

Review

Dynamic phospholipid signaling by G protein-coupled receptors

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Abstract

G protein-coupled receptors (GPCRs) control a variety of fundamental cellular processes by regulating phospholipid signaling pathways. Essential for signaling by a large number of receptors is the hydrolysis of the membrane phosphoinositide PIP₂ by phospholipase C (PLC) into the second messengers IP₃ and DAG. Many receptors also stimulate phospholipase D (PLD), leading to the generation of the versatile lipid, phosphatidic acid. Particular PLC and PLD isoforms take differential positions in receptor signaling and are additionally regulated by small GTPases of the Ras, Rho and ARF families. It is now recognized that the PLC substrate, PIP₂, has signaling capacity by itself and can, by direct interaction, affect the activity and subcellular localization of PLD and several other proteins. As expected, the synthesis of PIP₂ by phosphoinositide 5-kinases is tightly regulated as well. In this review, we present an overview of how these signaling pathways are governed by GPCRs, explain the molecular basis for the spatially and temporally organized, highly dynamic quality of phospholipid signaling, and point to the functional connection of the pathways.

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Keywords: GPCR; Phospholipase C; Phospholipase D; PIP₂; Phosphoinositide 5-kinase; Small GTPase

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Abbreviations: DAG, diacylglycerol; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; GTPγS, guanosine 5'-0-(3-thio)-triphosphate; IP₃, inositol-1,4,5-trisphosphate; LPA, lysophosphatidic acid; PA, phosphatidic acid; PDGF, platelet-derived growth factor; PH, pleckstrin homology; PX, phox homology; PI3K, phosphoinositide 3-kinase; PIP₂, phosphoinositide 5-kinase; PIP₃, phosphatidylinositol-4,5-bisphosphate; PIP₃, phosphatidylinositol-3,4,5-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; PTX, pertussis toxin; RA, Ras-binding domain; RGS, regulators of G protein signaling; RTK, receptor tyrosine kinase; SH, Src homology

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1. Introduction

GPCRs constitute a large group of membrane receptors known to modulate a wide range of biological responses, including cell growth, differentiation, migration, and inflammatory processes. Many cellular responses elicited by GPCRs are mediated by phospholipid signaling cascades, initiated by $G\alpha$ and $G\beta\gamma$ subunits of heterotrimeric G proteins. The hydrolysis of membrane phospholipids leads to the formation of various bioactive lipid mediators, acting either as extracellular signaling molecules or as intracellular second messengers. A crucial second messenger-forming system is the stimulation of phosphoinositide-specific phospholipase C (PLC) isoforms. Upon activation, PLC enzymes hydrolyze phosphatidylinositol-4,5-bisphosphate (PIP_2) at the inner face of the plasma membrane and thereby generate the messengers, diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). These two signaling molecules lead to the activation of several protein kinase C (PKC) isoforms and the release of calcium from intracellular stores, respectively. It is now well established that PLC stimulation plays a major role in many early and late cellular responses to GPCR activation, including smooth muscle contraction, secretion, neuronal signaling as well as fertilization, cell growth and differentiation [1,2]. Phospholipase D (PLD) is a distinct, phospholipid-specific phosphodiesterase that hydrolyzes phosphatidylcholine to phosphatidic acid (PA) and choline. PLD is rapidly activated in response to extracellular stimuli, and the generation of PA is considered to

mediate many of the biological functions attributed to PLD and to play important roles in the regulation of cell function and cell fate. Indeed, activation of PLD by GPCRs has now been established to 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 apoptosis [3–6].

The phosphoinositide PIP_2 takes a pivotal point in cellular signaling by both PLC and PLD. PIP_2 serves as the major substrate for PLC enzymes and, at the same time, profoundly affects the subcellular localization and activity of PLD enzymes and many other proteins via specific interaction with unique phosphoinositide-binding domains, including pleckstrin homology (PH), phox homology (PX), ENTH, FERM, FYVE and tubby domains. In this way, PIP_2 (and other phosphoinositides) modulate a remarkable number of cellular processes, such as actin cytoskeletal dynamics, vesicle trafficking, ion channel activity, gene expression and cell survival [7,8]. The activity and localization of phosphoinositide 5-kinase (PIP_5K) isoforms, which catalyze the formation of PIP_2 , are tightly regulated by monomeric GTPases. The regulation of PIP_5K by GTPases likely contribute to the spatial and temporal organization of PIP_2 metabolism and, thus, direct discrete and dynamic GPCR signaling by PLC and PLD. Consistent with their distinct structural organization, the PLC and PLD isoforms are susceptible to distinct modes of activation by membrane receptors and monomeric GTPases. This review will focus on

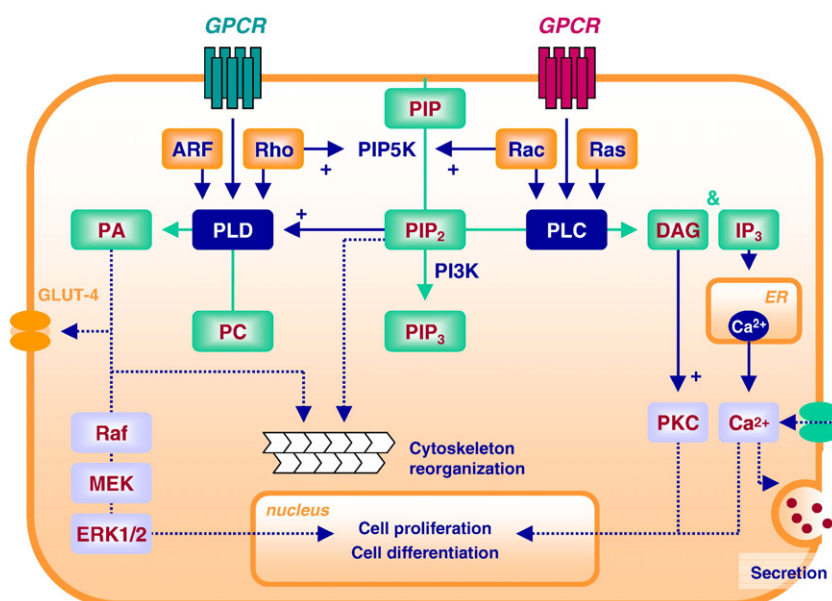


Fig. 1. Phospholipid signaling by GPCRs. GPCRs stimulate PLC isoforms that hydrolyze PIP_2 into the second messengers DAG and IP_3 , leading to the activation of PKC isoforms and the release of Ca^{2+} from intracellular stores, respectively. PLC stimulation plays a major role in many cellular responses, including smooth muscle contraction, secretion, and cell proliferation and differentiation. Activation of PLD leads to the hydrolysis of phosphatidylcholine, and GPCR-induced PA generation modulates a wide array of cellular responses, including GLUT-4 translocation, actin cytoskeleton rearrangement, activation of the Raf/MEK/ERK1/2 signaling cascade, and mitogenesis. Stimulation of PLC and PLD enzymes by GPCRs involve monomeric GTPases of the Rho, Ras, and ARF families. PIP_2 takes a pivotal point in cellular signaling by both PLC and PLD: it serves as the major substrate for PLC enzymes, profoundly affects the subcellular localization and enzyme activity of PLD enzymes, can be further phosphorylated to the lipid mediator PIP_3 by PI3K, but also directly interacts with actin-remodeling proteins and ion channels. The synthesis of PIP_2 by PIP_5K is regulated by Rho and ARF GTPases as well. PLC and PLD signaling is even more interconnected, as PLD activity is significantly regulated by PKC, and PA is a strong activator of PIP_5K .

the regulation of PLC and PLD enzymes by GPCRs, and on how signaling by these lipases is organized and connected by small GTPases and PIP₂ metabolism (Fig. 1).

2. The PLC family

At present, thirteen distinct mammalian PLC isoforms have been identified and grouped into six families: PLC-β (1–4), PLC-γ (1–2), PLC-δ (1,3,4), PLC-ε, PLC-ζ, and PLC-η (1–2). The PLC isoforms share four EF-hand motifs, the X and Y domains that form the catalytic core, and a C-terminal C2 domain [9–11]. Binding of calcium to the EF-hands of PLC-δ may be necessary for proper interaction of the PH domain with PIP₂ [12], whereas the C2 domain may differentially support plasma membrane localization and facilitate substrate hydrolysis [13]. All isoforms, with exception of PLC-ζ, have an N-terminal PH domain that localizes the enzymes to their substrate and other signaling components. In addition to these canonical domains, the isoforms contain family-specific regulatory domains to take differential positions in receptor signaling (Fig. 2).

PLC-β enzymes are activated by heterotrimeric G protein subunits after stimulation of GPCRs, and possess a C-terminal regulatory domain that interacts with GTP-bound α subunits of G_q proteins [14,15]. The C-terminus of PLC-β enzymes also bears a GTPase-activating protein (GAP) activity mimicking the Gα_q interaction site of regulators of G protein signaling (RGS) proteins, a family of GAP proteins for heterotrimeric G proteins [16]. The C-terminus further triggers membrane association and localization of PLC-β1 into the nucleus [17] and has a binding motif, which may interact with PDZ domain-containing proteins to promote scaffolding of signaling molecules [18]. PLC-γ enzymes contain Src homology 2 (SH2) domains and are regulated by nonreceptor and receptor tyrosine kinases (RTK). The second split PH domain of PLC-γ1 can interact with EF-1α, an activator of phosphoinositide 4-kinases [19], and the SH3 domain of PLC-γ1 exhibits guanine

nucleotide exchange factor (GEF) activity towards PIKE, a nuclear GTPase that activates phosphoinositide 3-kinase (PI3K) [20]. Both mechanisms may contribute to a rapid, highly dynamic phosphoinositide turnover in intact cells and, hence, the localization and functionality of PLC-γ1. The SH3 domain of PLC-γ1 also interacts with PLD2, and may therefore directly link the two signaling phospholipase families [21]. The archetypal PLC-δ enzymes are tightly regulated by intracellular [Ca²⁺]. Activation of PLC-δ isoforms by membrane receptors is only poorly understood, but it has been reported that the atypical high molecular weight G_h GTPase can directly activate PLC-δ1 *via* interaction with the C2 domain, and can mediate PLC-δ1 activation by the oxytocin receptor, the thromboxane A₂ receptor and the α_{1B}-adrenoceptor [9–11]. PLC-ε bears an N-terminal CDC25 domain with GEF activity for Ras-like GTPases as well as two C-terminal Ras-binding (RA) domains, qualifying PLC-ε for upstream and downstream interactions with Ras-like GTPases [22–24]. PLC-ζ from sperm was found to initiate calcium oscillations in the fertilized oocyte and trigger embryo development [25]. The latest isoform, PLC-η, was simultaneously described by four groups last year and likely plays a role in neuronal functioning [26]. The distinct regulatory domains and unique organization patterns of the mammalian PLC isoforms apparently allow differential positioning of PLC signaling in cellular processes.

3. Regulation of PLC-β isoforms

GPCRs can activate PLC-β isoforms by two distinct mechanisms. Pertussis toxin (PTX)-insensitive heterotrimeric G_q family proteins (G_q, G₁₁, G₁₄, G₁₅ and G₁₆) activate PLC-β isoforms, but not PLC-γ, PLC-δ and PLC-ε isoforms, *via* GTP-loaded Gα subunits [9–11,23]. Many GPCRs employ this classical mode of PLC-β activation, including the receptors for bradykinin, α₁-adrenergic agonists, angiotensin II, vasopressin, acetylcholine (muscarinic M₁ and M₃), thromboxane A₂, bombesin and endothelin-1. GTPγS-bound Gα_q subunits

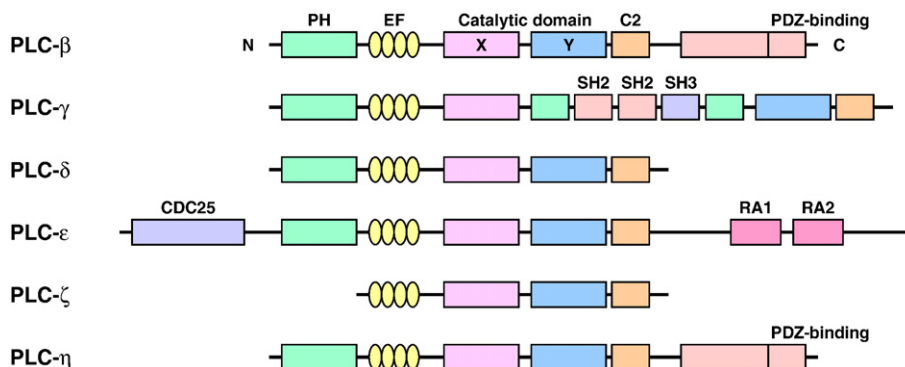


Fig. 2. The PLC family. The basic structure of the PLC isoforms comprehends an N-terminal PH domain (except PLC-ζ from sperm), four EF-hand motifs, the X and Y domains that form the catalytic core, and a C-terminal C2 domain. In addition to these canonical domains, the isoforms contain family-specific regulatory domains to take differential positions in receptor signaling. The PLC-β C-terminal domain interacts with Gα_q proteins and with PDZ domain-containing proteins. PLC-γ enzymes contain a second split PH, two SH2 and an SH3 domain and are regulated by tyrosine kinases. The PLC-δ enzymes are tightly regulated by intracellular [Ca²⁺]. PLC-ε bears an N-terminal CDC25 domain with GEF activity for Ras-like GTPases as well as two C-terminal RA domains, qualifying PLC-ε for upstream and downstream interactions with Ras-like GTPases. The latest isoform, PLC-η, contains a C-terminal sequence with a PDZ-binding motif, and likely plays a role in neuronal functioning.

activate PLC- β isoforms, with the order PLC- $\beta 1$ = PLC- $\beta 4$ \geq PLC- $\beta 3$ > PLC- $\beta 2$ [27]. The region required for PLC- $\beta 1$ activation by $G\alpha_q$ is localized in the C-terminus [17]. The crystal structure of PLC- β revealed that the C-terminus forms a long coiled coil that dimerizes along its long axis [14], and residues located at the outface of the helices were identified to specifically interact with $G\alpha_q$ [15]. Binding of $G\alpha_q$ to the C2 domains of PLC- β enzymes may additionally contribute to enzyme activation [28].

$G\beta\gamma$ subunits liberated from PTX-sensitive G_i family proteins likewise activate PLC- β (except PLC- $\beta 4$) isoforms, and also PLC- ϵ [9–11,29]. This mode of PLC- β activation includes the M_2 muscarinic receptors and receptors for chemoattractants. The efficiency of PLC- β activation by $G\beta\gamma$ depends on the subunit composition, $G\beta_1\gamma_{1/2}$ and $G\beta_5\gamma_2$ preferring PLC- $\beta 3$ and PLC- $\beta 2$, respectively [30]. $G\beta\gamma$ subunits can bind to the PH domain of PLC- β enzymes, but an additional site of interaction of $G\beta\gamma$ with PLC- $\beta 2$ was localized in the Y catalytic subdomain [31,32]. The distinct interaction sites of $G\beta\gamma$ subunits may promote membrane insertion of PLC- β enzymes. $G\alpha_q$ proteins were shown to permit PLC- β stimulation by receptors acting through $G\beta\gamma$ subunits of G_i proteins [33,34], suggesting that $G\alpha_q$ and $G\beta\gamma$ -dependent signals may converge on the level of PLC- β isozymes to gain full activation.

In addition to heterotrimeric G protein subunits, PLC- β isozymes are also activated by Rho family GTPases. PLC- $\beta 2$ is *in vitro* activated by Rac proteins (Rac1 and Rac2), to a lesser extent by Cdc42, but not by RhoA [30,35]. Regulation of PLC- β by Rho GTPases involves regions of PLC- β distinct from those required for interaction with $G\alpha_q$ and $G\beta\gamma$, and stimulation of PLC- $\beta 2$ by $G\beta\gamma$ subunits and Rac proteins was found to occur independently [30]. Activated Rac proteins bind to PLC- $\beta 2$ and PLC- $\beta 3$, but not to PLC- $\beta 1$, probably *via* the PH domain of the enzymes [30,36], and stimulate PLC- β with the rank order PLC- $\beta 2$ > PLC- $\beta 3$ \geq PLC- $\beta 1$. Rac2 also induces membrane association of PLC- $\beta 2$, which requires the PH domain but also depends on the C-terminal region of PLC- $\beta 2$ [37]. PLC- $\beta 2$ is highly expressed in myeloid cells, and Rac proteins may be involved in stimulation of the enzyme by chemoattractant receptors [38]. Thus, the PH domains of PLC- β are more than tethering devices and additionally mediate enzyme regulation by $G\beta\gamma$ subunits and Rac proteins.

4. Regulation of the PLC- ϵ isoform

Five years ago, PLC- ϵ has been identified as a novel mammalian PLC isoform with unique structural and regulatory features. PLC- ϵ is most abundant in the heart, followed by the kidney, lung and brain [22–24]. The presence of a CDC25 domain possessing Ras-GEF activity at the N-terminus and two RA domains (RA1 and RA2) at the C-terminus directly links PLC- ϵ with Ras signaling [39,40]. Indeed, PLC- ϵ is obviously under control of Ras-like GTPases, in particular H-Ras, but also Rap1A and Rap2B, as well as α subunits of G_{12} type proteins and $G\beta\gamma$ subunits.

H-Ras, and maybe Rap1A, directly binds to the RA domains of PLC- ϵ [22,24], and binding of H-Ras to the RA2 domain seems critical for enzyme activation [41]. Binding of Ras proteins to PLC- ϵ may not be sufficient to directly affect its lipase activity, but profoundly changes its intracellular localization. Stimulation of PLC- ϵ in intact cells seems to involve additional cellular components, which might be present at the plasma membranes or intracellular compartments. The CDC25 domain of PLC- ϵ exhibits significant GEF activity towards Rap1A, and perhaps H-Ras, but not to R-Ras and Ral [23,42], and this domain is required for a long lasting translocation of PLC- ϵ to the Golgi compartment. PLC- ϵ has no GEF activity towards Rap2 proteins, structurally close relatives of Rap1A, but both Rap2A and Rap2B can bind to PLC- ϵ and, similar to H-Ras, Rap1A and TC21, enhance PLC- ϵ activity upon cotransfection in intact cells in an RA2-dependent manner [43–45]. Activation of PLC- ϵ by Ras and Rap1A seems critical for cellular survival and proliferation [43], and PLC- ϵ was shown to be involved in Ras-mediated development of skin tumors in mice [46]. PLC- ϵ can also be activated by RhoA, Rac1 and Ral. Their mode of activation is RA2-independent, and activation by RhoA is likely mediated by a region within the Y catalytic box of PLC- ϵ [45,47].

Both $G\alpha_{12}$ and $G\alpha_{13}$, but not $G\alpha_q$ or $G\alpha_i$, can increase PLC- ϵ activity [23,29], and stimulation of PLC- ϵ by G_{12} proteins may contribute to the mitogenic potential of the G_{12} family proteins [48]. PLC- ϵ contains a domain very similar to the C-terminal regulatory domain of PLC- β enzymes that mediates their interaction with $G\alpha_q$ proteins. Also $G\beta\gamma$ subunits can activate PLC- ϵ [29], but it is presently unclear whether the effects of $G\beta\gamma$ are mediated *via* interaction with the PH domain of PLC- ϵ . $G\beta_1$ in combination with $G\gamma_1$, $G\gamma_2$, $G\gamma_3$ and $G\gamma_{13}$ all activated PLC- ϵ : $G\beta_2$ and $G\beta_4$ equally stimulated PLC- ϵ , whereas $G\beta_3$ containing dimers were less active and $G\beta_5$ was inactive [29].

Consistent with its diverse regulation by G proteins, PLC- ϵ was found to be stimulated by GPCRs. We have shown that stimulation of the typically G_s -coupled β_2 -adrenoceptor and the prostaglandin E_1 prostanoid receptor, but also the M_3 muscarinic receptor, can induce PLC- ϵ stimulation *via* $G\alpha_s$, Epac and Rap2B [49–51]. This PLC and calcium signaling pathway is dependent on cyclic AMP, but independent of protein kinase A (PKA). Stimulation of PLC- ϵ by these receptors was specifically mediated by Rap2B, but not by H-Ras, Rap1A and Rap2A, and involved the Rap-specific GEF, Epac. Like PLC- ϵ , Epac is abundantly expressed in the heart [52], and integration of Epac and PLC- ϵ in β_2 -adrenoceptor signaling may modulate heart physiology. Indeed, PLC- $\epsilon^{-/-}$ mice were shown to have cardiac developmental and functional deficits [53,54]. Alternatively, lysophosphatidic acid (LPA), sphingosine 1-phosphate and thrombin stimulate PLC- ϵ *via* $G\alpha_{12/13}$ and Rap or Rho proteins, but partly also *via* PTX sensitive G proteins [45,55]. Signaling to PLC- ϵ is clearly not restricted to GPCRs. PLC- ϵ is activated by the PDGF receptor mediated by Ras and Rap1A [43], and EGF receptor signaling to PLC- ϵ is mediated by Rap2B, and involves the Rap-specific

GEF, RasGRP3 [56]. Thus, several distinctly organized signaling pathways seem to converge on the level of PLC- ϵ .

5. PLC signaling by GPCRs

GPCRs can activate PLC- β as well as PLC- ϵ and, by cross-talk with RTKs, modulate PLC- γ isoforms as well. PLC- β and PLC- ϵ are diversely regulated by heterotrimeric G protein subunits as well as monomeric GTPases, and PLC- ϵ is even under control of both GPCRs and RTKs. The central position of the individual PLC isoforms in cellular signaling demands that their activation is tightly controlled both in space and time. Indeed, PLC- β 1 has GAP activity towards $G\alpha_q$ proteins, and this mechanism, probably in concert with RGS proteins, may ensure steady-state signal amplitude and accelerate signal termination on agonist removal [16,57]. PLC- ϵ has Ras-GEF activity and might function as an amplifier for Rap1A signaling, leading to cellular proliferation, differentiation, lymphocyte aggregation, T-cell anergy and platelet activation [58]. PKA and PKC modulate PLC signaling: both inhibition and potentiation of PLC signals have been observed probably depending on the cellular setting. These mechanisms may, together with calcium feedback onto PLC, contribute to oscillations in PLC-dependent second messenger generation by GPCRs, and may thereby control amplitude, frequency and duration of PLC signals [59,60].

Monomeric GTPases likely direct PLC- β and PLC- ϵ signaling by membrane receptors into diverse pathways at distinct subcellular locations [61]. These pathways can be timely dissected: the rapid and initial phase of PLC- ϵ activation by PDGF was mediated by Ras, while prolonged activation of PLC- ϵ was mediated by Rap1A and required the GEF domain of PLC- ϵ [43]. Ras and Rap1A induced translocation of PLC- ϵ to the plasma membrane and to perinuclear Golgi regions, respectively [24], and likewise, Ras and Rap1A differentially mediated activation of the extracellular signal-regulated kinase (ERK) pathway by PLC- ϵ via Raf-1 kinase and B-Raf kinase [23,24].

Multiple PLC isoforms can be activated by a single receptor and may act in succession or in adjacent signaling cascades. For instance, PLC- δ 1 likely functions as a calcium signal amplifier and is activated by capacitative calcium entry upon PLC- β activation [62], a process shown to involve PLC- γ [63], but PLC- β can also directly bind and regulate PLC- δ 1 [64]. GPCRs for endothelin, LPA and thrombin were shown to independently activate both PLC- β and PLC- ϵ , but with agonist- and isoform-specific profiles [65]. Thus, PLC- β was predominantly involved in acute, and PLC- ϵ in sustained PI hydrolysis. We found that the M_3 muscarinic receptor, which activates PLC- β by coupling to $G\alpha_q$, can additionally activate PLC- ϵ by two different mechanisms. Activation of PLC- ϵ by Rap2B could be achieved by the cyclic AMP-dependent GEF, Epac [49,50], but alternatively, by the calcium and DAG-sensitive exchange factors RasGRP1 or RasGRP3 (own unpublished data). Likewise, stimulation of PLC- ϵ by the EGF receptor depended on Rap2B stimulation by RasGRP3, which required tyrosine phosphorylation by Src as well as active PLC- γ and calcium

[56]. This exciting cascade positions PLC- ϵ downstream of PLC- γ (and maybe PLC- β) during single receptor action, suggesting specific and coordinated functions for these PLC isoforms.

Recruitment of PLC isozymes to specialized membrane compartments likely contributes to the spatial regulation of PLC signaling. The PH domain of PLC- δ 1 binds to both PIP_2 and IP_3 which may successively control the membrane localization and catalytic activity of PLC- δ 1, and the termination of this process by the formation of IP_3 . The PH domain of PLC- β 1 preferentially binds phosphatidylinositol-3-phosphate and membrane recruitment of PLC- β 1 is supported by PI3K activity [66], but also $G\beta\gamma$ subunits and Rac can tether PLC- β isoforms via their PH domains. In addition, PDZ domain-containing proteins induce clustering of PLC- β enzymes and scaffolding of signaling molecules and apparently enhance PLC- β activation by GPCRs [18].

6. PLD isoforms and phosphatidic acid

Two mammalian PLD genes, PLD1 and PLD2, both with two splice variants have been identified [67–70]. PLD1 and PLD2 share four highly conserved catalytic domains comprising two HKD motifs, which are essential for catalytic activity (Fig. 3). PLD1 and PLD2 further possess N-terminal PH and PX domains as well as a polybasic PIP_2 binding motif within the catalytic core, and PIP_2 profoundly affects the cellular localization and enzyme activity of both isoforms [71–73]. PLD1 is extensively regulated by PKC and by GTPases of the ARF and Rho families [5,74], but PLD2 responds to ARF and to PKC as well [75]. 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 intracellular compartments, including the Golgi apparatus, endosomes, and the perinuclear region [76–78]. PLD2 is almost exclusively present at the plasma membrane in lipid raft fractions [79]. Also PLD1 is found in lipid rafts, and both PLD enzymes have been shown to associate with membrane receptors.

PLD can modulate cellular signaling due to the formation of several bioactive lipids, but most cellular responses are likely mediated by the immediate PLD reaction product PA [6]. PA can be further metabolized to the GPCR agonist LPA by phospholipase A_2 and to DAG by phosphatidate phosphohydrolase. However, GPCR-induced DAG production seems to mainly result from PIP_2 breakdown by PLC [80]. PA directly binds to cellular proteins, such as Raf-1 kinase, protein phosphatase 1, and mTOR, and can affect both cellular localization and activity of these proteins. Quenching of PA synthesis by primary alcohols has proven useful to investigate the involvement of PLD enzymes in cell physiology, and demonstrated a role for PLD in a variety of signaling processes, such as activation of phosphoinositide (PI3K, PIP5K) and protein (Akt, ERK1/2) kinases, calcium mobilization, agonist-induced secretion and actin stress fibre formation, and ERK-driven mitogenesis. Whether the effects are directly mediated by PA, or via generation of LPA is not always definitively answered. Inactive

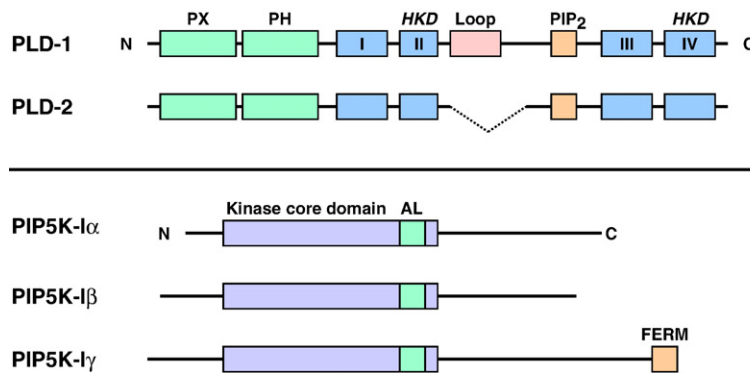


Fig. 3. Domain structure of PLD and PIP5K isoforms. The PLD isoforms PLD1 and PLD2, both with at least two alternate splice variants, contain N-terminal PX and PH domains and the highly conserved domains I–IV. The domains II and IV contain HKD sequence motifs that are necessary for catalytic activity. N-terminal to domain III is a well conserved basic sequence that binds PIP₂, whereas Gβγ subunits, PIP₃ and other phosphoinositides interact with the PX/PH region. PLD1 is distinguished by a loop region that seem to contribute to the regulation of PLD1 activity. The three PIP5K isoforms contain a conserved catalytic core with an activation loop (AL), that determines substrate specificity as well as subcellular targeting of the enzymes. Full-length PIP5K-γ, but not two shorter PIP5K-γ splice variants, comprises a C-terminal sequence that binds to the FERM domain of talin.

mutants and RNA interference are now used to further discriminate isoform-specific PLD functions and showed that PLD1 is implicated in agonist-induced secretion, actin organization, and cell adhesion and migration [81–84], and PLD2 in endocytosis and recycling of membrane receptors [85–87].

7. Regulation of PLD isoforms by GPCRs

Signal-dependent activation of PLD enzymes has been demonstrated in numerous cell types, and both PLD1 [88,89] and PLD2 [90,91] seem to respond to GPCRs. As nearly every membrane receptor known to stimulate PLC isoforms caused PLD stimulation, it was assumed that PLD activation might be secondary to PLC-initiated increase in cytosolic [Ca²⁺] and activation of PKC isoforms. Indeed, PKC inhibitors and down-regulation of DAG-dependent PKC reduced receptor-induced PLD responses. 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 [92]. Moreover, PLD1 mutants unresponsive to PKC did poorly respond to activation of GPCRs [88] or to active Gα_q [93]. However, stimulation of PLD in some receptor systems, including M₃ muscarinic and α₁-adrenergic receptors, were actually PKC-independent [94–97], suggesting that PLD stimulation must not necessarily be secondary to PLC stimulation.

Stimulation of PLD by GPCRs was shown to involve both PTX-insensitive [94,98] and PTX-sensitive [99,100] G proteins. Besides G_q, G₁₂ family proteins can stimulate PLD as well [101], and RGS proteins, that act as α subunit-specific GAPs, have been used to demonstrate that G₁₂ proteins mediate PLD activation by the M₃ muscarinic [102], the PAR1 [103], and the Ca²⁺-sensing receptor [104] as well as mechanical force [105]. As forskolin and cAMP were shown to activate PLD mediated by PKA and ERK1/2 [106,107] or, alternatively, by Epac and R-Ras [108], G_s proteins are also expected to couple to PLD. PLD activation is also controlled by βγ-subunits [109,110] and Gβγ can directly interact with PLD [111].

8. Regulation of PLD by monomeric GTPases

ARF proteins are involved in the regulation of intracellular transport and membrane trafficking, and have been identified as regulators of PLD activity. Although the interaction site on PLD for ARF has not yet been precisely defined, it is well established that ARF proteins, particularly ARF1 and ARF6, directly activate PLD1 and can also activate, to a lesser extent, PLD2 [67,68]. Initial evidence for participation of ARF proteins in receptor-mediated PLD stimulation was based on *in vitro* reconstitution studies with membranes and ARF-depleted permeabilized cells. In intact cells, receptor signaling to PLD was reduced with the ARF-GEF inhibitor Brefeldin A [99,112–114]. Likewise, sequestration of ARF-GEFs by the ARF-related protein ARP inhibited M₃ muscarinic receptor signaling to PLD [115]. Rho proteins, particularly RhoA, Rac1 and Cdc42, which control actin cytoskeleton reorganization, exclusively activate PLD1 by direct interaction with its C-terminus, and this activation can be triggered by PKC-α and ARF1 [5,74]. Clostridial toxins that specifically inactivate Rho proteins, and expression of inactive Rho mutants have been used to study the role of Rho proteins in signaling to PLD. Thus, RhoA was found to be relevant for PLD stimulation by both GPCR (bradykinin, sphingosine-1-phosphate and LPA) and RTK (PDGF) agonists. We found that Rho proteins, particularly RhoA, specifically affect signaling to PLD by the muscarinic M₃ receptor, without changing PLD stimulation by RTKs in HEK-293 cells [116–119]. Likewise, regulation of mTOR by LPA, but not PDGF, involved PLD1 activation by Rho GTPases [120]. PLD stimulation by RhoA may be mediated by direct interaction, but apparently also involve Rho-dependent serine/threonine kinases. Thus, Rho-kinase was found to mediate PLD activation by the M₃ muscarinic receptor [119] and PKN to directly interact with PLD [121] and mediate PLD activation by the α₁-adrenergic receptor [122].

The contribution of ARF and Rho proteins to GPCR-induced PLD stimulation likely depends on receptor function in specific cellular settings. For instance in HEK-293 cells, the PLD

response to the M₃ muscarinic receptor seems to be regulated in concerted action by independent ARF and Rho pathways, and both ARF1 and RhoA translocated to the membrane compartment after receptor activation [112,123]. In cardiomyocytes, Rho proteins affect signaling to PLD by both endothelin-1 and thrombin, apparently by controlling PIP₂ synthesis, whereas ARF selectively affects signaling by the PAR1 receptor [103]. The picture emerges that G₁₂ proteins induce PLD activation by Arf and Rho proteins, whereas G_q proteins control PLD in a PKC-dependent manner. ARF and Rho have been found to localize in PLD containing lipid rafts, and ARF proteins to directly interact with GPCRs [124]. The identification of ARAP proteins, which activate ARF and Rho signaling pathways and can be activated by Ras-like GTPases, suggest even more dynamic signaling towards PLD [125].

PLD1 can also directly interact with RalA, and a Ras/Ral signaling cascade was shown to regulate PLD responses. Ral apparently cooperates with ARF [126,127] and Rho proteins [128,129] to achieve full PLD activation. Experiments with clostridial toxins and inactive mutants located Ras and RalA in RTK, but not muscarinic M₃ receptor signaling to PLD, and this Ras/Ral-dependent signaling cascade was dependent on PKC- α and a Ral-specific GEF [117,118]. Likewise, Ras proteins were found to modulate PLD responses by PDGF [130], and RalA to affect EGF receptor signaling to PLD [131]. Ras-related R-Ras was recently found to be involved in GPCR signaling to PLD [108], but a contribution of Ral to GPCR-induced PLD activation seems unlikely [132].

9. Regulation of PIP₂ synthesis by GPCRs and GTPases

Although PIP₂ represents less than 1% of cellular phospholipid, this polyphosphoinositide directly affects an astonishing variety of fundamental cellular processes, including the regulation of the actin cytoskeleton, membrane trafficking and ion channel activity [7,8]. To execute these different tasks, PIP₂ is generally supposed not to be randomly distributed in cells, but to be organized in confined regions, for instance lipid rafts, that may control specific cellular functions [133,134]. The synthesis and turnover of the lipid in particular subcellular compartments is believed to be detailed and distinctly regulated, especially by monomeric GTPases [135]. PIP₂ is mainly generated *via* the phosphorylation of phosphatidylinositol-4-phosphate by PIP5K. Three isoforms of PIP5K (designated I α , I β and I γ) with alternative splice variants have been cloned and characterized (Fig. 3) [136–138]. The existence of PIP5K isoforms likely contributes to the organization of diverse structural and functional PIP₂ pools. For instance, the short PIP5K-I γ splice variant seems the major producer of the PIP₂ pool that supports GPCR-induced IP₃ generation [139]. PIP₂ is not only consumed by PLC-mediated hydrolysis, but can also be further phosphorylated by PI3K to PIP₃, which recruits and activates mediators involved in actin remodeling, mitogenesis and survival [140]. Dephosphorylation of PIP₂ by inositol polyphosphate 5-phosphatases, such as synaptojanin, is clearly involved in the regulation of local PIP₂ concentrations and is indispensable for PIP₂ recycling during vesicle trafficking [141].

The synthesis of PIP₂ can be stimulated by both GPCRs and RTKs [8]. Modulation of PIP₂ synthesis by GPCRs has been mainly studied in platelets, where stimulation with thrombin resulted in enhanced PIP₂ formation [142] and in increased association of PIP5K with the cytoskeleton [143]. The receptor-induced stimulation of PIP5K may be directly involved in actin cytoskeletal regulation and/or to guarantee efficient signaling, for instance by PLC. We found that pulse stimulation of various GPCRs induced a strong and long-lasting potentiation of agonist-induced PLC activation [144,145]. This PLC sensitization was apparently dependent on G_i proteins and PKC, and could be partly explained by an increase in the cellular level of PIP₂. In agreement, GPCR-induced stimulation of PIP₂ synthesis was found to be blocked by PTX [146], and stimulation of PKC isoforms by phorbol esters greatly enhances PIP₂ synthesis [147–149]. Indeed, LPA-induced PKC activation increased PIP5K activity, most probably by stimulating PIP5K dephosphorylation by protein phosphatase 1 [150].

PIP5K is, like PLD, under control of both Rho and ARF family GTPases. RhoA stimulates PIP₂ synthesis [151], and PIP5K functions downstream of RhoA in actin organization [152]. We have shown that Rho-kinase is involved in RhoA-dependent regulation of PIP5K activity [153], and PIP5K was found to play an essential role as downstream effector of RhoA and Rho-kinase in neurite remodeling [154,155] and to mediate the effects of RhoA on ERM (ezrin, radixin, moesin) activation and microvilli formation [156,157]. PIP5K isoforms are also activated by Rac proteins and seem critical mediators of Rac-dependent actin remodeling. PIP5K mediates actin filament assembly induced by thrombin or Rac1 [158] and membrane ruffle formation by PDGF [159], and PIP₂ functions downstream of Rac proteins in pollen tube elongation [160]. PIP5K isoforms were found to physically associate with both RhoA [161] and Rac1 [158]. Cdc42 does not interact with PIP5K [162,163], but markedly stimulates PIP₂ synthesis [163], and it is speculated that the interaction with the Rho GTPases serves the recruitment of PIP5K to specific cellular compartments. PIP5K is also activated by ARF proteins. ARF1 mediates PIP5K activation [164] and recruitment to the Golgi complex [165]. ARF6 colocalizes with PIP5K and PIP₂ on the plasma membrane and endosomal structures [166,167], and the ARF6-regulated PIP5K activity and organized PIP₂ turnover is apparently critical for regulated secretion [167–170], but also affects phagocytosis [171] as well as neurite formation [172].

10. Interaction of phosphoinositide metabolism with PLC and PLD

PIP₂ takes a strategic position in phospholipid signaling by GPCRs. PIP₂ is the major PLC substrate, and both PLD enzymes have an almost absolute requirement for the lipid. *Vice versa*, all PIP5K enzymes are specifically and potently stimulated by PA [138,173,174]. This stimulation may be essential for resynthesis of PIP₂ in response to PIP₂ hydrolysis by PLC and subsequent conversion of DAG to PA. Alternatively, PA is generated by PLD, and DGK- as well as PLD-derived PA was able to stimulate PIP5K activity *in vivo* [175].

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. Indeed, phosphoinositide kinases and PLD might directly cooperate in the control of cellular processes. Both PLD1 and PLD2 interact with PIP5K- α , and PLD2 recruits PIP5K- α to a submembrane vesicular compartment [176]. Quenching of PA production with primary alcohols inhibited PIP₂ synthesis and led to disruption of Golgi membranes [177], blockade of clathrin-coat assembly [178] and inhibition of ARF1-reconstituted secretion [179], demonstrating that PLD-induced PIP₂ synthesis is essential for maintaining the function of the Golgi apparatus and the endo- and exocytotic machinery. 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 the regulation of PIP₂ synthesis in intact cells [180].

We have to realize that receptor-induced hydrolysis of PIP₂ by PLC leads, in addition to second messenger generation, to the consumption of PIP₂ [181], which likely affects cellular processes. Agonist-induced PLC signaling (for instance by the muscarinic M₁ receptor or the α_1 -adrenoceptor) or expression of active G α_q resulted, most probably by causing PIP₂ depletion, in the inactivation of various ion channels [182–185], the release of gelsolin and actin remodeling [186,187], and apoptosis due to suppression of the PI3K protective pathway and attenuation of Akt phosphorylation [188]. Reversely, sequestering of PIP₂ by profilin and tubulin at high concentrations was shown to inhibit PLC signaling [189,190]. Reduction of cellular PIP₂ by the actin binding protein fodrin [191] or the PIP₂ phosphatase synaptojanin [192] likewise inhibited PLD activity. Inactivation of Rho GTPases also reduced cellular PIP₂ levels, resulting in inhibition of receptor-mediated inositol phosphate formation by PLC [193] as well as diminished PLD stimulation [194]. The inhibition of PLD responses after Rho inactivation could be largely rescued by the addition of PIP₂, indicating that Rho proteins actually affect PLD *via* PIP5K regulation [116,194]. These findings suggest that PLD enzymes may be implicated in various phosphoinositide-driven cellular transport processes.

In addition, PA can also modulate the activity of PLC enzymes. PLC- γ 1 binds specifically *via* its SH3 domain to the PX domain of PLD2 [21], and PIP₂-dependent membrane recruitment and activation of PLD2 may result in co-transport of PLC- γ 1 and PA-induced activation of PLC- γ 1. PA also enhances PLC- β 1 activity, and renders PLC- β 1 less sensitive to inhibition by PKC [195].

11. Concluding remarks

Our view of how GPCRs transduce signals into cells has fundamentally changed during the last decade. It is now appreciated that a particular receptor does not merely activate a single second messenger system in a fixed, preassigned manner. Dependent on the cellular context, GPCRs rather communicate with other GPCRs and RTKs as well, couple to

different heterotrimeric G protein families and can drive diverse signaling networks which are additionally directed by small GTPases. We presented an overview of the remarkable progress in our knowledge about the mechanisms of signal integration from GPCRs to the distinct PLC and PLD enzymes. Signaling by these enzymes contribute to very different processes to allow specific cellular tasking. Protein–protein and protein–lipid interactions mediated by distinct regulatory domains confer specificity into the highly dynamic organization of phospholipid signaling in space and time, and tightly controls transient or sustained phospholipase signaling. The appropriate intracellular localization of the signaling units is controlled by small GTPases, calcium, cAMP and phosphoinositides, and the turnover of PIP₂ and other polyphosphoinositides affect the quality of PLC and PLD signaling. Agonist-induced recruitment and compartmentalization of signaling components, for instance in lipid rafts and other microdomains, likely allows localized changes in effectors and initiation of cellular processes without global changes to avoid unwanted side-effects. On the other hand, it is becoming increasingly clear that the products generated (as well as consumed) by PLC, PLD, and phosphoinositide kinases and phosphatases act in close harmony to effectively accomplish cellular signaling. In the coming years, we expect a great expansion in our understanding of how membrane receptors control the temporal and spatial production of second messengers to regulate a diversity of cellular processes.

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References

- [1] Y. Nishizuka, Discovery and prospect of protein kinase C research: epilogue, *J. Biochem.* 133 (2003) 155–158.
- [2] M.J. Berridge, Unlocking the secrets of cell signaling, *Annu. Rev. Physiol.* 67 (2005) 1–21.
- [3] M. Liscovitch, M. Czarny, G. Fiucci, X. Tang, Phospholipase D: molecular and cell biology of a novel gene family, *Biochem. J.* 345 (2000) 401–415.
- [4] S. Cockcroft, Signalling roles of mammalian phospholipase D1 and D2, *Cell. Mol. Life Sci.* 58 (2001) 1674–1687.
- [5] J.H. Exton, Regulation of phospholipase D, *FEBS Lett.* 531 (2002) 58–61.
- [6] G.M. Jenkins, M.A. Frohman, Phospholipase D: a lipid centric review, *Cell. Mol. Life Sci.* 62 (2005) 2305–2316.
- [7] A. Toker, Phosphoinositides and signal transduction, *Cell. Mol. Life Sci.* 59 (2002) 761–779.
- [8] P.A. Oude Weernink, M. Schmidt, K.H. Jakobs, Regulation and cellular roles of phosphoinositide 5-kinases, *Eur. J. Pharmacol.* 500 (2004) 87–99.
- [9] M.J. Rebecchi, S.N. Pentyala, Structure, function, and control of phosphoinositide-specific phospholipase C, *Physiol. Rev.* 80 (2000) 1291–1335.

- [10] S.G. Rhee, Regulation of phosphoinositide-specific phospholipase C, *Annu. Rev. Biochem.* 70 (2001) 281–312.
- [11] M. Schmidt, P.A. Oude Weernink, F. vom Dorp, M.B. Stope, K.H. Jakobs, Mammalian phospholipase C, *Adv. Mol. Cell. Biol.* 33 (2004) 431–450.
- [12] T. Yamamoto, H. Takeuchi, T. Kanematsu, V. Allen, H. Yagisawa, U. Kikkawa, Y. Watanabe, A. Nakasima, M. Katan, M. Hirata, Involvement of EF hand motifs in the Ca^{2+} -dependent binding of the pleckstrin homology domain to phosphoinositides, *Eur. J. Biochem.* 265 (1999) 481–490.
- [13] B. Ananthanarayanan, S. Das, S.G. Rhee, D. Murray, W. Cho, Membrane targeting of C2 domains of phospholipase C- δ isoforms, *J. Biol. Chem.* 277 (2002) 3568–3575.
- [14] A.U. Singer, G.L. Waldo, T.K. Harden, J. Sondek, A unique fold of phospholipase C- β mediates dimerization and interaction with $\text{G}\alpha_q$, *Nat. Struct. Biol.* 9 (2002) 32–36.
- [15] O. Ilkaeva, L.N. Kinch, R.H. Paulsen, E.M. Ross, Mutations in the carboxyl-terminal domain of phospholipase C- β 1 delineate the dimer interface and a potential $\text{G}\alpha_q$ interaction site, *J. Biol. Chem.* 277 (2002) 4294–4300.
- [16] E.M. Ross, T.M. Wilkie, GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins, *Annu. Rev. Biochem.* 69 (2000) 795–827.
- [17] C.G. Kim, D. Park, S.G. Rhee, The role of carboxyl-terminal basic amino acids in $\text{G}\alpha_q$ -dependent activation, particulate association, and nuclear localization of phospholipase C- β 1, *J. Biol. Chem.* 271 (1996) 21187–21192.
- [18] P.G. Suh, J.I. Hwang, S.H. Ryu, M. Donowitz, J.H. Kim, The roles of PDZ-containing proteins in PLC- β -mediated signaling, *Biochem. Biophys. Res. Commun.* 288 (2001) 1–7.
- [19] J.S. Chang, H. Seok, T.K. Kwon, D.S. Min, B.H. Ahn, Y.H. Lee, J.W. Suh, J.W. Kim, S. Iwashita, A. Omori, S. Ichinose, O. Numata, J.K. Seo, Y.S. Oh, P.G. Suh, Interaction of elongation factor-1 α and pleckstrin homology domain of phospholipase C- γ 1 with activating its activity, *J. Biol. Chem.* 277 (2002) 19697–19702.
- [20] K. Ye, B. Aghdasi, H.R. Luo, J.L. Moriarity, F.Y. Wu, J.J. Hong, K.J. Hurt, S.S. Bae, P.G. Suh, S.H. Snyder, Phospholipase C γ 1 is a physiological guanine nucleotide exchange factor for the nuclear GTPase PIKE, *Nature* 415 (2002) 541–544.
- [21] I.H. Jang, S. Lee, J.B. Park, J.H. Kim, C.S. Lee, E.M. Hur, I.S. Kim, K.T. Kim, H. Yagisawa, P.G. Suh, S.H. Ryu, The direct interaction of phospholipase C- γ 1 with phospholipase D2 is important for epidermal growth factor signaling, *J. Biol. Chem.* 278 (2003) 18184–18190.
- [22] G.G. Kelley, S.E. Reks, J.M. Ondrako, A.V. Smrcka, Phospholipase C ϵ : a novel Ras effector, *EMBO J.* 20 (2001) 743–754.
- [23] I. Lopez, E.C. Mak, J. Ding, H.E. Hamm, J.W. Lomasney, A novel bifunctional phospholipase C that is regulated by $\text{G}\alpha_{12}$ and stimulates the Ras/mitogen-activated protein kinase pathway, *J. Biol. Chem.* 276 (2001) 2758–2765.
- [24] C. Song, C.D. Hu, M. Masago, K. Kariya, Y. Yamawaki-Kataoka, M. Shibatohe, D. Wu, T. Satoh, T. Kataoka, Regulation of a novel human phospholipase C, PLC ϵ , through membrane targeting by Ras, *J. Biol. Chem.* 276 (2001) 2752–2757.
- [25] C.M. Saunders, M.G. Larman, J. Parrington, L.J. Cox, J. Royse, L.M. Blayney, K. Swann, F.A. Lai, PLC ζ : a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development, *Development* 129 (2002) 3533–3544.
- [26] S. Cockcroft, The latest phospholipase C, PLC η , is implicated in neuronal function, *Trends Biochem. Sci.* 31 (2006) 4–7.
- [27] L.W. Runnels, S.F. Scarlata, Determination of the affinities between heterotrimeric G protein subunits and their phospholipase C- β effectors, *Biochemistry* 38 (1999) 1488–1496.
- [28] T. Wang, S. Pentyala, J.T. Elliott, L. Dowal, E. Gupta, M.J. Rebecchi, S. Scarlata, Selective interaction of the C2 domains of phospholipase C- β 1 and - β 2 with activated $\text{G}\alpha_q$ subunits: an alternative function for C2-signaling modules, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 7843–7846.
- [29] M.R. Wing, D. Houston, G.G. Kelley, C.J. Der, D.P. Siderovski, T.K. Harden, Activation of phospholipase C- ϵ by heterotrimeric G protein $\beta\gamma$ -subunits, *J. Biol. Chem.* 276 (2001) 48257–48261.
- [30] D. Illenberger, C. Walliser, B. Nürnberg, M. Diaz Lorente, P. Gierschik, Specificity and structural requirements of phospholipase C- β stimulation by Rho GTPases versus G protein by dimers, *J. Biol. Chem.* 278 (2003) 3006–3014.
- [31] B. Sankaran, J. Osterhout, D. Wu, A.V. Smrcka, Identification of a structural element in phospholipase C β 2 that interacts with G protein $\beta\gamma$ subunits, *J. Biol. Chem.* 273 (1998) 7148–7154.
- [32] T. Wang, L. Dowal, M.R. El-Maghrabi, M. Rebecchi, S. Scarlata, The pleckstrin homology domain of phospholipase C- β 2 links the binding of $\text{G}_{\beta\gamma}$ to activation of the catalytic core, *J. Biol. Chem.* 275 (2000) 7466–7469.
- [33] S. Offermanns, C.F. Toombs, Y.H. Hu, M.I. Simon, Defective platelet activation in $\text{G}\alpha_q$ -deficient mice, *Nature* 389 (1997) 183–186.
- [34] J.S.C. Chan, J.W.M. Lee, M.K.C. Ho, Y.H. Wong, Preactivation permits subsequent stimulation of phospholipase C by G_i -coupled receptors, *Mol. Pharmacol.* 57 (2000) 700–708.
- [35] D. Illenberger, F. Schwald, D. Pimmer, W. Binder, G. Maier, A. Dietrich, P. Gierschik, Stimulation of phospholipase C- β 2 by the Rho GTPases Cdc42Hs and Rac1, *EMBO J.* 17 (1998) 6241–6249.
- [36] J.T. Snyder, A.U. Singer, M.R. Wing, T.K. Harden, J. Sondek, The pleckstrin homology domain of phospholipase C- β 2 as an effector site for Rac, *J. Biol. Chem.* 278 (2003) 21099–21104.
- [37] D. Illenberger, C. Walliser, J. Strobel, O. Gutman, H. Niv, V. Gaidzik, Y. Kloog, P. Gierschik, Y.I. Henis, Rac2 regulation of phospholipase C- β 2 activity and mode of membrane interactions in intact cells, *J. Biol. Chem.* 278 (2003) 8645–8652.
- [38] M.J. Rabiet, M. Tardif, L. Braun, F. Boulay, Inhibitory effects of a dominant-interfering form of the Rho-GTPase Cdc42 in the chemoattractant-elicited signaling pathways leading to NADPH oxidase activation in differentiated HL-60 cells, *Blood* 100 (2002) 1835–1844.
- [39] L.A. Quilliam, J.F. Rebhun, A.F. Castro, A growing family of guanine nucleotide exchange factors is responsible for activation of ras-family GTPases, *Prog. Nucleic Acids Res. Mol. Biol.* 71 (2002) 391–444.
- [40] P.J. Cullen, P.J. Lockyer, Integration of calcium and Ras signalling, *Nat. Rev., Mol. Cell Biol.* 3 (2002) 339–348.
- [41] T.D. Bunney, R. Harris, N. Lamuño Garillas, M.B. Josephs, S.M. Roe, S.C. Sorli, H.F. Paterson, F. Rodrigues-Lima, D. Esposito, C.P. Ponting, P. Gierschik, L.H. Pearl, P.C. Driscoll, M. Katan, Structural and mechanistic insights into Ras association domains of phospholipase C epsilon, *Mol. Cell* 21 (2006) 495–507.
- [42] T.G. Jin, T. Satoh, Y. Liao, C. Song, X. Gao, K. Kariya, C.D. Hu, T. Kataoka, Role of the CDC25 homology domain of phospholipase C ϵ in amplification of Rap1-dependent signaling, *J. Biol. Chem.* 276 (2001) 30301–30307.
- [43] C. Song, T. Satoh, H. Edamatsu, D. Wu, M. Tadano, X. Gao, T. Kataoka, Differential roles of Ras and Rap1 in growth factor-dependent activation of phospholipase C ϵ , *Oncogene* 21 (2002) 8105–8113.
- [44] G.A. Murphy, S.M. Graham, S. Morita, S.E. Reks, K. Rogers-Graham, A. Vojtek, G.G. Kelley, C.J. Der, Involvement of phosphatidylinositol 3-kinase, but not RalGDS, in TC21/R-Ras2-mediated transformation, *J. Biol. Chem.* 277 (2002) 9966–9975.
- [45] G.G. Kelley, S.E. Reks, A.V. Smrcka, Hormonal regulation of phospholipase C ϵ through distinct and overlapping pathways involving G_{12} and Ras family G-proteins, *Biochem. J.* 378 (2004) 129–139.
- [46] Y. Bai, H. Edamatsu, S. Maeda, H. Saito, N. Suzuki, T. Satoh, T. Kataoka, Crucial role of phospholipase C ϵ in chemical carcinogen-induced skin tumor development, *Cancer Res.* 64 (2004) 8808–8810.
- [47] J.P. Seifert, M.R. Wing, J.T. Snyder, S. Gershburg, J. Sondek, T.K. Harden, RhoA activates purified phospholipase C- ϵ by a guanine nucleotide-dependent mechanism, *J. Biol. Chem.* 279 (2004) 47992–47997.
- [48] H. Kurose, $\text{G}\alpha_{12}$ and $\text{G}\alpha_{13}$ as key regulatory mediator in signal transduction, *Life Sci.* 74 (2003) 155–161.
- [49] M. Schmidt, S. Evellin, P.A. Oude Weernink, F. vom Dorp, H. Rehmann, J.W. Lomasney, K.H. Jakobs, A new phospholipase-C-calcium signalling pathway mediated by cyclic AMP and a Rap GTPase, *Nat. Cell Biol.* 3 (2001) 1020–1024.

- [50] S. Evellin, J. Nolte, K. Tysack, F. vom Dorp, M. Thiel, P.A. Oude Weernink, K.H. Jakobs, E.J. Webb, J.W. Lomasney, M. Schmidt, Stimulation of phospholipase C- ϵ by the M3 muscarinic acetylcholine receptor mediated by cyclic AMP and the GTPase Rap2B, *J. Biol. Chem.* 277 (2002) 16805–16813.
- [51] M. Keiper, M.B. Stope, D. Szatkowski, A. Böhm, K. Tysack, F. vom Dorp, O. Saur, P.A. Oude Weernink, S. Evellin, K.H. Jakobs, M. Schmidt, Epac- and Ca^{2+} -controlled activation of Ras and extracellular signal-regulated kinases by G_s -coupled receptors, *J. Biol. Chem.* 279 (2004) 46497–46508.
- [52] J. de Rooij, F.J.T. Zwartkruis, M.H.G. Verheijen, R.H. Cool, S.M.B. Nijman, A. Wittinghofer, J.L. Bos, Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP, *Nature* 396 (1998) 474–477.
- [53] M. Tadano, H. Edamatsu, S. Minamisawa, U. Yokoyama, Y. Ishikawa, N. Suzuki, H. Saito, D. Wu, M. Masago-Toda, Y. Yamawaki-Kataoka, T. Setzu, T. Terashima, S. Maeda, T. Satoh, T. Kataoka, Congenital semilunar valvulogenesis defect in mice deficient in phospholipase C ϵ , *Mol. Cell. Biol.* 25 (2005) 2191–2199.
- [54] H. Wang, E.A. Oestreich, N. Maekawa, T.A. Bullard, K.L. Vikstrom, R.T. Dirksen, G.G. Kelley, B.C. Blaxall, A.V. Smrcka, Phospholipase C ϵ modulates β -adrenergic receptor-dependent cardiac contraction and inhibits cardiac hypertrophy, *Circ. Res.* 97 (2005) 1305–1313.
- [55] M.D. Hains, M.R. Wing, S. Maddileti, D.P. Siderovski, T.K. Harden, $\text{G}\alpha_{12/13}$ - and Rho-dependent activation of phospholipase C- ϵ by lysophosphatidic acid and thrombin receptors, *Mol. Pharmacol.* 69 (2006) 2068–2075.
- [56] M.B. Stope, F. vom Dorp, D. Szatkowski, A. Böhm, M. Keiper, J. Nolte, P.A. Oude Weernink, D. Rosskopf, S. Evellin, K.H. Jakobs, M. Schmidt, Rap2B-dependent stimulation of phospholipase C- ϵ by epidermal growth factor receptor mediated by c-Src phosphorylation of RasGRP3, *Mol. Cell. Biol.* 24 (2004) 4664–4676.
- [57] S. Mukhopadhyay, E.M. Ross, Rapid GTP binding and hydrolysis by G_q promoted by receptor and GTPase-activating proteins, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 9539–9544.
- [58] J.L. Bos, J. de Rooij, K.A. Reedquist, Rap1 signalling: adhering to new models, *Nat. Rev., Mol. Cell Biol.* 2 (2002) 369–377.
- [59] M.S. Nash, K.W. Young, R.A.J. Challiss, S.R. Nahorski, Intracellular signalling. Receptor-specific messenger oscillations, *Nature* 413 (2001) 381–382.
- [60] K.W. Young, M.S. Nash, R.A.J. Challiss, S.R. Nahorski, Role of Ca^{2+} feedback on single cell inositol 1,4,5-trisphosphate oscillations mediated by G-protein-coupled receptors, *J. Biol. Chem.* 278 (2003) 20753–20760.
- [61] T.K. Harden, J. Sondek, Regulation of phospholipase C isozymes by Ras superfamily GTPases, *Annu. Rev. Pharmacol. Toxicol.* 46 (2006) 355–379.
- [62] Y.H. Kim, T.J. Park, Y.H. Lee, K.J. Baek, P.G. Suh, S.H. Ryu, K.T. Kim, Phospholipase C- δ 1 is activated by capacitative calcium entry that follows phospholipase C- β activation upon bradykinin stimulation, *J. Biol. Chem.* 274 (1999) 26127–26134.
- [63] R.L. Patterson, D.B. van Rossum, D.L. Ford, K.J. Hurt, S.S. Bae, P.G. Suh, T. Kurosaki, S.H. Snyder, D.L. Gill, Phospholipase C- γ is required for agonist-induced Ca^{2+} entry, *Cell* 111 (2002) 529–541.
- [64] Y. Guo, M. Rebecchi, S. Scarlata, Phospholipase C β_2 binds to and inhibits phospholipase C δ_1 , *J. Biol. Chem.* 280 (2005) 1438–1447.
- [65] G.G. Kelley, K.A. Kaproth-Joslin, S.E. Reks, A.V. Smrcka, R.J.H. Wojcikiewicz, G-protein-coupled receptor agonists activate endogenous phospholipase C ϵ and phospholipase C β 3 in a temporally distinct manner, *J. Biol. Chem.* 281 (2006) 2639–2648.
- [66] G. Razzini, A. Brancaccio, M.A. Lemmon, S. Guarnieri, M. Falasca, The role of pleckstrin homology domain in membrane targeting and activation of phospholipase C β_1 , *J. Biol. Chem.* 275 (2000) 14873–14881.
- [67] S.M. Hammond, Y.M. Altshuller, T.C. Sung, S.A. Rudge, K. Rose, J. Engebrecht, A.J. Morris, M.A. Frohman, Human ADP-ribosylation factor-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved gene family, *J. Biol. Chem.* 270 (1995) 29640–29643.
- [68] S.M. Hammond, J.M. Jenco, S. Nakashima, K. Cadwallader, Q. Gu, S. Cook, Y. Nozawa, G.D. Prestwich, M.A. Frohman, A.J. Morris, Characterization of two alternately spliced forms of phospholipase D1. Activation of the purified enzymes by phosphatidylinositol 4,5-bisphosphate, ADP-ribosylation factor, and Rho family monomeric GTP-binding proteins and protein kinase C- α , *J. Biol. Chem.* 272 (1997) 3860–3868.
- [69] W.C. Colley, T.C. Sung, R. Roll, J. Jenco, S.M. Hammond, Y. Altshuller, D. Bar-Sagi, A.J. Morris, M.A. Frohman, Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization, *Curr. Biol.* 7 (1997) 191–201.
- [70] P.M. Steed, K.L. Clark, W.C. Boyar, D.J. Lasala, Characterization of human PLD2 and the analysis of PLD isoform splice variants, *FASEB J.* 12 (1998) 1309–1317.
- [71] M.N. Hodgkin, M.R. Masson, D. Powner, K.M. Saqib, C.P. Ponting, M.J.O. Wakelam, Phospholipase D regulation and localisation is dependent upon a phosphatidylinositol 4,5-bisphosphate-specific PH domain, *Curr. Biol.* 10 (2000) 43–46.
- [72] V.A. Sciorra, S.A. Rudge, J. Wang, S. McLaughlin, J. Engebrecht, A.J. Morris, Dual role for phosphoinositides in regulation of yeast and mammalian phospholipase D enzymes, *J. Cell Biol.* 159 (2002) 1039–1049.
- [73] G. Du, Y.M. Altshuller, N. Vitale, P. Huang, S. Chasserot-Golaz, A.J. Morris, M.-F. Bader, M.A. Frohman, Regulation of phospholipase D1 subcellular cycling through coordination of multiple membrane association motifs, *J. Cell Biol.* 162 (2003) 305–315.
- [74] D.J. Powner, M.J.O. Wakelam, The regulation of phospholipase D by inositol phospholipids and small GTPases, *FEBS Lett.* 531 (2002) 62–64.
- [75] J.S. Chen, J.H. Exton, Regulation of phospholipase D2 activity by protein kinase C α , *J. Biol. Chem.* 279 (2004) 22076–22083.
- [76] F.D. Brown, N. Thompson, K.M. Saqib, J.M. Clark, D. Powner, N.T. Thompson, R. Solari, M.J.O. Wakelam, Phospholipase D1 localises to secretory granules and lysosomes and is plasma-membrane translocated on cellular stimulation, *Curr. Biol.* 8 (1998) 835–838.
- [77] Z. Freyberg, D. Sweeney, A. Siddhanta, S. Bourgoin, M. Frohman, D. Shields, Intracellular localization of phospholipase D1 in mammalian cells, *Mol. Biol. Cell* 12 (2001) 943–955.
- [78] W.E. Hughes, P.J. Parker, Endosomal localization of phospholipase D 1a and 1b is defined by the C-termini of the proteins, and is independent of activity, *Biochem. J.* 356 (2001) 727–736.
- [79] M. Czarny, Y. Lavie, G. Fiucci, M. Liscovitch, Localization of phospholipase D in detergent-insoluble, caveolin-rich membrane domains, *J. Biol. Chem.* 274 (1999) 2717–2724.
- [80] L.S. Nilssen, O. Dajani, T. Christoffersen, D. Sandnes, Sustained diacylglycerol accumulation resulting from prolonged G protein-coupled receptor agonist-induced phosphoinositide breakdown in hepatocytes, *J. Cell. Biochem.* 94 (2005) 389–402.
- [81] N. Vitale, A.-S. Caumont, S. Chasserot-Golaz, G. Du, S. Wu, V.A. Sciorra, A.J. Morris, M.A. Frohman, M.-F. Bader, Phospholipase D1: a key factor for the exocytotic machinery in neuroendocrine cells, *EMBO J.* 20 (2001) 2424–2434.
- [82] J.H. Exton, Phospholipase D—structure, regulation and function, *Rev. Physiol., Biochem. Pharmacol.* 144 (2002) 1–94.
- [83] S.S. Iyer, R.S. Agrawal, C.R. Thompson, S. Thompson, J.A. Barton, D.J. Kusner, Phospholipase D1 regulates phagocyte adhesion, *J. Immunol.* 176 (2006) 3686–3696.
- [84] J.H. Kim, H.W. Kim, H. Jeon, P.G. Suh, S.H. Ryu, Phospholipase D1 regulates cell migration in a lipase activity-independent manner, *J. Biol. Chem.* 281 (2006) 15747–15756.
- [85] G. Du, P. Huang, B.T. Liang, M.A. Frohman, Phospholipase D2 localizes to the plasma membrane and regulates angiotensin II receptor endocytosis, *Mol. Biol. Cell* 15 (2004) 1024–1030.
- [86] T. Koch, D.F. Wu, L.Q. Yang, L.O. Brandenburg, V. Höllt, Role of phospholipase D2 in the agonist-induced and constitutive endocytosis of G-protein coupled receptors, *J. Neurochem.* 97 (2006) 365–372.
- [87] D. Padrón, R.D. Tall, M.G. Roth, Phospholipase D2 is required for efficient endocytic recycling of transferrin receptors, *Mol. Biol. Cell* 17 (2006) 598–606.

- [88] Y. Zhang, Y.M. Altshuller, S.M. Hammond, A.J. Morris, M.A. Frohman, Loss of receptor regulation by a phospholipase D1 mutant unresponsive to protein kinase C, *EMBO J.* 18 (1999) 6339–6348.
- [89] G. Du, Y.M. Altshuller, Y. Kim, J.M. Han, S.H. Ryu, A.J. Morris, M.A. Frohman, Dual requirement for Rho and protein kinase C in direct activation of phospholipase D1 through G protein-coupled receptor signaling, *Mol. Biol. Cell* 11 (2000) 4359–4368.
- [90] L. Wang, R. Cummings, Y. Zhao, A. Kazlauskas, J.K.S. Sham, A. Morris, S. Georas, D.N. Brindley, V. Natarajan, Involvement of phospholipase D2 in lysophosphatidate-induced transactivation of platelet-derived growth factor receptor- β in human bronchial epithelial cells, *J. Biol. Chem.* 278 (2003) 39931–39940.
- [91] J.H. Parmentier, Z. Pavicevic, K.U. Malik, ANG II stimulates phospholipase D through PKC ζ activation in VSMC: implications in adhesion, spreading, and hypertrophy, *Am. J. Physiol. Heart Circ. Physiol.* 290 (2006) H46–H54.
- [92] S.K. Park, D.S. Min, J.H. Exton, Definition of the protein kinase C interaction site of phospholipase D, *Biochem. Biophys. Res. Commun.* 244 (1998) 364–367.
- [93] Z. Xie, W.T. Ho, R. Spellman, S. Cai, J.H. Exton, Mechanisms of regulation of phospholipase D1 and D2 by the heterotrimeric G proteins G₁₃ and G_q, *J. Biol. Chem.* 277 (2002) 11979–11986.
- [94] M. Schmidt, S.M. Hüwe, B. Fasselt, D. Homann, U. Rümenapp, J. Sandmann, K.H. Jakobs, Mechanisms of phospholipase D stimulation by m3 muscarinic acetylcholine receptors. Evidence for involvement of tyrosine phosphorylation, *Eur. J. Biochem.* 225 (1994) 667–675.
- [95] U. Rümenapp, M. Schmidt, F. Wahn, E. Tapp, A. Grannass, K.H. Jakobs, Characteristics of protein-kinase-C- and ADP-ribosylation-factor-stimulated phospholipase D activities in human embryonic kidney cells, *Eur. J. Biochem.* 248 (1997) 407–414.
- [96] M.A. Balboa, P.A. Insel, Stimulation of phospholipase D via α_1 -adrenergic receptors in Madin–Darby canine kidney cells is independent of PKC α and - ϵ activation, *Mol. Pharmacol.* 53 (1998) 221–227.
- [97] M.M. Muthalif, J.H. Parmentier, I.F. Benter, N. Karzoun, A. Ahmed, Z. Khandekar, M.Z. Adl, S. Bourgoin, K.U. Malik, Ras/mitogen-activated protein kinase mediates norepinephrine-induced phospholipase D activation in rabbit aortic smooth muscle cells by a phosphorylation-dependent mechanism, *J. Pharmacol. Exp. Ther.* 293 (2000) 268–274.
- [98] N. Gosau, M. Fahimi-Vahid, C. Michalek, M. Schmidt, T. Wieland, Signalling components involved in the coupling of α_1 -adrenoceptors to phospholipase D in neonatal rat cardiac myocytes, *Naunyn-Schmiedeberg's Arch. Pharmacol.* 365 (2002) 468–476.
- [99] A. Fensome, J. Whatmore, C. Morgan, D. Jones, S. Cockcroft, ADP-ribosylation factor and Rho proteins mediate fMLP-dependent activation of phospholipase D in human neutrophils, *J. Biol. Chem.* 273 (1998) 13157–13164.
- [100] R.J. Cummings, N.L. Parinandi, A. Zaiman, L. Wang, P.V. Usatyuk, J.G.N. Garcia, V. Natarajan, Phospholipase D activation by sphingosine 1-phosphate regulates interleukin-8 secretion in human bronchial epithelial cells, *J. Biol. Chem.* 277 (2002) 30227–30235.
- [101] S.G. Plonk, S.K. Park, J.H. Exton, The α -subunit of the heterotrimeric G protein G₁₃ activates a phospholipase D isozyme by a pathway requiring Rho family GTPases, *J. Biol. Chem.* 273 (1998) 4823–4826.
- [102] U. Rümenapp, M. Asmus, H. Schabowski, M. Woznicki, L. Han, K.H. Jakobs, M. Fahimi-Vahid, C. Michalek, T. Wieland, M. Schmidt, The M₃ muscarinic acetylcholine receptor expressed in HEK-293 cell signals to phospholipase D via G₁₂ but not G_q-type G proteins. Regulators of G proteins as tools to dissect pertussis toxin-resistant G proteins in receptor-effector coupling, *J. Biol. Chem.* 276 (2001) 2474–2479.
- [103] M. Fahimi-Vahid, N. Gosau, C. Michalek, L. Han, K.H. Jakobs, M. Schmidt, N. Roberts, M. Avkiran, T. Wieland, Distinct signaling pathways mediate cardiomyocyte phospholipase D stimulation by endothelin-1 and thrombin, *J. Mol. Cell. Cardiol.* 34 (2002) 441–453.
- [104] C. Huang, K.M. Hujer, Z. Wu, R.T. Miller, The Ca²⁺-sensing receptor couples to G $\alpha_{12/13}$ to activate phospholipase D in Madin–Darby canine kidney cells, *Am. J. Physiol., Cell Physiol.* 286 (2004) C22–C30.
- [105] J. Ziembicki, R. Tandon, J.R. Schelling, J.R. Sedor, R.T. Miller, C. Huang, Mechanical force-activated phospholipase D is mediated by G $\alpha_{12/13}$ -Rho and calmodulin-dependent kinase in renal epithelial cells, *Am. J. Physiol., Renal Fluid Electrolyte Physiol.* 289 (2005) F826–F834.
- [106] J. Ginsberg, S. Gupta, W.C. Matowe, L. Kline, D.N. Brindley, Activation of phospholipase D in FRTL-5 thyroid cells by forskolin and dibutyl-cyclic adenosine monophosphate, *Endocrinology* 138 (1997) 3645–3651.
- [107] M.S. Yoon, J.B. Koo, J.H. Hwang, K.S. Lee, J.S. Han, 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 (2005) 5635–5642.
- [108] M. López De Jesús, M.B. Stope, P.A. Oude Weernink, Y. Mahlke, C. Börgemann, V.N. Ananaba, C. Rimbach, D. Roskopf, M.C. Michel, K.H. Jakobs, M. Schmidt, Cyclic AMP-dependent and Epac-mediated activation of R-Ras by G protein-coupled receptors leads to phospholipase D stimulation, *J. Biol. Chem.* 281 (2006) 21837–21847.
- [109] M. Ushio-Fukai, R.W. Alexander, M. Akers, P.R. Lyons, B. Lassègue, K.K. Griendling, Angiotensin II receptor coupling to phospholipase D is mediated by the $\beta\gamma$ subunits of heterotrimeric G proteins in vascular smooth muscle cells, *Mol. Pharmacol.* 55 (1999) 142–149.
- [110] H. Le Stunff, L. Dokhac, S. Bourgoin, M.F. Bader, S. Harbon, Phospholipase D in rat myometrium: occurrence of a membrane-bound ARF6 (ADP-ribosylation factor 6)-regulated activity controlled by $\beta\gamma$ subunits of heterotrimeric G-proteins, *Biochem. J.* 352 (2000) 491–499.
- [111] A.M. Preininger, L.G. Henage, W.M. Oldham, E.J. Yoon, H.E. Hamm, H.A. Brown, Direct modulation of phospholipase D activity by G $\beta\gamma$, *Mol. Pharmacol.* 70 (2006) 311–318.
- [112] U. Rümenapp, M. Geiszt, F. Wahn, M. Schmidt, K.H. Jakobs, Evidence for ADP-ribosylation-factor-mediated activation of phospholipase D by m3 muscarinic acetylcholine receptor, *Eur. J. Biochem.* 234 (1995) 240–244.
- [113] R. Mitchell, D. McCulloch, E. Lutz, M. Johnson, C. MacKenzie, M. Fennell, G. Fink, W. Zhou, S.C. Sealfon, Rhodopsin-family receptors associate with small G proteins to activate phospholipase D, *Nature* 392 (1998) 411–414.
- [114] K. Shome, M.A. Rizzo, C. Vasudevan, B. Andresen, G. Romero, 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 (2000) 2200–2208.
- [115] A. Schürmann, M. Schmidt, M. Asmus, S. Bayer, F. Fliegert, S. Koling, S. Maßmann, C. Schilf, M.C. Subauste, M. Voß, K.H. Jakobs, H.G. Joost, The ADP-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 (1999) 9744–9751.
- [116] M. Schmidt, U. Rümenapp, C. Bienek, J. Keller, C. von Eichel-Streiber, K.H. Jakobs, Inhibition of receptor signaling to phospholipase D by *Clostridium difficile* toxin B. Role of Rho proteins, *J. Biol. Chem.* 271 (1996) 2422–2426.
- [117] M. Schmidt, M. Voss, M. Thiel, B. Bauer, A. Grannass, E. Tapp, R.H. Cool, J. de Gunzburg, C. von Eichel-Streiber, K.H. Jakobs, 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 (1998) 7413–7422.
- [118] M. Voss, P.A. Oude Weernink, S. Hauptenthal, U. Möller, R.H. Cool, B. Bauer, J.H. Camonis, K.H. Jakobs, M. Schmidt, Phospholipase D stimulation by receptor tyrosine kinases mediated by protein kinase C and a Ras/Ral signaling cascade, *J. Biol. Chem.* 274 (1999) 34691–34698.
- [119] M. Schmidt, M. Voss, P.A. Oude Weernink, J. Wetzel, M. Amano, K. Kaibuchi, K.H. Jakobs, A role for Rho-kinase in Rho-controlled phospholipase D stimulation by the m3 muscarinic acetylcholine receptor, *J. Biol. Chem.* 274 (1999) 14648–14654.
- [120] Y. Kam, J.H. Exton, Role of phospholipase D1 in the regulation of mTOR activity by lysophosphatidic acid, *FASEB J.* 18 (2004) 311–319.
- [121] K. Oishi, M. Takahashi, H. Mukai, Y. Banno, S. Nakashima, Y. Kanaho, Y. Nozawa, Y. Ono, PKN regulates phospholipase D1 through direct interaction, *J. Biol. Chem.* 276 (2001) 18096–18101.

- [122] J.H. Parmentier, A. Ahmed, Y. Ruan, G.K. Gandhi, A.E. Saeed, K.U. Malik, Calcium and protein kinase C (PKC)-related kinase mediate α_{1A} -adrenergic receptor-stimulated activation of phospholipase D in rat-1 cells, independent of PKC, *J. Pharmacol. Exp. Ther.* 303 (2002) 1206–1215.
- [123] J. Keller, M. Schmidt, B. Hussein, U. R umenapp, K.H. Jakobs, Muscarinic receptor-stimulated cytosol-membrane translocation of RhoA, *FEBS Lett.* 403 (1997) 299–302.
- [124] M.S. Johnson, D.N. Robertson, P.J. Holland, E.M. Lutz, R. Mitchell, Role of the conserved NPxxY motif of the 5-HT_{2A} receptor in determining selective interaction with isoforms of ADP-Ribosylation Factor (ARF), *Cell Signal.* 18 (2006) 1793–1800.
- [125] S. Krugmann, R. Williams, L. Stephens, P.T. Hawkins, ARAP3 is a PI3K- and rap-regulated GAP for RhoA, *Curr. Biol.* 14 (2004) 1380–1384.
- [126] J.H. Kim, S.D. Lee, J.M. Han, T.G. Lee, Y. Kim, J.B. Park, J.D. Lambeth, P.G. Suh, S.H. Ryu, Activation of phospholipase D1 by direct interaction with ADP-ribosylation factor 1 and RalA, *FEBS Lett.* 430 (1998) 231–235.
- [127] L. Xu, P. Frankel, D. Jackson, T. Rotunda, R.L. Boshans, C. D'Souza-Schorey, D.A. Foster, Elevated phospholipase D activity in H-Ras- but not K-Ras-transformed cells by the synergistic action of RalA and ARF6, *Mol. Cell. Biol.* 23 (2003) 645–654.
- [128] P. Frankel, M. Ramos, J. Flom, S. Bychenok, T. Joseph, E. Kerkhoff, U.R. Rapp, L.A. Feig, D.A. Foster, Ral and Rho-dependent activation of phospholipase D in v-Raf-transformed cells, *Biochem. Biophys. Res. Commun.* 255 (1999) 502–507.
- [129] C. Wilde, H. Barth, P. Sehr, L. Han, M. Schmidt, I. Just, K. Aktories, Interaction of the Rho-ADP-ribosylating C3 exoenzyme with RalA, *J. Biol. Chem.* 277 (2002) 14771–14776.
- [130] L. Lucas, L. del Peso, P. Rodriguez, V. Penalva, J.C. Lacal, Ras protein is involved in the physiological regulation of phospholipase D by platelet-derived growth factor, *Oncogene* 19 (2000) 431–437.
- [131] Z.M. Lu, A. Hornia, T. Joseph, T. Sukezane, P. Frankel, M. Zhong, S. Bychenok, L.Z. Xu, L.A. Feig, D.A. Foster, Phospholipase D and RalA cooperate with the epidermal growth factor receptor to transform 3Y1 rat fibroblasts, *Mol. Cell. Biol.* 20 (2000) 462–467.
- [132] E. Meacci, L. Becciolini, F. Nuti, C. Donati, F. Cencetti, M. Farnararo, P. Bruni, A role for calcium in sphingosine 1-phosphate-induced phospholipase D activity in C2C12 myoblasts, *FEBS Lett.* 521 (2002) 200–204.
- [133] S. Huang, L. Lifshitz, V. Patki-Kamath, R. Tuft, K. Fogarty, M.P. Czech, Phosphatidylinositol 4,5-bisphosphate-rich plasma membrane patches organize active zones of endocytosis and ruffling in cultured adipocytes, *Mol. Cell. Biol.* 24 (2004) 9102–9123.
- [134] J. van Rheenen, E.M. Achame, H. Janssen, J. Calafat, K. Jalink, PIP₂ signaling in lipid domains: a critical re-evaluation, *EMBO J.* 24 (2005) 1664–1673.
- [135] M. Santarius, C.H. Lee, R.A. Anderson, Supervised membrane swimming: small G-protein lifeguards regulate PIPK signalling and monitor intracellular PtdIns(4,5)P₂ pools, *Biochem. J.* 398 (2006) 1–13.
- [136] H. Ishihara, Y. Shibasaki, N. Kizuki, H. Katagiri, Y. Yazaki, T. Asano, Y. Oka, Cloning of cDNAs encoding two isoforms of 68-kDa type I phosphatidylinositol 4-phosphate 5-kinase, *J. Biol. Chem.* 271 (1996) 23611–23614.
- [137] J.C. Loijens, R.A. Anderson, Type I phosphatidylinositol 4-phosphate 5-kinases are distinct members of this novel lipid kinase family, *J. Biol. Chem.* 271 (1996) 32937–32943.
- [138] H. Ishihara, Y. Shibasaki, N. Kizuki, T. Wada, Y. Yazaki, T. Asano, Y. Oka, Type I phosphatidylinositol 4-phosphate 5-kinases, *J. Biol. Chem.* 273 (1998) 8741–8748.
- [139] Y.J. Wang, W.H. Li, J. Wang, K. Xu, P. Dong, X. Luo, H.L. Yin, Critical role of PIP5KI γ 87 in InsP₃-mediated Ca²⁺ signaling, *J. Cell Biol.* 167 (2004) 1005–1010.
- [140] B. Vanhaesebroeck, S.J. Leever, K. Ahmadi, J. Timms, R. Katso, P.C. Driscoll, R. Woscholski, P.J. Parker, M.D. Waterfield, Synthesis and function of 3-phosphorylated inositol lipids, *Annu. Rev. Biochem.* 70 (2001) 535–602.
- [141] P.W. Majerus, M.V. Kisseleva, F.A. Norris, The role of phosphatases in inositol signaling reactions, *J. Biol. Chem.* 274 (1999) 10669–10672.
- [142] R.D. Nolan, E.G. Lapetina, Thrombin stimulates the production of a novel polyphosphoinositide in human platelets, *J. Biol. Chem.* 265 (1990) 2441–2445.
- [143] P. Grondin, M. Plantavid, C. Sultan, M. Breton, G. Mauco, H. Chap, Interaction of pp60^{c-src}, phospholipase C, inositol-lipid, and diacylglycerol kinases with the cytoskeletons of thrombin-stimulated platelets, *J. Biol. Chem.* 266 (1991) 15705–15709.
- [144] M. Schmidt, C. Nehls, U. R umenapp, K.H. Jakobs, m3 Muscarinic receptor-induced and G_i-mediated heterologous potentiation of phospholipase C stimulation: role of phosphoinositide synthesis, *Mol. Pharmacol.* 50 (1996) 1038–1046.
- [145] M. Schmidt, M. Frings, M.-L. Mono, Y. Guo, P.A. Oude Weernink, S. Evellin, L. Han, K.H. Jakobs, G protein-coupled receptor-induced sensitization of phospholipase C stimulation by receptor tyrosine kinases, *J. Biol. Chem.* 275 (2000) 32603–32610.
- [146] L. Stephens, T.R. Jackson, P.T. Hawkins, Activation of phosphatidylinositol 4,5-bisphosphate supply by agonists and non-hydrolysable GTP analogues, *Biochem. J.* 296 (1993) 481–488.
- [147] D. de Chaffoy de Courcelles, P. Roevens, H. van Belle, 12-*O*-Tetradecanoylphorbol 13-acetate stimulates inositol lipid phosphorylation in intact human platelets, *FEBS Lett.* 173 (1984) 389–393.
- [148] S.P. Halenda, M.B. Feinstein, Phorbol myristate acetate stimulates formation of phosphatidyl inositol 4-phosphate and phosphatidyl inositol 4,5-bisphosphate in human platelets, *Biochem. Biophys. Res. Commun.* 124 (1984) 507–513.
- [149] A.M. Boon, B.J. Beresford, A. Mellors, A tumor promoter enhances the phosphorylation of polyphosphoinositides while decreasing phosphatidylinositol labelling in lymphocytes, *Biochem. Biophys. Res. Commun.* 129 (1985) 431–438.
- [150] S.J. Park, T. Itoh, T. Takenawa, Phosphatidylinositol 4-phosphate 5-kinase type I is regulated through phosphorylation response by extracellular stimuli, *J. Biol. Chem.* 276 (2001) 4781–4787.
- [151] L.D. Chong, A. Traynor-Kaplan, G.M. Bokoch, M.A. Schwartz, The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate 5-kinase in mammalian cells, *Cell* 79 (1994) 507–513.
- [152] Y. Shibasaki, H. Ishihara, N. Kizuki, T. Asano, Y. Oka, Y. Yazaki, Massive actin polymerization induced by phosphatidylinositol 4-phosphate 5-kinase in vivo, *J. Biol. Chem.* 272 (1997) 7578–7581.
- [153] P.A. Oude Weernink, P. Schulte, Y. Guo, J. Wetzel, M. Amano, K. Kaibuchi, S. Haverland, M. Voss, M. Schmidt, G.W. Mayr, K.H. Jakobs, Stimulation of phosphatidylinositol 4-phosphate 5-kinase by Rho-kinase, *J. Biol. Chem.* 275 (2000) 10168–10174.
- [154] F.P.G. van Horck, E. Lavazais, B.J. Eickholt, W.H. Moolenaar, N. Divecha, Essential role of type I α phosphatidylinositol 4-phosphate 5-kinase in neurite remodeling, *Curr. Biol.* 12 (2002) 241–245.
- [155] M. Yamazaki, H. Miyazaki, H. Watanabe, T. Sasaki, T. Maehama, M.A. Frohman, Y. Kanaho, Phosphatidylinositol 4-phosphate 5-kinase is essential for ROCK-mediated neurite remodeling, *J. Biol. Chem.* 277 (2002) 17226–17230.
- [156] T. Matsui, S. Yonemura, S. Tsukita, S. Tsukita, Activation of ERM proteins *in vivo* by Rho involves phosphatidylinositol 4-phosphate 5-kinase and not ROCK kinases, *Curr. Biol.* 9 (1999) 1259–1262.
- [157] S. Yonemura, T. Matsui, S. Tsukita, S. Tsukita, Rho-dependent and -independent activation mechanisms of ezrin/radixin/moesin proteins: an essential role for polyphosphoinositides in vivo, *J. Cell Sci.* 115 (2002) 2569–2580.
- [158] K.F. Tolias, J.H. Hartwig, H. Ishihara, Y. Shibasaki, L.C. Cantley, C.L. Carpenter, Type I α phosphatidylinositol 4-phosphate 5-kinase mediates Rac-dependent actin assembly, *Curr. Biol.* 10 (2000) 153–156.
- [159] R.L. Doughman, A.J. Firestone, M.L. Wojtasiak, M.W. Bunce, R.A. Anderson, Membrane ruffling requires coordination between type I α phosphatidylinositol phosphate kinase and rac signaling, *J. Biol. Chem.* 278 (2003) 23036–23045.
- [160] B. Kost, E. Lemichez, P. Spielhofer, Y. Hong, K.F. Tolias, C.L. Carpenter, N.-H. Chua, Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth, *J. Cell Biol.* 145 (1999) 317–330.
- [161] X.-D. Ren, G.M. Bokoch, A. Traynor-Kaplan, G.H. Jenkins, R.A.

- Anderson, M.A. Schwartz, Physical association of the small GTPase Rho with a 68-kDa phosphatidylinositol 4-phosphate 5-kinase in Swiss 3T3 cells, *Mol. Biol. Cell* 7 (1996) 435–442.
- [162] P.B. van Hennik, J.P. ten Klooster, J.R. Halstead, C. Voermans, E.C. Anthony, N. Divecha, P.L. Hordijk, The C-terminal domain of Rac1 contains two motifs that control targeting and signaling specificity, *J. Biol. Chem.* 278 (2003) 39166–39175.
- [163] P.A. Oude Weernink, K. Meletiadis, S. Hommeltenberg, M. Hinz, H. Ishihara, M. Schmidt, K.H. Jakobs, Activation of type I phosphatidylinositol-4-phosphate 5-kinase isoforms by the Rho GTPases, RhoA, Rac1, and Cdc42, *J. Biol. Chem.* 279 (2004) 7840–7849.
- [164] D.H. Jones, J.B. Morris, C.P. Morgan, H. Kondo, R.F. Irvine, S. Cockcroft, Type I phosphatidylinositol 4-phosphate 5-kinase directly interacts with ADP-ribosylation factor I and is responsible for phosphatidylinositol 4,5-bisphosphate synthesis in the Golgi compartment, *J. Biol. Chem.* 275 (2000) 13962–13966.
- [165] A. Godi, P. Pertile, R. Meyers, P. Marra, G. Di Tullio, C. Iurisci, A. Luini, D. Corda, M.A. De Matteis, 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 (1999) 280–287.
- [166] A. Honda, M. Nogami, T. Yokozeki, M. Yamazaki, H. Nakamura, H. Watanabe, K. Kawamoto, K. Nakayama, A.J. Morris, M.A. Frohman, Y. Kanaho, Phosphatidylinositol 4-phosphate 5-kinase α is a downstream effector of the small G protein ARF6 in membrane ruffle formation, *Cell* 99 (1999) 521–532.
- [167] F.D. Brown, A.L. Rozelle, H.L. Yin, T. Balla, J.G. Donaldson, Phosphatidylinositol 4,5-bisphosphate and Arf6-regulated membrane traffic, *J. Cell Biol.* 154 (2001) 1007–1017.
- [168] Y. Aikawa, T.F.J. Martin, ARF6 regulates a plasma membrane pool of phosphatidylinositol(4,5)bisphosphate required for regulated exocytosis, *J. Cell Biol.* 162 (2003) 647–659.
- [169] M. Krauss, M. Kinuta, M.R. Wenk, P. De Camilli, K. Takei, V. Haucke, ARF6 stimulates clathrin/AP-2 recruitment to synaptic membranes by activating phosphatidylinositol phosphate kinase type I γ , *J. Cell Biol.* 162 (2003) 113–124.
- [170] J.T.R. Lawrence, M.J. Birnbaum, ADP-ribosylation factor 6 regulates insulin secretion through plasma membrane phosphatidylinositol 4,5-bisphosphate, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 13320–13325.
- [171] K.W. Wong, R.R. Isberg, Arf6 and phosphoinositol-4-phosphate-5-kinase activities permit bypass of the Rac1 requirement for β 1 integrin-mediated bacterial uptake, *J. Exp. Med.* 198 (2003) 603–614.
- [172] D.J. Hernández-Deviez, M.G. Roth, J.E. Casanova, J.M. Wilson, ARNO and ARF6 regulate axonal elongation and branching through downstream activation of phosphatidylinositol 4-phosphate 5-kinase α , *Mol. Biol. Cell* 15 (2004) 111–120.
- [173] A. Moritz, P.N.E. De Graan, W.H. Gispen, K.W.A. Wirtz, Phosphatidic acid is a specific activator of phosphatidylinositol-4-phosphate kinase, *J. Biol. Chem.* 267 (1992) 7207–7210.
- [174] G.H. Jenkins, P.L. Fiset, R.A. Anderson, Type I phosphatidylinositol 4-phosphate 5-kinase isoforms are specifically stimulated by phosphatidic acid, *J. Biol. Chem.* 269 (1994) 11547–11554.
- [175] D.R. Jones, M.A. Sanjuan, I. Mérida, Type I α phosphatidylinositol 4-phosphate 5-kinase is a putative target for increased intracellular phosphatidic acid, *FEBS Lett.* 476 (2000) 160–165.
- [176] N. Divecha, M. Roefs, J.R. Halstead, S. D'Andrea, M. Fernandez-Borga, L. Oomen, K.M. Saqib, M.J.O. Wakelam, C. D'Santos, Interaction of the type Ia PIPkinase with phospholipase D: a role for the local generation of phosphatidylinositol 4,5-bisphosphate in the regulation of PLD2 activity, *EMBO J.* 19 (2000) 5440–5449.
- [177] D.A. Sweeney, A. Siddhanta, D. Shields, 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 (2002) 3030–3039.
- [178] L.S. Ameson, J. Kunz, R.A. Anderson, L.M. Traub, Coupled inositide phosphorylation and phospholipase D activation initiates clathrin-coat assembly on lysosomes, *J. Biol. Chem.* 274 (1999) 17794–17805.
- [179] G. Way, N. O'Lunaigh, S. Cockcroft, 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 (2000) 63–70.
- [180] A. Skippen, D.H. Jones, C.P. Morgan, M. Li, S. Cockcroft, Mechanism of ADP ribosylation factor-stimulated phosphatidylinositol 4,5-bisphosphate synthesis in HL60 cells, *J. Biol. Chem.* 277 (2002) 5823–5831.
- [181] T.P. Stauffer, S. Ahn, T. Meyer, Receptor-induced transient reduction in plasma membrane PtdIns(4,5)P₂ concentration monitored in living cells, *Curr. Biol.* 8 (1998) 343–346.
- [182] L.-H. Xie, M. Horie, M. Takano, Phospholipase C-linked receptors regulate the ATP-sensitive potassium channel by means of phosphatidylinositol 4,5-bisphosphate metabolism, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 15292–15297.
- [183] T. Haruna, H. Yoshida, T.Y. Nakamura, L.-H. Xie, H. Otani, T. Ninomiya, M. Takano, W.A. Coetzee, M. Horie, α_1 -Adrenoceptor-mediated breakdown of phosphatidylinositol 4,5-bisphosphate inhibits pinacidil-activated ATP-sensitive K⁺ currents in rat ventricular myocytes, *Circ. Res.* 91 (2002) 232–239.
- [184] E. Kobrinsky, T. Mirshahi, H. Zhang, T. Jin, D.E. Logothetis, Receptor-mediated hydrolysis of plasma membrane messenger PIP₂ leads to K⁺-current desensitization, *Nat. Cell Biol.* 2 (2000) 507–514.
- [185] L.W. Runnels, L. Yue, D.E. Clapham, The TRPM7 channel is inactivated by PIP₂ hydrolysis, *Nat. Cell Biol.* 4 (2002) 329–336.
- [186] J. Chou, D.B. Stolz, N.A. Burke, S.C. Watkins, A. Wells, Distribution of gelsolin and phosphoinositol 4,5-bisphosphate in lamellipodia during EGF-induced motility, *Int. J. Biochem. Cell Biol.* 34 (2002) 776–790.
- [187] C.C. Scott, W. Dobson, R.J. Botelho, N. Coody-Osberg, P. Chavrier, D.A. Knecht, C. Heath, P. Stahl, S. Grinstein, Phosphatidylinositol-4,5-bisphosphate hydrolysis directs actin remodeling during phagocytosis, *J. Cell Biol.* 169 (2005) 139–149.
- [188] A.L. Howes, J.F. Arthur, T. Zhang, S. Miyamoto, J.W. Adams, G.W. Dorn, E.A. Woodcock, J.H. Brown, Akt-mediated cardiomyocyte survival pathways are compromised by G α_q -induced phosphoinositide 4,5-bisphosphate depletion, *J. Biol. Chem.* 278 (2003) 40343–40351.
- [189] P.J. Goldschmidt-Clermont, L.M. Machesky, J.J. Baldassare, T.D. Pollard, The actin-binding protein profilin binds to PIP₂ and inhibits its hydrolysis by phospholipase C, *Science* 247 (1990) 1575–1578.
- [190] J.S. Popova, A.K. Greene, J. Wang, M.M. Rasenick, Phosphatidylinositol 4,5-bisphosphate modifies tubulin participation in phospholipase C β_1 signaling, *J. Neurosci.* 22 (2002) 1668–1678.
- [191] S. Lukowski, J.P. Mira, A. Zachowski, B. Geny, Fodrin inhibits phospholipases A2, C, and D by decreasing polyphosphoinositide cell content, *Biochem. Biophys. Res. Commun.* 248 (1998) 278–284.
- [192] J.K. Chung, F. Sekiya, H.S. Kang, C. Lee, J.S. Han, S.R. Kim, Y.S. Bae, A.J. Morris, S.G. Rhee, Synaptotagmin inhibition of phospholipase D activity by hydrolysis of phosphatidylinositol 4,5-bisphosphate, *J. Biol. Chem.* 272 (1997) 15980–15985.
- [193] M. Schmidt, C. Bienek, U. Rumenapp, C. Zhang, G. Lümmer, K.H. Jakobs, I. Just, K. Aktories, M. Moos, C. von Eichel-Streiber, A role for Rho in receptor- and G protein-stimulated phospholipase C. Reduction in phosphatidylinositol 4,5-bisphosphate by *Clostridium difficile* toxin B, Naunyn-Schmiedeberg's Arch. Pharmacol. 354 (1996) 87–94.
- [194] M. Schmidt, U. Rumenapp, C. Nehls, S. Ott, J. Keller, C. von Eichel-Streiber, K.H. Jakobs, Restoration of *Clostridium difficile* toxin-B-inhibited phospholipase D by phosphatidylinositol 4,5-bisphosphate, *Eur. J. Biochem.* 240 (1996) 707–712.
- [195] I. Litosch, Regulation of phospholipase C- β activity by phosphatidic acid: isoform dependence, role of protein kinase C, and G protein subunits, *Biochemistry* 42 (2003) 1618–1623.