998) Fodrin inhibits phospholipases A2, C, and D by decreasing polyphosphoinositide cell content. Biochem Biophys Res Commun 248:278–284

Majerus PW, Kisseleva MV, Norris FA (1999) The role of phosphatases in inositol signalings reactions. J Biol Chem 274:10669–10672

Matsui T, Yonemura S, Tsukita S, Tsukita S (1999) Activation of ERMs in vivo by Rho involves phosphatidylinositol 4-phosphate 5-kinase and not ROCK kinases. Curr Biol 9:1295–1262

Meacci E, Becciolini L, Nuti F, Donati C, Cencetti F, Farnararo M, Brunì P (2002) A role for calcium in sphingosine 1-phosphate-induced phospholipase D activity in C2C12 myoblasts. FEBS Lett 521:200–204

Mitchell R, McCulloch D, Lutz E, Johnson M, MacKenzie C, Fennell P, Pike LJ, Eakes AT (1987) Epidermal growth factor stimulates the production of phosphatidylinositol monophosphate and the breakdown of polyphosphoinositides in A431 cells. J Biol Chem 262:1644–1651
Plonk SG, Park SK, Exton JH (1998) The α-subunit of the heterotrimeric G protein G_{13} activates a phospholipase D isozyme by a pathway requiring Rh family GTPases. J Biol Chem 273:4823–4826

Porcelli AM, Ghelli A, Hrelia S, Rugolo M (2002) Phospholipase D stimulation is required for sphingosine-1-phosphate activation of actin stress fibre assembly in human airway epithelial cells. Cell Signal 14:75–81

Pownier DJ, Walealm MJO (2002) The regulation of phospholipase D by inositol phospholipids and small GTPases. FEBs Lett 531:62–64

Pownier DJ, Payne RM, Pettit TR, Giudici ML, Irvine RF, Walealm MJO (2005) Phospholipase D2 stimulates integrin-mediated adhesion via phosphatidylinositol 4-phosphate 5-kinase Iβ. J Cell Sci 118:2975–2986

Preininger AM, Henage LG, Oldham WM, Yoon EJ, Hamm HE, Powner DJ, Payne RM, Pettit TR, Giudici ML, Irvine RF, Walealm MJO, Schmidt M, Voss M, Thiel M, Bauer B, Grannass A, Tapp E, Cool Schmidt M, Nehls C, Rümenapp U, Jakobs KH (1996b) m3 Muscarinic acetylcholine receptor expressed in HEK-293 kidney cells. Eur J Biochem 248:407

Preininger AM, Henage LG, Oldham WM, Yoon EJ, Hamm HE, Powner DJ, Payne RM, Pettit TR, Giudici ML, Irvine RF, Walealm MJO, Schmidt M, Voss M, Thiel M, Bauer B, Grannass A, Tapp E, Cool Schmidt M, Nehls C, Rümenapp U, Jakobs KH (1996b) m3 Muscarinic acetylcholine receptor expressed in HEK-293 kidney cells. Eur J Biochem 248:407

Rümenapp U, Asmus M, Schablowski H, Woznicki M, Han L, Jakobs KH (1995) Evidence for ADP-ribosylation-factor-mediated activation of phospholipase D by m3 muscarinic acetylcholine receptor. Eur J Biochem 234:240–244

Rümenapp U, Schmidt M, Wahn F, Tapp E, Grannass A, Jakobs KH (1995) Characteristics of protein-kinase-C- and ADP-ribosylation-factor-stimulated phospholipase D activities in human embryonic kidney cells. Eur J Biochem 248:407–414

Rümenapp U, Asmus M, Schablowlski W, Woznicki M, Han L, Jakobs KH, Fahimi-Vahid M, Michalek C, Wieland T, Schmidt M (2001) The M3 muscarinic acetylcholine receptor expressed in HEK-293 cell signals to phospholipase D via G12 but not Gq-type G proteins. Regulators of G proteins as tools to dissect pertussis toxin-resistant G proteins in receptor-effector coupling. J Biol Chem 276:2977–2981

Rümenapp U, Schmidt M, Wahn F, Tapp E, Grannass A, Jakobs KH (1995) Evidence for ADP-ribosylation-factor-mediated activation of phospholipase D by m3 muscarinic acetylcholine receptor. Eur J Biochem 234:240–244

Santarius M, Lee CH, Anderson RA (2006) Supervised membrane swimming: small G-protein lifeguard regulates PIPK signalling and monitor intracellular PtdIns(4,5)P2 pools. Biochem J 398:1–13

Schmidt M, Hiiwe SM, Fassett B, Homann D, Rümenapp U, Sandmann J, Jakobs KH (1994) Mechanisms of phospholipase D stimulation by m3 muscarinic acetylcholine receptors. Evidence for involvement of tyrosine phosphorylation. Eur J Biochem 225:667–675

Schmidt M, Bieneck C, Rümenapp U, Zhang C, Lümmen G, Jakobs KH, Just I, Aktories K, Moos M, von Eichel-Streiber C (1996a) A role for Rho in receptor- and G protein-stimulated phospholipase C. Reduction in phosphatidylinositol 4,5-bisphosphate by Clostridium difficile toxin B. Naunyn Schmiedebers Arch Pharmacol 354:87–81

Schmidt M, Nehls C, Rümenapp U, Jakobs KH (1996b) m3 Muscarinic receptor-induced and G,-mediated heterologous potentiation of phospholipase C stimulation: role of phosphoinositide synthesis. Mol Pharmacol 50:1038–1046

Schmidt M, Rümenapp U, Bieneck C, Keller J, von Eichel-Streiber C, Jakobs KH (1996c) Inhibition of receptor signaling to phospholipase D by Clostridium difficile toxin B. Role of Rho proteins. J Biol Chem 271:2422–2426

Schmidt M, Rümenapp U, Nehls C, Ott S, Keller J, von Eichel-Streiber C, Jakobs KH (1996d) Restoration of Clostridium difficile toxin-B inhibited phospholipase D by phosphatidylinositol 4,5-bisphosphate. Eur J Biochem 240:707–712

Schmidt M, Voss M, Thié M, Bauer B, Grannass A, Tapp E, Cool RH, de Gunzburg J, von Eichel-Streiber C, Jakobs KH (1998) Specific inhibition of phorbol ester-stimulated phospholipase D by Clostridium sordellii lethal toxin and Clostridium difficile toxin B:1470 in HEK-293 cells. J Biol Chem 273:7413–7422

Schmidt M, Voss M, Oude Wernink PA, Wetzel J, Amano M, Kaibuchi K, Jakobs KH (1999) A role for Rho-kinase in Rho-controlled phospholipase D stimulation by the m3 muscarinic acetylcholine receptor. J Biol Chem 274:14648–14654

Schmidt M, Oude Wernink PA, vom Dorf F, Stope MB, Jakobs KH (2004) Mammalian phospholipase C. Adv Mol Cell Biol 33:431–450

Schürmann A, Schmidt M, Asmus M, Bayer S, Fliegert F, Koling S, Maßmann S, Schiffl C, Subausta MC, Vol M, Jakobs KH, Joost HG (1999) The ARF-ribosylation factor (ARF)-related GTPase ARF-related protein binds to the ARF-specific guanine nucleotide exchange factor cytohesin and inhibits the ARF-dependent activation of phospholipase D. J Biol Chem 274:9744–9751

Sciore VA, Rudge SA, Prestwich GD, Frohman MA, Engbrecht J, Morris AJ (1999) Identification of a phosphoinositide binding motif that mediates activation of mammalian and yeast phospholipase D isozymes. EMBO J 20:5911–5921

Sciore VA, Rudge SA, Wang J, McLaughlin S, Engbrecht J, Morris AJ (2002) Dual role for phosphoinositides in regulation of yeast and mammalian phospholipase D enzymes. J Cell Biol 159:1039–1049

Scott CC, Dobson W, Botelho RJ, Coady-Osberg N, Chavrier P, Knecht DA, Heath C, Stahl P, Grinstein S (2005) Phosphatidylinositol-4,5-bisphosphate hydrolysis directs actin remodeling during phagocytosis. J Cell Biol 169:139–149

Shibasaki Y, Ishihara H, Kizuki N, Asano T, Oka Y, Yazaki Y (1997) Massive act polymerization induced by phosphatidylinositol-4-phosphate 5-kinase in vivo. J Biol Chem 272:7578–7581

Sheme K, Rizzo MA, Vasudevan C, Andresen B, Romero G (2000) The activation of phospholipase D by endothelin-1, angiotensin II, and platelet-derived growth factor in vascular smooth muscle A10 cells is mediated by small G proteins of the ADP-ribosylation factor family. Endocrinology 141:2200–2208

Skippen A, Jones DH, Morgan CP, Li M, Cockcroft S (2002) Mechanism of ARD ribosylation factor-stimulated phosphatidylinositol 4,5-bisphosphate synthesis in HL60 cells. J Biol Chem 277:5823–5831

Stahelin RV, Ananthanarayanan B, Blatner NR, Singh S, Bruzik KS, Murray D, Cho W (2004) Mechanism of membrane binding of the phospholipase D1 PX domain. J Biol Chem 279:54918–54926

Steed PM, Clark KL, Boyar WC, Lasala DJ (1998) Characterization of human PLD2 and the analysis of PLD isofrom splice variants. FASEB J 12:1309–1317

Stephens L, Jackson TR, Hawkins PT (1993) Activation of phosphatidylinositol 4,5-bisphosphate supply by agonists and non-hydrolysable GTP analogues. Biochem J 296:407–414

Suh BC, Hille B (2005) Regulation of ion channels by phosphatidylinositol 4,5-bisphosphate. Curr Opin Neurobiol 15:370–378

Sung TC, Zhang Y, Morris AJ, Frohman MA (1999) Structural analysis of human phospholipase D1. J Biol Chem 274:3659–3666

Sweeney DA, Siddhanta A, Cockcroft S (2002) Fragmentation and re-assembly of the Golgi apparatus in vitro. A requirement for phosphatidic acid and phosphatidylinositol 4,5-bisphosphate synthesis. J Biol Chem 277:3030–3039

Tall EG, Spector I, Pentyala SN, Bitter I, Rebecchi MJ (2000) Dynamics of phosphatidylinositol 4,5-bisphosphate in actin-rich structures. Curr Biol 10:743–746

Toker A (2002) Phosphoinositides and signal transduction. Cell Mol Life Sci 59:761–779

Tolias KF, Hartwig JH, Ishihara H, Shibasaki Y, Cantley LC, Carpenter CL (2000) Type Ix phosphatidylinositol-4-phosphate 5-kinase mediates Rac-dependent actin assembly. Curr Biol 10:153–156

Ushio-Fukai M, Alexander RW, Akers M, Lyons PR, Lassègue B, Griendling KK (1999) Angiotensin II receptor coupling to phospholipase D is mediated by the bg subunits of heterotrimeric G proteins in vascular smooth muscle cells. Mol Pharmacol 55:142–149

van Hennek PB, ten Klooster JP, Halstead JR, Voermans C, Anthony EC, Divecha N, Jordijk PL (2003) The C-terminal domain of...
Rac1 contains two motifs that control targeting and signaling specificity. J Biol Chem 278:39166–39175
van Horck FPG, Lavazais E, Eickholt BJ, Moolenaar WH, Divecha N (2002) Essential role of type 1α phosphatidylinositol 4-phosphate 5-kinase in neurite remodeling. Curr Biol 12:241–245
van Rheenen J, Achame EM, Janssen H, Calafat J, Jalink K (2005) PIP2 signaling in lipid domains: a critical re-evaluation. EMBO J 24:1664–1673
Vanhaesebroeck B, Leevers SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, Waterfield MD (2001) Synthesis and function of 3-phosphorylated inositol lipids. Annu Rev Biochem 70:535–602
Vitale N, Caumont A-S, Chasserot-Golaz S, Du G, Wu S, Sciorra VA, Morris AJ, Frohman MA, Bader M-F (2001) Phospholipase D1: a key factor for the exocytotic machinery in neuroendocrine cells. EMBO J 20:2424–2434
Voss M, Oude Weernink PA, Haupenthal S, Möller U, Cool RH, Bauer B, Camonis JH, Jakobs KH, Schmidt M (1999) Phospholipase D stimulation by receptor tyrosine kinases mediated by protein kinase C and a Ras/Ral signaling cascade. J Biol Chem 274:34691–34698
Wang YJ, Li WH, Wang J, Xu K, Dong P, Luo X, Yin HL (2004) Critical role of PIP5K1y87 in InsP7-mediated Ca2+ signaling. J Cell Biol 167:1005–1010
Waselle L, Gerona RRL, Vitale N, Martin TFJ, Bader MF, Regazzi R (2005) Role of phosphoinositide signaling in the control of insulin exocytosis. Mol Endocrinol 19:3097–3106
Way G, O’Luanaigh N, Cockcroft S (2000) Activation of exocytosis by cross-linking of the IgE receptor is dependent on ADP-ribosylation factor 1-regulated phospholipase D in RBL-2H3 mast cells: evidence that the mechanism of activation is via regulation of phosphatidylinositol 4,5-bisphosphate. Biochem J 346:63–70
Wenk MR, Pellegrini L, Klenchin VA, Di Paolo G, Chang S, Daniell L, Arioka M, Martin TF, De Camilli P (2001) PIP kinase IIy is the major PI(4,5)P2 synthesizing enzyme at the synapse. Neuron 32:79–88
Wilde C, Barth H, Sehr P, Han L, Schmidt M, Just I, Aktories K (2002) Interaction of the Rho-ADP-ribosylating C3 exoenzyme with RasA. J Biol Chem 277:14771–14776
Wong KW, Isberg RR (2003) Arf6 and phosphoinositide-4-phosphate-5-kinase activities permit bypass of the Rac1 requirement for β1 integrin-mediated bacterial uptake. J Exp Med 198:603–614
Xie Z, Ho WT, Exton JH (2000) Association of the N- and C-terminal domains of phospholipase D. J Biol Chem 275:24962–24969
Xie Z, Ho WT, Spellman R, Cai S, Exton JH (2002) Mechanisms of regulation of phospholipase D1 and D2 by the heterotrimeric G proteins G13 and Gq. J Biol Chem 277:11979–11986
Xu L, Frankel P, Jackson D, Rotunda T, Boshans RL, D’Souza-Schorey C, Foster DA (2003) Elevated phospholipase D activity in H-Ras- but not K-Ras-transformed cells by the synergistic action of RasA and ARF6. Mol Cell Biol 23:645–654
Yamazaki M, Miyazaki H, Watanabe H, Sasaki T, Maehama T, Frohman MA, Kanaho Y (2002) Phosphatidylinositol 4-phosphate 5-kinase is essential for ROCK-mediated neurite remodeling. J Biol Chem 277:17226–17230
Yang S-A, Carpenter CL, Abrams CS (2004) Rho and Rho-kinase mediate thrombin-induced phosphatidylinositol 4-phosphate 5-kinase trafficking in platelets. J Biol Chem 279:42331–42336
Yin HL, Janmey PA (2003) Phosphoinositide regulation of the actin cytoskeleton. Annu Rev Physiol 65:761–789
Yoon MS, Koo JB, Hwang JH, Lee KS, Han JS (2005) Activation of phospholipase D by 8-Br-cAMP occurs through novel pathway involving Src, Ras, and ERK in human endometrial stromal cells. FEBS Lett 579:5635–5642
Zhang Y, Altschuller YM, Hammond SM, Morris AJ, Frohman MA (1999) Loss of receptor regulation by a phospholipase D1 mutant unresponsive to protein kinase C. EMBO J 18:6339–6348
Ziemnicki J, Tandon R, Schilling JR, Sedor JR, Miller RT, Huang C (2005) Mechanical force-activated phospholipase D is mediated by Gα12/13-Rho and calmodulin-dependent kinase in renal epithelial cells. Am J Physiol Renal Physiol 289:F826–F834