CMLS, Cell. Mol. Life Sci. 62 (2005) 1448–1461
1420-682X/05/131448-14
DOI 10.1007/s00018-005-4531-7

CMLS Cellular and Molecular Life Sciences

Review

Protein kinase C and phospholipase D: intimate interactions in intracellular signaling

K. P. Becker and Y. A. Hannun*

Department of Biochemistry and Molecular Biology, Medical University of South Carolina, 173 Ashley Avenue, P.O. Box 250509, Charleston, South Carolina 29425 (USA), Fax: +1 843 792 4322, e-mail: hannun@musc.edu

Received 29 November 2004; received after revision 18 January 2005; accepted 4 March 2005
Online First 28 May 2005

Abstract. Diacylglycerol (DAG) was discovered as a potent lipid second messenger with protein kinase C (PKC) as its major cellular target more than 25 years ago. There is increasing evidence of significant complexity within lipid signaling, and the classical DAG-PKC model no longer stands alone but is part of a larger bioactive lipid universe involving glycerolipids and sphingolipids. Multiple layers of regulation exist among PKC- and DAG-metabolizing enzymes such as phosphatidylcholine (PC)-specific phospholipase D, and cross-talk exists between the glycerolipid and sphingolipid pathways, with PKC at the center. Currently, there is intense interest in the question of whether DAG derived from PC can function as a lipid second messenger and regulate PKC analogous to DAG derived from phosphatidylinositol-4,5-bisphosphate (PIP_2). To address these issues and incorporate DAG-PKC and other signaling pathways into an expanded view of cell biology, it will be necessary to go beyond the classical approaches and concepts.

Key words. Phospholipase D; protein kinase C; diacylglycerol; ceramide; signal transduction.

Overview

The discovery that certain classes of membrane lipids could be broken down in a regulated fashion to produce biologically active molecules constituted a turning point in understanding how the cell transduces extracellular signals to the interior of the cell. The phosphatidylinositol (PI) cycle, first described in the 1950s [1], was a well-studied but largely unexplained phenomenon for several decades until the finding that the newly identified serine-threonine kinase, protein kinase C (PKC), was in fact the major cellular target for the PI breakdown product diacylglycerol (DAG) [2]. Contemporaneously, inositol-1,4,5-trisphosphate (IP_3), the other product of phosphatidylinositol-4,5-bisphosphate (PIP_2) breakdown, was shown to effect intracellular calcium release [3]. Since these early observations, DAG and PKC have held central roles in the conceptual and experimental paradigm of lipid-dependent signaling.

In the classical model of PKC activation, PI-specific phospholipase C (PI-PLC) is activated by G proteins or tyrosine kinases dispatched by agonist-bound cell surface receptors. Activated PI-PLC cleaves PIP_2 at the inner face of the plasma membrane to generate membrane-bound DAG and soluble IP_3, with a subsequent increase in intracellular calcium. Membrane-bound DAG and calcium act in concert to recruit cytosolic PKC to the plasma membrane, where it can bind to and become activated by DAG and acidic phospholipids in the presence of local substrates.

Although this is the most well studied and best-understood model of lipid regulation of PKC, it is now recognized that PKC regulation is much more diverse than the

* Corresponding author.
This work is dedicated to the memory of Dr. Yasutomi Nishizuka, the discoverer of protein kinase C, who was both a gentleman and a scientist.

above model portrays. Regulation of PKC involves a multitude of activators, inhibitors and phosphorylation events that determine catalytic competency and access to substrates through the modulation of PKC subcellular localization to a variety of membrane surfaces. Further, DAG arising from phosphatidylcholine (PC) hydrolysis as well as numerous fatty acids (e.g. oleic acid), glycerolipids and sphingolipid molecules have also been reported to modulate PKC. Additionally, it is no longer just the calcium-dependent PKCs featured in the classical model that must be considered: a large number of PKC family members with significant regulatory diversity have been cloned and identified [4–6].

Certainly one of the most enigmatic relationships involving PKC is that with the PC-hydrolyzing enzyme phospholipase D (PC-PLD), which acts on membrane PC to generate phosphatidic acid (PA) and free choline. PC-PLD serves not only as a target of PKC but also as a potential source of effector lipid products for various PKC isoenzymes. Evidence now exists that products of PC hydrolysis (PA itself or its subsequent breakdown to DAG) can regulate PKC. Although our understanding of these relationships is in its relatively early stages, a number of conclusions may be drawn and specific possibilities raised. This review will summarize key regulatory mechanisms of PKC and PLD and then address the interactions of these two signaling pathways, with a focus on emerging principles and evolving concepts.

The PKC family

PKC is not a single protein but a large group of lipid-dependent kinases that phosphorylate protein substrates on serine and threonine residues. Currently, 11 PKC isoenzymes have been identified in mammals, and these can be divided into three subgroups based on sequence homology. The classical, or conventional, isoenzymes, PKC α, βI, βII and γ, are calcium-dependent PKCs that are activated by DAG and DAG-mimicking phorbol esters. The novel PKC isoenzymes, PKC δ, ε, θ, η and μ/PKD (protein kinase D), are calcium-independent and are stimulated by DAG/phorbol esters. The third subgroup contains the atypical PKC ζ and λ/ι isoenzymes. These are the most structurally divergent of the PKC family members and have been shown to be activated by acidic phospholipids such as phosphatidylserine (PS), polyphosphoinositides and PA [4–6], but not by DAG or phorbol esters (see fig. 1A, B for lipid structures).

Discovery of PKC

PKC was first described in 1977 by Nishizuka and colleagues as a novel kinase isolated from rat brain that was

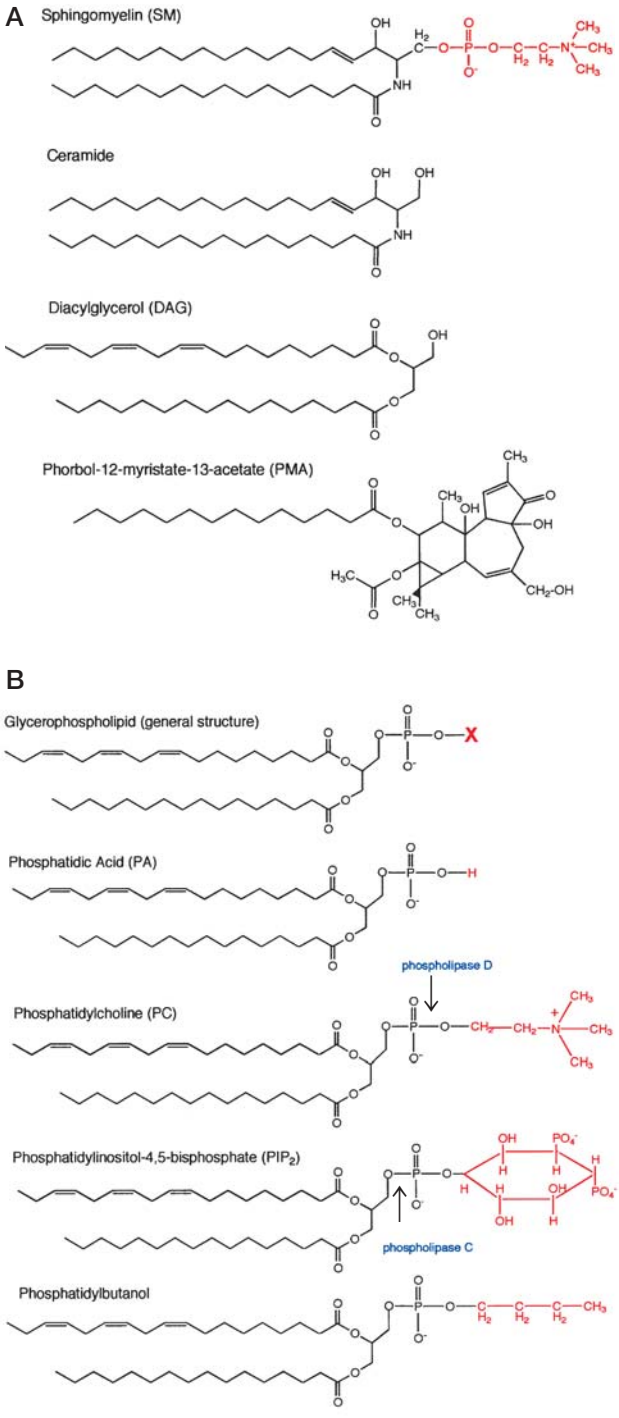

Figure 1. Structures of key lipids. (*A*) The sphingolipids ceramide and sphingomyelin (SM) are shown along with the glycerolipid diacylglycerol (DAG) and phorbol-12-myristate-13-acetate (PMA). Ceramide consists of a long chain alcohol (sphingosine) and an amide-linked fatty acid. SM is similar to ceramide but has a choline phosphate head group (highlighted in red) present at the 1-hydroxyl position. The structure of DAG is provided to demonstrate similarities to sphingolipids and to illustrate the fundamental structure of all glycerophospholipids. (*B*) 'X' denotes the position of head group moieties. Blue arrows show sites of action for phospholipase C (PLC) and phospholipase D (PLD). Phosphatidylbutanol is not thought to occur under physiological conditions but rather is the product of PLD when it is incubated in the presence of 1-butanol.

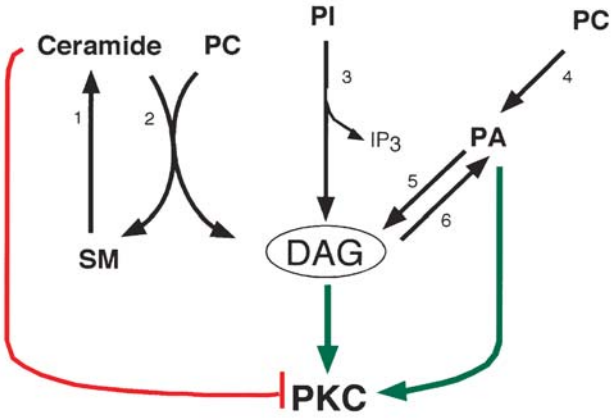

Figure 2. Multiple sources of diacylglycerol (DAG) in the cell and the enzymes that are involved. (1) sphingomyelinase; (2) sphingomyelin (SM) synthase; (3) phosphatidylinositol (PI)-specific phospholipase C; (4) phosphatidylcholine (PC)-specific phospholipase D; (5) phosphatidic acid (PA)-phosphohydrolyase; (6) diacylglycerol (DAG) kinase. PKC, protein kinase C.

activated upon treatment with calcium-dependent proteases with subsequent phosphorylation of serine and threonine residues on histone [7, 8]. It was later demonstrated that this novel protein kinase was actually a proenzyme that was fully active in the absence of proteolysis but that required physiological concentrations of calcium and a 'membrane factor'. This membrane factor was revealed as the neutral glycerolipid DAG and acidic phospholipids such as PS [2, 9]. This stunning observation led to the landmark concept that DAG, which up to then was a well-known by-product of regulated PI turnover without signaling significance, was in fact a potent second messenger molecule that utilized PKC as a major cellular effector (fig. 2). These findings were advanced by subsequent reports that tumor-promoting phorbol esters, such as phorbol-12-myristate-13-acetate (PMA), whose cellular target had been sought for more than 20 years, could serve as structural mimetics of DAG and activate PKC in the presence of PS [10].

Taken together, these observations offered the first insight that PKC could play a role in major cellular processes and that it could mediate these functions either as a transducer of acute DAG signals generated at the plasma membrane or as a regulator of more long term functions such as tumor promotion or cell differentiation [10, 11].

Structural features of PKC and in vitro lipid regulation

All PKC family members can be structurally divided into two regions: a carboxy terminus segment that contains the highly conserved C3 ATP-binding domain and the C4 kinase, or catalytic, domain. All PKC isoenzymes have a common C3 and C4 domain; however, the various complements of lipid- and calcium-binding regulatory domains found in the amino terminus are what give each individual isoenzyme, and also each PKC subfamily, its unique regulatory properties.

Early on, it was demonstrated that DAG could compete with phorbol esters for a common binding site on PKC [12–14]. This site was later shown to be the C1 domain, which consists of two cysteine-rich (C1a and C1b) zinc finger subdomains that are found intact and in tandem only in the amino terminus of the classical and novel PKC family members [15–18]. In contrast, the atypical PKCs contain a modified C1 domain with only a single cysteine-rich domain that does not bind DAG or PMA [19]. Interestingly, the binding sites for proposed lipid activators of the atypical isoenzymes have not been definitively identified, but unpublished results from our laboratory suggest that there may be an interplay of both amino and carboxy sites (see discussion below). Calcium dependence is conferred upon the classical PKCs through the presence of the anionic phospholipid and calcium-binding C2 domain, whereas modification of this domain or its absence from the novel and atypical isoenzymes renders these PKCs calcium-independent [20–22].

Data from numerous studies have led to a detailed understanding of the mechanism for lipid activation of the classical PKCs (for general PKC reviews, see [4, 5]). In brief, initial studies demonstrated that the addition of small quantities of unsaturated DAG to membrane-bound PKC increased the affinity of PKC for calcium, suggesting a cooperative effect of calcium and phospholipids in PKC activation [2, 23]. Using a Triton X-100 detergent-based mixed micelle assay, it was later shown that a single molecule of DAG was sufficient to activate monomeric PKC and that activation occurred with the cooperative effect of four or more molecules of PS in the presence of micromolar calcium [24, 25]. A similar approach revealed that the binding of DAG and PS to PKC is highly stereospecific and requires the physiologically relevant *sn*-1,2-DAG and *sn*-1,2-phosphatidyl-L-serine [26–28]. An understanding of this mechanism was further advanced by reports that the stereospecific interaction of PKC with DAG and PS serves to induce a conformational change in the enzyme that leads to the expulsion of an autoinhibitory pseudosubstrate domain sequence from the active site, thereby allowing substrates to bind [29]. This model of lipid activation is regarded as the prototypical mechanism and has served as a platform from which to study other PKC family members as well as a large number of other lipid-signaling proteins.

Spatiotemporal regulation of PKC activity

In 1983, Kraft and Anderson [30] reported that treatment of parietal yolk sac cells with phorbol esters could induce

the recruitment or 'translocation' of cytosolic PKC to the membrane fraction. It was later shown that PMA mimics DAG by intercalating into the plasma membrane and binding to PKC at the membrane's inner surface. This observation of stimulus-induced alteration of subcellular localization led to a conceptual model of how a soluble protein such as PKC could function as an effector for a hydrophobic activator such as DAG or PMA. The translocation of PKC from the cytosol to the membrane fraction has stood as the experimental hallmark of PKC activation since these early observations and has been widely used to define an enormous variety of PKC functions in the cell. Importantly, some PKC isoenzymes are predominantly localized to the membrane fraction under basal conditions, making assessment of activation through translocation problematic in these circumstances.

Advances in molecular biology, particularly the ability to rapidly generate full-length, deletional and single-site mutations of various PKC isoenzymes have contributed significantly to delineating the molecular regulation of PKC. It was reported early on that PKC is a phosphoprotein [31]; we now appreciate that PKC is subject to several post-translational events involving transphosphorylation [32, 33], autophosphorylation [34, 35] and dephosphorylation [36, 37]. Elegant studies revealed that nascent PKC is processed to a catalytically competent enzyme through the phosphorylation of residues within the activation loop of PKC by phosphinositide-dependent kinase-1 [38–40] and that this is followed by autophosphorylation events at conserved residues in the carboxy terminus [34]. It is now clear that the phosphorylation state of PKC determines catalytic activity as well as interactions with subcellular binding partners that can regulate subcellular localization [41].

In the late 1990s, several groups utilized green fluorescent protein (GFP) fusion proteins of PKC to observe the cellular movements of PKC in response to various agonists [42–45]. These studies unequivocally confirmed that in response to receptor stimulation and phorbol ester treatment, PKC translocates to the plasma membrane (up to that time, translocation had been demonstrated only with biochemical fractionation, which does not allow the distinction of individual membrane compartments). Further, these studies revealed that PKC is recruited to the plasma membrane through an interplay of both the C1 and the C2 domains and that kinase activity and subsequent autophosphorylation at serine 660 is necessary to drive classical PKC (cPKC) back into the cytosol [46] (fig. 3A). This requirement for autophosphorylation at the carboxy terminus in order to preserve the mechanism of reverse translocation has been supported by other studies, which found that the phosphorylation status at carboxy terminus sites determines subcellular localization [41, 47].

More recently, the study of GFP fusion proteins of PKC isoenzymes has allowed identification of alternate sites

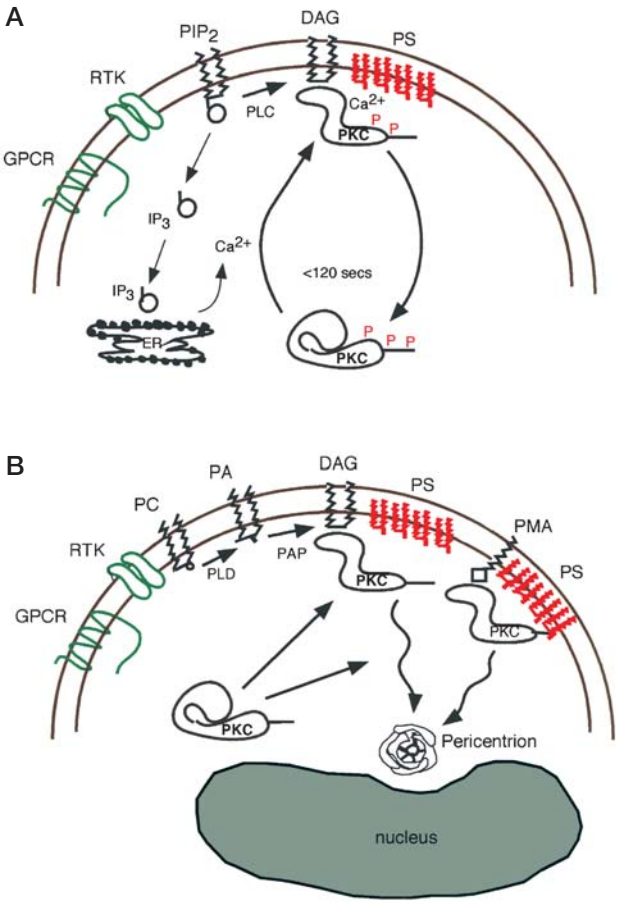

Figure 3. Translocation of protein kinase C (PKC) in response to short- and long-term exposure to agonists. (*A*) The classical model of PKC activation. Occupancy of G protein-coupled or tyrosine kinase receptors leads to the activation of phospholipase Cs (PLC) and the subsequent hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) to produce membrane-bound diacylglycerol (DAG) and soluble inositol-1,4,5-trisphosphate (IP_3). IP_3 mediates an elevation of calcium (Ca^{2+}), which leads to the recruitment of cytosolic PKC to the plasma membrane, where it binds DAG and phosphatidylserine (PS) in the presence of Ca^{2+} and becomes activated. Activated PKC can then target local substrates with phosphorylation. PKC returns to the cytosol within 120 s, which has been shown to correlate with the metabolism and loss of DAG from the membrane and the autophosphorylation of residues in the carboxy terminus of PKC. (*B*) The model of chronic PKC stimulation. Stimulation of G protein or tyrosine kinase receptors leads to the activation of phospholipase D (PLD). Activated PLD cleaves phosphatidylcholine (PC) to generate phosphatidic acid (PA) and free choline. PA can be converted to DAG through the actions of a PA phosphohydrolase (PAP). DAG from this source can be sustained (>1 h) in the membrane and has been shown [156] to induce prolonged PKC translocation. Phorbol-12-myristate-13-acetate (PMA) can be used to mimic acute or sustained DAG signals, dependent upon length of exposure. It has recently been reported that sustained DAG stimulated by platelet-derived growth factor or long-term PMA leads to the translocation of PKC to the plasma membrane and to a novel juxtanuclear compartment, the pericentrion [48].

for membrane translocation of PKC. Recent data from our laboratory suggest that under conditions where PKC is stimulated in a sustained manner (i.e. 30–60 min), select PKC isoenzymes translocate to the plasma membrane early on and at later time points translocate to a juxtanuclear site concentrated around the microtubule-organizing center (MTOC)/centrosome [48] (fig. 3B). Translocation of PKC to this compartment, which we have named the 'pericentrion', leads to the retention of recycling membrane proteins. In addition, other studies using GFP fusions of PKC have revealed that, depending on the type of agonist and length of exposure to it, PKC can translocate to the plasma membrane, the nuclear membrane or the Golgi network [49].

PKC as a mediator of short- and long-term cellular processes

In its classical role, PKC functions as a sensor and mediator of transient elevations of DAG at the plasma membrane. In this model, the calcium-dependent PKCs complete a rapid round-trip, dependent upon the presence of DAG in the membrane, from the cytosol to the membrane and back again, all within 120 s [44, 46]. Through this general mechanism, PKC can regulate a variety of acute signaling events, including regulation of receptor desensitization, cell adhesion, membrane transport, neurotransmitter release, blood cell activation and possibly various metabolic effects such as lipogenesis and glycogenolysis [6].

The role of PKC as a major cellular receptor for tumor-promoting phorbol esters distinctly suggested that PKC can function in a sustained manner and carry out more long term cellular processes, such as tumor promotion and carcinogenesis. It is now recognized that PKC mediates a variety of cellular events that are measured not in seconds and minutes but in hours, days and, in some cases, even years. Long-term cellular functions attributed to PKC include cell differentiation, long-term potentiation, mitogenesis, cell motility, cancer metastasis, multidrug resistance and microvascular pathology [50–52]. While some of these effects may be influenced or regulated by acute activation of PKC, evidence has shown additional mechanisms for sustained activation of PKC that could be mediated either through upregulation of PKC expression, and a subsequent increase in basal PKC activity, or through the sustained elevation of PKC activators, such as DAG.

Phospholipase D

PLD activity was first described in plants [53, 54], then later in mammals [55], as a novel phospholipid-specific phosphodiesterase that hydrolyzes membrane PC to generate PA and free choline. The next major breakthrough was the finding that PLD activity could be induced through a variety of extracellular stimuli [56]. Currently, it is known that many of the agonists that activate PC-PLD also activate PI-PLC and include a large number of neurotransmitters, hormones and growth factors [57].

To date, two mammalian PLD isoenzymes, PLD1 [58–60] and PLD2 [61, 62], have been cloned and shown to contain conserved sequences that are also found in PLDs isolated from plants, yeast and bacteria, thus defining an evolutionary conserved gene family and enzymatic function [63, 64]. Human PLD1 and PLD2 are 51% homologous, and splice variants of both enzymes have been described [65, 66]. PLD activity was initially isolated in the particulate fraction and is believed to be primarily a membrane-associated enzyme. Whereas PLD1 is localized to perinuclear endosomes [67] and trans-Golgi sites [68, 69], PLD2 is found predominantly at the plasma membrane [62, 70].

The most extensively studied PLD activity is PLD that is specific for PC. Upon stimulation, PC-PLD hydrolyzes membrane PC to generate PA and free choline. PA can then be metabolized directly to DAG through the actions of PA phosphohydrolase (PAP). DAG can be converted back to PA through phosphorylation via DAG kinase (fig. 2). PLD can also catalyze a transphosphatidylation reaction utilizing primary alcohols such as 1-butanol to generate phosphatidylbutanol, which has served as a convenient approach to assay PLD activity as well as to inhibit the formation of the PA product in cells. The relative predominance of PA or DAG formation in any particular cell after PLD activation is likely determined by the associated activity of PAP, such that in cells where there is high activity of PAP, PLD activity results in relatively more accumulation of DAG than PA [57, 71].

Additionally, PLD that can hydrolyze lipids other than PC have been described. Kiss and Anderson have reported the PLD-mediated hydrolysis of phosphatidylethanolamine in MCF-7 human breast carcinoma cells [72] and in NIH 3T3 fibroblasts [73, 74]. In addition, Daniel and colleagues described a PLD activity that catalyzed the hydrolysis of phosphatidylinositol in Madin-Darby canine kidney cells [75]. The contribution and significance of these lipid products to lipid signaling is unknown and is currently an open area of investigation.

A variety of cytosolic and membrane-associated factors have been demonstrated to regulate PLD in vivo and in vitro, and upregulation of PLD activity is associated with several cellular functions, including vesicle transport, endocytosis, cell migration and mitosis (for a general review, see [76, 77]). PLD1 is activated by the small guanosine triphosphatases (GTPases) ARF, RhoA and RalA and by the cPKC isoenzymes PKC α and βII. On the other hand, PLD2, which exhibits a higher basal level

of cellular activity than PLD1, was initially thought to be regulated primarily through an inhibitory mechanism mediated by α-synuclein, β-synuclein or fodrin. It has now been reported that PLD2 also responds to ARF with a modest increase in activity, and a very recent paper revealed that cPKC can activate PLD2 as well [78].

Regulation of PLD by PKC

One of the major cellular targets for PKC is PLD. The first hints of a link between PKC and PLD came from early studies that showed that phorbol esters such as PMA could induce PC hydrolysis, resulting in the accumulation of PA and DAG [79–82]. Prior to the cloning of PLD and the production of recombinant enzymes for study, a variety of indirect approaches built the case for a link between PKC and PLD [57]. It was found that downregulation of PKC (via high concentrations of phorbol esters or antisense oligomers) or inhibition of PKC activity could block the effect of phorbol esters on PC hydrolysis. Also, in studies where PLC was overexpressed and DAG levels were elevated, PKC activity was found to be upregulated, leading to an increase in PLD activity [83].

Regulation of PLD by PKC is a complex and incompletely understood interaction. There is much evidence to suggest that, at least in the cell, PLD activation by PKC requires an initial hydrolysis of PI lipids and generation of DAG. Presumably, DAG generation serves to recruit PKC to the inner face of the plasma membrane, where it is subsequently activated in the locale of PLD. This leads to a direct interaction between PKC and PLD (which may or may not be ATP-dependent) and, possibly, to other indirect effects mediated by PKC phosphorylation of substrates. That PKC and PLD associate upon activation has been fully demonstrated [78, 84, 85]. Moreover, early in vitro studies with recombinant PLD1 revealed that it is the calcium and the DAG-dependent PKC isoenzymes PKC α and β that activate PLD [65, 86]. These data are further supported by cellular studies in which overexpression of either cPKC isoenzyme served to enhance phorbol ester stimulation of PLD activity [87, 88].

Because PKC is a protein kinase, it is logical to predict that it mediates much of its cellular effects through ATP-dependent mechanisms. Surprisingly, there is evidence for both ATP-dependent and ATP-independent activation of PLD by PKC. Evidence for ATP-independent interaction between PKC and PLD comes from both in vitro and cellular data, which showed that the regulatory domain of PKC by itself is sufficient to activate PLD [89]. Moreover, residues within PLD that are not subject to phosphorylation have been identified as necessary for PKC [90]. Recently, Hu and Exton reported a specific residue in the catalytic domain of PKC a (phe663) that is required for PLD1 activation [91]. In this study, binding and early activation of PLD1 by PKC did not correlate with the phosphorylation status of PLD; in fact, phosphorylation was even found to reduce PLD activity. Thus, there is a strong case for ATP-independent interaction of PKC and PLD.

On the other hand, evidence suggesting a possible role for phosphorylation by PKC in the activation of PLD derives from two major lines of study: (i) the requirement for ATP in a cell-free system for PMA activation of PLD [92], and (ii) the ability of PKC to phosphorylate PLD as shown by two studies from the same group [93, 94].

Of course, it is possible that PKC has additional indirect effects on PLD. For example, there are data concerning an agonist-induced complex of PLD and PKC, along with a 220-kDa protein that becomes phosphorylated on serine and threonine residues when activated [85]. Thus, interaction of PKC and PLD in the cell may involve multiple mechanisms that may serve to fine-tune PLD activation.

Regulation of PLD by ceramide

Ceramide is a potent lipid second messenger that mediates a variety of cellular effects, including cellular differentiation and apoptosis (for reviews, see [95–97]). Ceramide can be derived in the cell from either the breakdown of sphingomyelin (SM) by sphingomyelinase (SMase) or synthesis by ceramide synthase. Conceptually, ceramide represents one end of the spectrum of antagonistic bioactive lipids that determine cell fate, with promitogenic DAG on the other end. It is being discovered that the glycerolipid and sphingolipid pathways intersect at numerous points, and PKC and PKC-regulating enzymes are at the forefront of this interaction.

Modulation of PLD activity by ceramide has been demonstrated to be a key mechanism in the antiproliferative actions of ceramide; however, the workings of this mechanism are unclear. Ceramide inhibition of PLD could occur by several mechanisms: (i) ceramide could exert its effects on PLD activators, such as PKC α and β, ARF-1, RhoA and CDC42. Importantly, this could include either direct inhibition of PKC activity or alteration of PKC subcellular localization (i.e. inhibition of translocation to the membrane); (ii) ceramide could have a direct effect on PLD, such as alteration of PLD trafficking or subcellular localization; or (iii) ceramide could disrupt the interaction of PLD with its activators, for example, PKC or PIP_2.

In the mid-1990s, several groups published the first reports on ceramide inhibition of PLD. While studying young versus senescent Wi38 fibroblast cells, Venable et al. came across a defect in PLD signaling in the senescent fibroblasts that correlated with an increase in SMase activity and an elevation of ceramide levels [98, 99]. The group found that C_6-ceramide action on young fibroblasts could recapitulate the signaling defects seen in the senes-

cent fibroblasts and that inhibition of PLD occurred through a disruption of the PLD-PKC interaction [100]. Around the same time, a partial inhibition of phorbol ester-induced PLD activity by C_2-ceramide was reported to occur in human neutrophils [101], as well as a partial inhibition of PLD activity by C_2- and C_6-ceramide in fibroblasts stimulated with phorbol esters and thrombin [102]. These results have been substantiated by numerous other studies that showed inhibition of PLD by C_2-ceramide or by C_6-ceramide and ceramide generated by exogenous SMases [103–116].

Stimulation of cells with agonists that activate PLD has been shown to correlate with the translocation or association of PLD activators to membrane surfaces. Indeed, data show that ceramide can inhibit the translocation of PKC to the plasma membrane in response to PMA [103, 117]. Currently, there is uncertainty about the ability of ceramide to inhibit PLD through its effects on the translocation of PKC α and β, ARF-1 and RhoA. Several investigations have reported that ceramide can mediate its inhibition of PLD, at least in part, by inhibiting translocation or membrane association of one or more of these activators [103, 104, 107]. Other data suggest that ceramide may mediate its effects on PLD through alternative mechanisms: Singh et al. reported that in a cell-free system, ceramide reversibly and directly inhibited PLD by competing with PIP_2 [112]. Another series of reports found that ceramide mediates its effects not by inhibiting the translocation of PLD activators but through the disruption of protein-protein interactions in which PLD participates in the plasma membrane [114, 116]. There is an intriguing finding that ceramide may mediate its effects through the disruption of lipid rafts, leading to an alteration of PLD membrane localization and normal protein-protein interactions [118]. While these studies clearly show the inhibitory effects of ceramide on PKC and PLD, the specific mechanisms operating in vivo have not been clearly established.

Regulation of PKC through PA

PA can be produced in the cell as a product of PC hydrolysis mediated by PLD or by the direct phosphorylation of DAG by DAG kinases (fig. 2). Although the physiological function of PA per se is not well understood, several in vitro and in vivo targets of PA have been identified, including PIP 5-kinase [119], PI-PLCβ [120], Raf-1 [121], protein phosphatase 1 [122] and PKC itself [28, 123, 124]. In addition, several groups have described PA-responsive protein kinases that to date remain unidentified but that have been determined as separate from known members of the PKC superfamily [125–127].

Early in vitro studies investigating lipid regulation of the classical PKC isoenzymes demonstrated an ability of PA to substitute for PS in the activation of PKC [28, 123, 124]. Importantly, the potency and specificity of PA substitution for PS were dependent upon whether the assay was performed with Triton X-100 mixed micelles or with sonicated lipid vesicles containing lipid bilayers [28, 128]. The greater specificity of PKC activation by PS over PA in the Triton X-100-based assay suggests that the electrostatic properties of the acidic PA head group may have accounted for the ability of PA to substitute for PS under some circumstances. Importantly, the concentrations of PA necessary for this activation are unlikely to be achieved in the cell. However, it remains to be determined whether PA may function as a lipid activator/cofactor of the classical PKCs.

On the other hand, evidence for a possible role for PA as a primary lipid activator for members of the atypical subfamily of PKC isoenzymes is accumulating. Activation of atypical PKC ζ, as measured by autophosphorylation, has been demonstrated in cell extracts of COS cells overexpressing PKC ξ treated with PA [129]. In addition, PA was seen to induce phosphorylation of an artificial PKC substrate, ε-pseudosubstrate, by purified PKC ζ in an in vitro assay [124]. This is further substantiated by unpublished studies from our laboratory using Triton X-100 mixed micelles with PA, which demonstrated that PA activates recombinant PKC ζ to a maximum at 5 mol% PA. Moreover, in cell studies where PLD was overexpressed and then stimulated with PMA in the presence of GFP-tagged PKC ζ, there was a shift in subcellular localization of PKC ζ from the cytosol to endocytic vesicles, suggesting an effect by PA. Thus, PA may directly regulate the activity of the atypical PKCs.

Regulation of PKC by PLD-derived DAG

It was first reported in the mid 1980s that cells stimulated with agonists that bind to G protein- and growth factor-coupled receptors generated two peaks of DAG. The initial peak, which appeared within 15 s of stimulation, was followed by a second peak that started at 5 min and was sustained at 1 h and later [130–136]. The initial peak of DAG corresponded with the production of IP_3 and was shown to arise from PLC-mediated hydrolysis of PI lipids, whereas the second peak was independent of IP_3 levels, and its source was unknown. Analysis of the fatty acyl chains of the second peak of DAG confirmed that these DAG species arose from PC hydrolysis, which is enriched with saturated fatty acids [133, 134, 137, 138]. These early results suggested the operation of either PC-specific PLC or PC-PLD. Over the ensuing years, evidence has pointed toward PC-PLD and away from PC-PLC as a source of the second peak of DAG, especially since no such mammalian enzyme has been identified at a molecular level. A role for PC-PLD as a producer of sustained DAG in the cell is well established. In a study

by Pettitt et al. [139], the authors examined the biphasic production of DAG and DAG species with mass spectrometry following agonist stimulation. They demonstrated that when 1-butanol was added to stimulated cells, the early peak of DAG was unchanged, but formation of the later peak (30 min) was completely blocked. In contrast, incubation of cells with 2-butanol, which is not utilized by PLD, had no effect on either peak of DAG.

Importantly, sustained elevations of DAG have been reported in a variety of pathological conditions, such as oncogenic Ras transformation [140–144], chronic angiotensin stimulation [135], lung cancer progression [145] and hyperglycemia [52]. Thus, understanding the mechanisms of sustained DAG is critical, specifically, whether it is capable of activating PKC.

Interestingly, data regarding PKC activation by sustained DAG derived from PC hydrolysis is scarce. Cellular PI lipids are predominantly composed of acyl chains that are saturated with stearate (18:0) in the *sn*-1 position and with polyunsaturated moieties, such as arachidonyl (20:4), in the *sn*-2 position [146] (fig. 1). As such, hydrolysis of PIP_2 leads to an accumulation of DAG species that have a corresponding composition. In contrast, PA and DAG species derived from PC hydrolysis are generally composed of saturated/monounsaturated acyl chains that have a predominance of palmitate, oleate and linoleate [133, 134, 147]. Initial in vitro biochemical analysis of DAG regulation of PKC revealed that unsaturated DAGs were most effective as activators of PKC in the presence of calcium and PS [2]. Subsequent studies revealed a strong requirement for polyunsaturation at the *sn*-2 position of DAG, with a gradation of decreasing potency that is proportional to saturation at this position. Saturated DAGs such as dipalmitoyl and distearoyl were the least effective, diunsaturated dioleoyl was more effective and polyunsaturated DAGs were the most potent [148–150]. Despite these studies and the requirement for polyunsaturation at the *sn*-2 position, some in vitro studies have demonstrated that naturally occurring and saturated species of DAG can activate PKC with similar effectiveness as polyunsaturated DAG [13, 151].

Although the purpose of analyzing PKC lipid regulation in vitro is to understand what occurs at the cell membrane level, studying regulation by DAG species that have different acyl compositions is inherently difficult. It is unknown whether differences observed between saturated and polyunsaturated DAG in vitro reflect true variations in potencies or whether they are due to different methods of presentation to PKC (although using detergent lipid mixed micelles tends to mitigate this concern). Moreover, in regard to PKC regulation by PC-derived DAG, mechanisms beyond the 'classical' concepts of PKC activation need to be explored in order to understand the effects of PLD-generated DAG on PKC. For instance, whereas polyunsaturated DAG is required for plasma membrane recruitment and activation of cPKCs, other DAGs at alternative membrane surfaces and with cofactors other than PS may be needed to activate PKC in a sustained manner.

In cells, there are data arguing both for and against a role for PLD-derived DAG in the activation of PKC. Early studies of PKC activation, as determined by PKC translocation or substrate phosphorylation in GH3 pituitary cells [152], IIC9 fibroblasts [153], GH4C1 pituitary cells [154] and RBL 2H3 mast cells [155] failed to reveal any effect of the sustained peak of DAG on PKC. These observations were followed by a study in porcine aortic endothelial cells wherein the authors devised a system using lysophosphatidic acid to stimulate PLD without concomitant activation of PLC. Based on a lack of translocation or enhancement of membrane-associated PKC, they concluded that PLD-derived DAG was unable to activate PKC [139]. Of note, this study recorded significant basal levels of membrane association of PKC isoenzymes in the absence of stimulation, which in our opinion makes evaluation of translocation difficult. Moreover, as mentioned above, it is conceivable that PLD-activated PKC may relocate to other compartments.

Alternatively, there are data suggesting that sustained DAG can activate PKC. Exton and colleagues demonstrated that in IIC9 fibroblasts stimulated with α-thrombin, which induces a biphasic accumulation of DAG, PKC α was transiently associated with the membrane (<1 min), but PKC ε was translocated to the plasma membrane in a sustained manner (60 min) [156]. Other cell studies substantiate these findings, including a study in which stimulation with a combination of bombesin and transforming growth factor β led to sustained accumulation of DAG and activation of PKC isoenzymes [157].

Although the above studies employed a traditional approach in evaluating PKC activation by PLD-derived DAG through biochemical fractionation methods, the use of GFP-tagged and confocal microscopy has provided additional perspectives. Recent data from our laboratory identified a novel site of translocation for GFP-PKC α and βII localized to a subset of recycling endosomes concentrated around the MTOC/centrosome during sustained activation [48], and this translocation has now been shown to occur in a PLD-dependent manner [158]. Hu and Exton recently reported the association of PKC α with PLD at a similar juxtanuclear compartment and to the subsequent activation of PLD [159]. This compartment thus defines a novel system in which to further study the relationship of PKC and PLD.

Concluding remarks and future directions

The discovery that DAG could function in the cell as a potent second messenger revolutionized our understanding

of signal transduction and thrust PKC and DAG-metabolizing enzymes (e.g. PI-PLC, SMS, PLD, DAG kinase) into the spotlight of cell biological research. The classical model of lipid signaling born of these early findings continues to serve as a powerful theoretical and experimental paradigm. On the other hand, recent technological advances in molecular biology and microscopy, coupled with a greater understanding of cell biology, have challenged the conceptual boundaries of this model. Certainly one of the largest tasks in the coming years will be to define the function of various lipid molecules and the regulation of lipid metabolizing enzymes and to incorporate these findings into an expanded model of lipid signaling.

This is exemplified by the complicated relationship between PKC and PLD. There is substantial evidence accumulated over the last 20 years to suggest that PLD is a major target for DAG/PMA-sensitive PKCs. Despite these significant advances, data regarding several aspects of this interaction are insufficient; for example, (i) where in the cell do PKC and PLD interact? The classical model of lipid signaling envisions the recruitment of PKC to the plasma membrane, where it can become activated and target downstream substrates such as PLD. In this model, PKC and PLD are thought to interact at the inner face of the plasma membrane. The recent discoveries of the pericentrion and the localization of PKC and PLD to a similar juxtanuclear compartment may constitute a cornerstone finding in our understanding of lipid signaling at intracellular membrane surfaces other than the plasma membrane; (ii) what role does phosphorylation of PLD by PKC play in its regulation? Large discrepancies among different studies suggest a highly complex regulation of PLD by PKC that may ultimately be revealed as both phosphorylation-dependent and phosphorylation-independent, and contextually determined (e.g. subcellular localization and protein or lipid cofactors); and (iii) what is the role of sphingolipids in PLD regulation? Glycerolipids (e.g. DAG) and sphingolipids (e.g. ceramide) have been suggested to be two functionally antagonistic molecules, and recent data have revealed that multiple sites of interaction may exist between these two pathways. Both ceramide and sphingosine have been shown to modulate PKC and PLD. It will be important in the coming years to dissect the significance of this interaction and its role in determining cell fate.

From the standpoint of DAG as a bioactive molecule, one of the most pressing questions is whether DAG derived from PC hydrolysis (via either PC-PLD or SMS) has signaling properties and can modulate any of the DAG/PMA-binding proteins (e.g. PKC, chimaerins). Although a comprehensive answer would require an exhaustive in vitro approach, ultimately the greatest insight will be derived from cell-based studies. Definitive studies in cells are needed to address whether PLD can produce local and sustained concentrations of DAG that, either alone or in conjunction with other lipid or protein cofactors, can effect PKC signaling. These questions await the development of cellular lipid probes that will allow the localization of distinct lipid species in live cells. Further, the recent development of small interfering RNA (siRNA) may make help answer these questions by providing the ability to isolate PLD-derived DAG through downregulation of other phospholipases and DAG kinase.

Although PLD/PKC interactions are mechanistically more complex than those of PI-PLC/PKC, technologies in cell biology are maturing at such a rapid rate that we are poised to witness several breakthroughs in lipid signaling.

Acknowledgement. This work was supported in part by the National Institutes of Health (NIH) grant HL 43707. We also thank Dr. Ashley Cowart for her careful review of this manuscript.

1 Hokin M. R. and Hokin L. E. (1953) Enzyme secretion and the incorporation of P32 into phospholipides of pancreas slices. J. Biol. Chem. **203:** 967–977
2 Takai Y., Kishimoto A., Kikkawa U., Mori T. and Nishizuka Y. (1979) Unsaturated diacylglycerol as a possible messenger for the activation of calcium-activated, phospholipid-dependent protein kinase system. Biochem. Biophys. Res. Commun. **91:** 1218–1224
3 Streb H., Irvine R. F., Berridge M. J. and Schulz I. (1983) Release of Ca2+ from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. Nature **306:** 67–69
4 Mellor H. and Parker P. J. (1998) The extended protein kinase C superfamily. Biochem. J. **332:** 281–292
5 Newton A. C. (2001) Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. Chem. Rev. **101:** 2353–2364
6 Ohno S. and Nishizuka Y. (2002) Protein kinase C isotypes and their specific functions: prologue. J. Biochem. **132:** 509–511
7 Inoue M., Kishimoto A., Takai Y. and Nishizuka Y. (1977) Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. II. Proenzyme and its activation by calcium-dependent protease from rat brain. J. Biol. Chem. **252:** 7610–7616
8 Takai Y., Yamamoto M., Inoue M., Kishimoto A. and Nishizuka Y. (1977) A proenzyme of cyclic nucleotide-independent protein kinase and its activation by calcium-dependent neutral protease from rat liver. Biochem. Biophys. Res. Commun. **77:** 542–550
9 Takai Y., Kishimoto A., Iwasa Y., Kawahara Y., Mori T. and Nishizuka Y. (1979) Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. J. Biol. Chem. **254:** 3692–3695
10 Castagna M., Takai Y., Kaibuchi K., Sano K., Kikkawa U. and Nishizuka Y. (1982) Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J. Biol. Chem. **257:** 7847–7851
11 Nishizuka Y. (1984) The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature **308:** 693–698
12 Sharkey N. A., Leach K. L. and Blumberg P. M. (1984) Competitive inhibition by diacylglycerol of specific phorbol ester binding. Proc. Natl. Acad. Sci. USA **81:** 607–610
13 Ebeling J. G., Vandenbark G. R., Kuhn L. J., Ganong B. R., Bell R. M. and Niedel J. E. (1985) Diacylglycerols mimic phorbol diester induction of leukemic cell differentiation. Proc. Natl. Acad. Sci. USA **82:** 815–819

14 Hannun Y. A. and Bell R. M. (1986) Phorbol ester binding and activation of protein kinase C on triton X-100 mixed micelles containing phosphatidylserine. J. Biol. Chem. **261:** 9341–9347
15 Ono Y., Fujii T., Igarashi K., Kuno T., Tanaka C., Kikkawa U. et al. (1989) Phorbol ester binding to protein kinase C requires a cysteine-rich zinc-finger-like sequence. Proc. Natl. Acad. Sci. USA **86:** 4868–4871
16 Burns D. J. and Bell R. M. (1991) Protein kinase C contains two phorbol ester binding domains. J. Biol. Chem. **266:** 18330–18338
17 Quest A. F., Bardes E. S. and Bell R. M. (1994) A phorbol ester binding domain of protein kinase C gamma. Deletion analysis of the Cys2 domain defines a minimal 43-amino acid peptide. J. Biol. Chem. **269:** 2961–2970
18 Quest A. F., Bardes E. S. and Bell R. M. (1994) A phorbol ester binding domain of protein kinase C gamma. High affinity binding to a glutathione-S-transferase/Cys2 fusion protein. J. Biol. Chem. **269:** 2953–2960
19 Hurley J. H., Newton A. C., Parker P. J., Blumberg P. M. and Nishizuka Y. (1997) Taxonomy and function of C1 protein kinase C homology domains. Prot. Sci. **6:** 477–480
20 Ohno S., Akita Y., Konno Y., Imajoh S. and Suzuki K. (1988) A novel phorbol ester receptor/protein kinase, nPKC, distantly related to the protein kinase C family. Cell **53:** 731–741
21 Saido T. C., Mizuno K., Konno Y., Osada S., Ohno S. and Suzuki K. (1992) Purification and characterization of protein kinase C epsilon from rabbit brain. Biochemistry **31:** 482–490
22 Medkova M. and Cho W. (1998) Mutagenesis of the C2 domain of protein kinase C-alpha. Differential roles of Ca^{2+} ligands and membrane binding residues. J. Biol. Chem. **273:** 17544–17552
23 Kishimoto A., Takai Y., Mori T., Kikkawa U. and Nishizuka Y. (1980) Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. J. Biol. Chem. **255:** 2273–2276
24 Hannun Y. A., Loomis C. R. and Bell R. M. (1985) Activation of protein kinase C by Triton X-100 mixed micelles containing diacylglycerol and phosphatidylserine. J. Biol. Chem. **269:** 10039–10043
25 Hannun Y. A., Loomis C. R. and Bell R. M. (1986) Protein kinase C activation in mixed micelles. Mechanistic implications of phospholipid, diacylglycerol, and calcium interdependencies. J. Biol. Chem. **261:** 7184–7190
26 Rando R. R. and Young N. (1984) The stereospecific activation of protein kinase C. Biochem. Biophys. Res. Commun. **122:** 818–823
27 Ganong B. R., Loomis C. R., Hannun Y. A. and Bell R. M. (1986) Specificity and mechanism of protein kinase C activation by sn-1,2-diacylglycerols. Proc. Natl. Acad. Sci. USA **83:** 1184–1188
28 Lee M. H. and Bell R. M. (1989) Phospholipid functional groups involved in protein kinase C activation, phorbol ester binding and binding to mixed micelles. J. Biol. Chem. **264:** 14797–14805
29 Orr J. W., Keranen L. M. and Newton A. C. (1992) Reversible exposure of the pseudosubstrate domain of protein kinase C by phosphatidylserine and diacylglycerol. J. Biol. Chem. **267:** 15263–15266
30 Kraft A. S. and Anderson W. B. (1983) Phorbol esters increase the amount of Ca2+, phospholipid-dependent protein kinase associated with plasma membrane. Nature **301:** 621–623
31 Borner C., Filipuzzi I., Wartmann M., Eppenberger U. and Fabbro D. (1989) Biosynthesis and posttranslational modifications of protein kinase C in human breast cancer cells. J. Biol. Chem. **264:** 13902–13909
32 Dutil E. M., Keranen L. M., DePaoli-Roach A. A. and Newton A. C. (1994) In vivo regulation of protein kinase C by trans-phosphorylation followed by autophosphorylation. J. Biol. Chem. **269:** 29359–29362
33 Pears C., Stabel S., Cazaubon S. and Parker P. J. (1992) Studies on the phosphorylation of protein kinase C-alpha. Biochem. J. **283:** 515–518
34 Edwards A. S. and Newton A. C. (1997) Phosphorylation at conserved carboxyl-terminal hydrophobic motif regulates the catalytic and regulatory domains of protein kinase C. J. Biol. Chem. **272:** 18382–18390
35 Flint A. J., Paladini R. D. and Koshland D. E. Jr (1990) Autophosphorylation of protein kinase C at three separated regions of its primary sequence. Science **249:** 408–411
36 Hansra G., Bornancin F., Whelan R., Hemmings B. A. and Parker P. J. (1996) 12-O-Tetradecanoylphorbol-13-acetate-induced dephosphorylation of protein kinase Calpha correlates with the presence of a membrane-associated protein phosphatase 2A heterotrimer. J. Biol. Chem. **271:** 32785–32788
37 Hansra G., Garcia-Paramio P., Prevostel C., Whelan R. D., Bornancin F. and Parker P. J. (1999) Multisite dephosphorylation and desensitization of conventional protein kinase C isotypes. Biochem. J. **342:** 337–344
38 Dutil E. M., Toker A. and Newton A. C. (1998) Regulation of conventional protein kinase C isozymes by phosphoinositide-dependent kinase 1 (PDK-1). Curr. Biol. **8:** 1366–1375
39 Chou M. M., Hou W., Johnson J., Graham L. K., Lee M. H., Chen C. S. et al. (1998) Regulation of protein kinase C zeta by PI 3-kinase and PDK-1. Curr. Biol. **8:** 1069–1077
40 Le Good J. A., Ziegler W. H., Parekh D. B., Alessi D. R., Cohen P. and Parker P. J. (1998) Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. Science **281:** 2042–2045
41 Nakhost A., Dyer J. R., Pepio A. M., Fan X. and Sossin W. S. (1999) Protein kinase C phosphorylated at a conserved threonine is retained in the cytoplasm. J. Biol. Chem. **274:** 28944–28949
42 Sakai N., Sasaki K., Ikegaki N., Shirai Y., Ono Y. and Saito N. (1998) Direct visualization of the translocation of the gamma-subspecies of protein kinase C in living cells using fusion proteins with green fluorescent protein. J. Cell Biol. **139:** 1465–1476
43 Oancea E., Teruel M. N., Quest A. F. and Meyer T. (1998) Green fluorescent protein (GFP)-tagged cysteine-rich domains from protein kinase C as fluorescent indicators for diacylglycerol signaling in living cells. J. Cell Biol. **140:** 485–498
44 Oancea E. and Meyer T. (1998) Protein kinase C as a molecular machine for decoding calcium and diacylglycerol signals. Cell **95:** 307–318
45 Feng X., Zhang J., Barak L. S., Meyer T., Caron M. G. and Hannun Y. A. (1998) Visualization of dynamic trafficking of a protein kinase C betaII/green fluorescent protein conjugate reveals differences in G protein-coupled receptor activation and desensitization. J. Biol. Chem. **273:** 10755–10762
46 Feng X., Becker K. P., Stribling S. D., Peters K. G. and Hannun Y. A. (2000) Regulation of receptor-mediated protein kinase C membrane trafficking by autophosphorylation. J. Biol. Chem. **275:** 17024–17034
47 England K. and Rumsby M. G. (2000) Changes in protein kinase C epsilon phosphorylation status and intracellular localization as 3T3 and 3T6 fibroblasts grow to confluency and quiescence: a role for phosphorylation at ser-729? Biochem. J. **1:** 19–26
48 Becker K. P. and Hannun Y. A. (2003) cPKC-dependent sequestration of membrane-recycling components in a subset of recycling endosome. J. Biol. Chem. **278:** 52747–52754
49 Wang Q. J., Bhattacharyya D., Garfield S., Nacro K., Marquez V. E. and Blumberg P. M. (1999) Differential localization of protein kinase C delta by phorbol esters and related compounds using a fusion protein with green fluorescent protein. J. Biol. Chem. **274:** 37233–37239
50 Nishizuka Y. (1995) Protein kinase C and lipid signaling for sustained cellular responses. FASEB J. **9:** 484–496

51 Blobe G. C., Stribling S., Obeid L. M. and Hannun Y. A. (1996) Protein kinase C isoenzymes: regulation and function. Cancer Sur. **27:** 213–248

52 Way K. J., Katai N. and King G. L. (2001) Protein kinase C and the development of diabetic vascular complications. Diabetic Med. **18:** 945–959

53 Hanahan D. J. and Chaikoff I. L. (1947) The phosphorus-containing lipides of the carrot. J. Biol. Chem. **168:** 233–240

54 Hanahan D. J. and Chaikoff I. L. (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. **172:** 191–198

55 Saito M. and Kanfer J. (1975) Phosphatidohydrolase activity in a solubilized preparation from rat brain particulate fraction. Arch. Biochem. Biophys. **169:** 318–323

56 Pai J. K., Siegel M. I., Egan R. W. and Billah M. M. (1988) Phospholipase D catalyzes phospholipid metabolism in chemotactic peptide-stimulated HL-60 granulocytes. J. Biol. Chem. **263:** 12472–12477

57 Exton J. H. (1997) Phospholipase D: enzymology, mechanisms of regulation and function. Phys. Rev. **77:** 303–320

58 Hammond S. M., Altshuller Y. M., Sung T. C., Rudge S. A., Rose K., Engebrecht J. et al. (1995) Human ADP-ribosylation factor-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved gene family. J. Biol. Chem. **270:** 29640–29643

59 Park S. K., Provost J. J., Bae C. D., Ho W. T. and Exton J. H. (1997) Cloning and characterization of phospholipase D from rat brain. J. Biol. Chem. **272:** 29263–29271

60 Colley W. C., Altshuller Y. M., Sue-Ling C. K., Copeland N. G., Gilbert D. J., Jenkins N. A. et al. (1997) Cloning and expression analysis of murine phospholipase D1. Biochem. J. **326:** 745–753

61 Lopez I., Arnold R. S. and Lambeth J. D. (1998) Cloning and initial characterization of a human phospholipase D2 (hPLD2). ADP-ribosylation factor regulates hPLD2. J. Biol. Chem. **273:** 12846–12852

62 Colley W. C., Sung T. C., Roll R., Jenco J., Hammond S. M., Altshuller Y. et al. (1997) Phospholipase D2, a distinct phospholipase D isoform with novel regulatory properties that provokes cytoskeletal reorganization. Curr. Biol. **7:** 191–201

63 Morris A. J., Engebrecht J. and Frohman M. A. (1996) Structure and regulation of phospholipase D. Trends Pharmacol. Sci. **17:** 182–185

64 Sung T. C., Roper R. L., Zhang Y., Rudge S. A., Temel R., Hammond S. et al. (1997) Mutagenesis of phospholipase D defines a superfamily including a trans-Golgi viral protein required for poxvirus pathogenicity. EMBO J. **16:** 4519–4530

65 Hammond S. M., Jenco J. M., Nakashima S., Cadwallader K., Gu Q., Cook S. et al. (1997) 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-alpha. J. Biol. Chem. **272:** 3860–3868

66 Redina O. E. and Frohman M. A. (1998) Organization and alternative splicing of the murine phospholipase D2 gene. Biochem. J. **331:** 845–851

67 Du G., Altshuller Y. M., Vitale N., Huang P., Chasserot-Golaz S., Morris A. et al. (2003) Regulation of phospholipase D1 subcellular cycling through coordination of multiple membrane association motifs. J. Cell Biol. **162:** 305–315

68 Strand A. M., Lauritzen L., Vinggaard A. M. and Hansen H. S. (1999) The subcellular localization of phospholipase D activities in rat Leydig cells. Mol. Cell. Endocrinol. **152:** 99–110

69 Freyberg Z., Sweeney D., Siddhanta A., Bourgoin S., Frohman M. and Shields D. (2001) Intracellular localization of phospholipase D1 in mammalian cells. Mol. Biol. Cell. **12:** 943–955

70 Slaaby R., Jensen T., Hansen H. S., Frohman M. A. and Seedorf K. (1998) PLD2 complexes with the EGF receptor and undergoes tyrosine phosphorylation at a single site upon agonist stimulation. J. Biol. Chem. **273:** 33722–33727

71 Mullmann T. J., Siegel M. I., Egan R. W. and Billah M. M. (1990) Phorbol-12-myristate-13-acetate activation of phospholipase D in human neutrophils leads to the production of phosphatides and diglycerides. Biochem. Biophys. Res. Commun. **170:** 1197–1202

72 Kiss Z., Tomono M. and Anderson W. B. (1994) Phorbol ester selectively stimulates the phospholipase D-mediated hydrolysis of phosphatidylethanolamine in multidrug-resistant MCF-7 human breast carcinoma cells. Biochem. J. **302:** 649–654

73 Kiss Z. and Anderson W. B. (1989) Alcohols selectively stimulate phospholipase D-mediated hydrolysis of phosphatidylethanolamine in NIH 3T3 cells. FEBS Lett. **257:** 45–48

74 Kiss Z. and Anderson W. H. (1994) Hydrogen peroxide regulates phospholipase D-mediated hydrolysis of phosphatidylethanolamine and phosphatidylcholine by different mechanisms in NIH 3T3 fibroblasts. Arch. Biochem. Biophys. **311:** 430–436

75 Huang C., Wykle R. L., Daniel L. W. and Cabot M. C. (1992) Identification of phosphatidylcholine-selective and phosphatidylinositol-selective phospholipases D in Madin-Darby canine kidney cells. J. Biol. Chem. **267:** 16859–16865

76 Exton J. H. (2002) Regulation of phospholipase D. FEBS Lett. **531:** 58–61

77 Exton J. H. (2002) Phospholipase D-structure, regulation and function. Rev. Phys. Biochem. Pharm. **144:** 1–94

78 Chen J. S. and Exton J. H. (2004) Regulation of phospholipase D2 activity by protein kinase C alpha. J. Biol. Chem. **279:** 22076–22083

79 Mufson R. A., Okin E. and Weinstein I. B. (1981) Phorbol esters stimulate the rapid release of choline from prelabelled cells. Carcinogenesis **2:** 1095–1102

80 Tettenborn C. S. and Mueller G. C. (1988) 12-O-tetradecanoylphorbol-13-acetate activates phosphatidylethanol and phosphatidylglycerol synthesis by phospholipase D in cell lysates. Biochem. Biophys. Res. Commun. **155:** 249–255

81 Exton J. H. (1988) Mechanisms of action of calcium-mobilizing agonists: some variations on a young theme. FASEB J. **2:** 2670–2676

82 Mullmann T. J., Siegel M. I., Egan R. W. and Billah M. M. (1990) Phorbol-12-myristate-13-acetate activation of phospholipase D in human neutrophils leads to the production of phosphatides and diglycerides. Biochem. Biophys. Res. Commun. **170:** 1197–1202

83 Lee Y. H., Kim H. S., Pai J. K., Ryu S. H. and Suh P. G. (1994) Activation of phospholipase D induced by platelet-derived growth factor is dependent upon the level of phospholipase C-gamma 1. J. Biol. Chem. **269:** 26842–26847

84 Lee T. G., Park J. B., Lee S. D., Hong S., Kim J. H., Kim Y. et al. (1997) Phorbol myristate acetate-dependent association of protein kinase C alpha with phospholipase D1 in intact cells. Biochim. Biophys. Acta **1347:** 199–204

85 Min D. S. and Exton J. H. (1998) Phospholipase D is associated in a phorbol ester-dependent manner with protein kinase C-alpha and with a 220-kDa protein which is phosphorylated on serine and threonine. Biochem. Biophys. Res. Commun. **248:** 533–537

86 Min D. S., Park S. K. and Exton J. H. (1998) Characterization of a rat brain phospholipase D isozyme. J. Biol. Chem. **273:** 7044–7051

87 Pai J. K., Pachter J. A., Weinstein I. B. and Bishop W. R. (1991) Overexpression of protein kinase C beta 1 enhances phospholipase D activity and diacylglycerol formation in phorbol ester-stimulated rat fibroblasts. Proc. Natl. Acad. Sci. USA **88:** 598–602

88 Eldar H., Ben-Av P., Schmidt U. S., Livneh E. and Liscovitch M. (1993) Up-regulation of phospholipase D activity induced by overexpression of protein kinase C-alpha. Studies in intact Swiss/3T3 cells and in detergent-solubilized membranes in vitro. J. Biol. Chem. **268:** 12560–12564

89 Singer W. D., Brown H. A., Jiang X. and Sternweis P. C. (1996) Regulation of phospholipase D by protein kinase C is synergistic with ADP-ribosylation factor and independent of protein kinase activity. J. Biol. Chem. **271:** 4504–4510

90 Zhang Y., Altshuller Y. M., Hammond S. M., Hayes F., Morris A. J. and Frohman M. A. (1999) Loss of receptor regulation by a phospholipase D1 mutant unresponsive to protein kinase C [erratum appears in EMBO J 2000 Jan 17;19(2):315]. EMBO J. **18:** 6339–6348

91 Hu T. and Exton J. H. (2003) Mechanisms of regulation of phospholipase D1 by protein kinase Calpha. J. Biol. Chem. **278:** 2348–2355

92 Lopez I., Burns D. J. and Lambeth J. D. (1995) Regulation of phospholipase D by protein kinase C in human neutrophils. Conventional isoforms of protein kinase C phosphorylate a phospholipase D-related component in the plasma membrane. J. Biol. Chem. **270:** 19465–19472

93 Kim Y., Han J. M., Han B. R., Lee K. A., Kim J. H., Lee B. D. et al. (2000) Phospholipase D1 is phosphorylated and activated by protein kinase C in caveolin-enriched microdomains within the plasma membrane. J. Biol. Chem. **275:** 13621–13627

94 Kim Y., Han J. M., Park J. B., Lee S. D., Oh Y. S., Chung C. et al. (1999) Phosphorylation and activation of phospholipase D1 by protein kinase C in vivo: determination of multiple phosphorylation sites. Biochemistry **38:** 10344–10351

95 Obeid L. M. and Hannun Y. A. (2003) Ceramide, stress and a 'LAG' in aging. Sci. Aging Know. Environ. **39:** 27.

96 Ogretmen B. and Hannun Y. A. (2004) Biologically active sphingolipids in cancer pathogenesis and treatment. Nat. Rev. Can. **4:** 604–616

97 Pettus B. J., Chalfant C. E. and Hannun Y. A. (2002) Ceramide in apoptosis: an overview and current perspectives. Biochim. Biophys. Acta **1585:** 114–125

98 Venable M. E., Blobe G. C. and Obeid L. M. (1994) Identification of a defect in the phospholipase D/diacylglycerol pathway in cellular senescence. J. Biol. Chem. **269:** 26040–26044

99 Venable M. E., Lee J. Y., Smyth M. J., Bielawska A. and Obeid L. M. (1995) Role of ceramide in cellular senescence. J. Biol. Chem. **270:** 30701–30708

100 Venable M. E., Bielawska A. and Obeid L. M. (1996) Ceramide inhibits phospholipase D in a cell-free system. J. Biol. Chem. **271:** 24800–24805

101 Nakamura T., Abe A., Balazovich K. J., Wu D., Suchard S. J., Boxer L. A. and Shayman J. A. (1994) Ceramide regulates oxidant release in adherent human neutrophils. J. Biol. Chem. **269:** 18384–18389

102 Gomez-Munoz A., Martin A., O'Brien L. and Brindley D. N. (1994) Cell-permeable ceramides inhibit the stimulation of DNA synthesis and phospholipase D activity by phosphatidate and lysophosphatidate in rat fibroblasts. J. Biol. Chem. **269:** 8937–8943

103 Jones M. J. and Murray A. W. (1995) Evidence that ceramide selectively inhibits protein kinase C-alpha translocation and modulates bradykinin activation of phospholipase D. J. Biol. Chem. **270:** 5007–5013

104 Nakamura Y., Nakashima S., Ojio K., Banno Y., Miyata H. and Nozawa Y. (1996) Ceramide inhibits IgE-mediated activation of phospholipase D, but not of phospholipase C, in rat basophilic leukemia (RBL-2H3) cells. J. Immunol. **156:** 256–262

105 Olivera A., Romanowski A., Rani C. S. and Spiegel S. (1997) Differential effects of sphingomyelinase and cell-permeable ceramide analogs on proliferation of Swiss 3T3 fibroblasts. Biochim. Biophys. Acta **3:** 311–323

106 Yoshimura S., Sakai H., Ohguchi K., Nakashima S., Banno Y., Nishimura Y. et al. (1997) Changes in the activity and mRNA levels of phospholipase D during ceramide-induced apoptosis in rat C6 glial cells. J. Neurochem. **69:** 713–720

107 Abousalham A., Liossis C., O'Brien L. and Brindley D. N. (1997) Cell-permeable ceramides prevent the activation of phospholipase D by ADP-ribosylation factor and RhoA. J. Biol. Chem. **272:** 1069–1075

108 Iwasaki-Bessho Y., Banno Y., Yoshimura S., Ito Y., Kitajima Y. and Nozawa Y. (1998) Decreased phospholipase D (PLD) activity in ceramide-induced apoptosis of human keratinocyte cell line HaCaT. J. Invest. Derm. **110:** 376–382

109 Kim J. H., Yoon Y. D., Shin I. and Han J. S. (1999) Effects of ceramide, the Fas signal intermediate, on apoptosis and phospholipase D activity in mouse ovarian granulosa cells in vitro. IUBMB Life **48:** 445–452

110 Park B. J., Kim J. H., Han J. S. and Jung P. M. (1999) Effect of ceramide on apoptosis and phospholipase D activity in FRTL-5 thyroid cells. Exp. Mol. Med. **31:** 142–150

111 Chen J. S., Chai M. Q., Chen H. H., Zhao S. and Song J. G. (2000) Regulation of phospholipase D activity and ceramide production in daunorubicin-induced apoptosis in A-431 cells. Biochim. Biophys. Acta **3:** 219–232

112 Singh I. N., Stromberg L. M., Bourgoin S. G., Sciorra V. A., Morris A. J. and Brindley D. N. (2001) Ceramide inhibition of mammalian phospholipase D1 and D2 activities is antagonized by phosphatidylinositol 4,5-bisphosphate. Biochemistry **40:** 11227–11233

113 Abousalham A., Hobman T. C., Dewald J., Garbutt M. and Brindley D. N. (2002) Cell-permeable ceramides preferentially inhibit coated vesicle formation and exocytosis in Chinese hamster ovary compared with Madin-Darby canine kidney cells by preventing the membrane association of ADP-ribosylation factor. Biochem. J. **361:** 653–661

114 Mansfield P. J., Hinkovska-Galcheva V., Carey S. S., Shayman J. A. and Boxer L. A. (2002) Regulation of polymorphonuclear leukocyte degranulation and oxidant production by ceramide through inhibition of phospholipase D. Blood **99:** 1434–1441

115 Kim K. O., Lee K. H., Kim Y. H., Park S. K. and Han J. S. (2003) Anti-apoptotic role of phospholipase D isozymes in the glutamate-induced cell death. Exper. Mol. Med. **35:** 38–45

116 Mansfield P. J., Carey S. S., Hinkovska-Galcheva V., Shayman J. A. and Boxer L. A. (2004) Ceramide inhibition of phospholipase D and its relationship to RhoA and ARF1 translocation in GTP gamma S-stimulated polymorphonuclear leukocytes. Blood **103:** 2363–2368

117 Signorelli P., Luberto C. and Hannun Y. A. (2001) Ceramide inhibition of NF-kappaB activation involves reverse translocation of classical protein kinase C (PKC) isoenzymes: requirement for kinase activity and carboxyl-terminal phosphorylation of PKC for the ceramide response. FASEB J. **15:** 2401–2414

118 Gidwani A., Brown H. A., Holowka D. and Baird B. (2003) Disruption of lipid order by short-chain ceramides correlates with inhibition of phospholipase D and downstream signaling by FcepsilonRI. J. Cell Sci. **116:** 3177–3187

119 Moritz A., De Graan P. N., Gispen W. H. and Wirtz K. W. (1992) Phosphatidic acid is a specific activator of phosphatidylinositol-4-phosphate kinase. J. Biol. Chem. **267:** 7207–7210

120 Jackowski S. and Rock C. O. (1989) Stimulation of phosphatidylinositol 4,5-bisphosphate phospholipase C activity by phosphatidic acid. Arch. Biochem. Biophys. **268:** 516–524

121 Rizzo M. A., Shome K., Vasudevan C., Stolz D. B., Sung T. C., Frohman M. A. et al. (1999) Phospholipase D and its product, phosphatidic acid, mediate agonist-dependent raf-1 translocation to the plasma membrane and the activation of the mitogen-activated protein kinase pathway. J. Biol. Chem. **274:** 1131–1139

122 Jones J. A. and Hannun Y. A. (2002) Tight binding inhibition of protein phosphatase-1 by phosphatidic acid. Specificity of inhibition by the phospholipid. J. Biol. Chem. **277:** 15530–15538
123 Epand R. M., Stafford A. R. and Lester D. S. (1992) Lipid vesicles which can bind to protein kinase C and activate the enzyme in the presence of EGTA. Eur. J. Biochem. **208:** 327–332
124 Nakanishi H. and Exton J. H. (1992) Purification and characterization of the zeta isoform of protein kinase C from bovine kidney. J. Biol. Chem. **267:** 16347–16354
125 Bocckino S. B., Wilson P. B. and Exton J. H. (1991) Phosphatidate-dependent protein phosphorylation. Proc. Natl. Acad. Sci. USA **88:** 6210–6213
126 Khan W. A., Blobe G. C., Richards A. L. and Hannun Y. A. (1994) Identification, partial purification and characterization of a novel phospholipid-dependent and fatty acid-activated protein kinase from human platelets. J. Biol. Chem. **269:** 9729–9735
127 McPhail L. C., Qualliotine-Mann D. and Waite K. A. (1995) Cell-free activation of neutrophil NADPH oxidase by a phosphatidic acid-regulated protein kinase. Proc. Natl. Acad. Sci. USA **92:** 7931–7935
128 Mosior M. and Epand R. M. (1993) Mechanism of activation of protein kinase C: roles of diolein and phosphatidylserine. Biochemistry **32:** 66–75
129 Limatola C., Schaap D., Moolenaar W. H. and van Blitterswijk W. J. (1994) Phosphatidic acid activation of protein kinase C-zeta overexpressed in COS cells: comparison with other protein kinase C isotypes and other acidic lipids. Biochem. J. **304:** 1001–1008
130 Kojima I., Kojima K., Kreutter D. and Rasmussen H. (1984) The temporal integration of the aldosterone secretory response to angiotensin occurs via two intracellular pathways. J. Biol. Chem. **259:** 14448–14457
131 Griendling K. K., Rittenhouse S. E., Brock T. A., Ekstein L. S., Gimbrone M. A. Jr and Alexander R. W. (1986) Sustained diacylglycerol formation from inositol phospholipids in angiotensin II-stimulated vascular smooth muscle cells. J. Biol. Chem. **261:** 5901–5906
132 Wright T. M., Rangan L. A., Shin H. S. and Raben D. M. (1988) Kinetic analysis of 1,2-diacylglycerol mass levels in cultured fibroblasts. Comparison of stimulation by alpha-thrombin and epidermal growth factor. J. Biol. Chem. **263:** 9374–9380
133 Pessin M. S. and Raben D. M. (1989) Molecular species analysis of 1,2-diglycerides stimulated by alpha-thrombin in cultured fibroblasts. J. Biol. Chem. **264:** 8729–8738
134 Pettitt T. R. and Wakelam M. J. (1993) Bombesin stimulates distinct time-dependent changes in the sn-1,2-diradylglycerol molecular species profile from Swiss 3T3 fibroblasts as analysed by 3,5-dinitrobenzoyl derivatization and h.p.l.c. separation. Biochem. J. **289:** 487–495
135 Tuominen R. K., Werner M. H., Ye H., McMillian M. K., Hudson P. M., Hannun Y. A. et al. (1993) Biphasic generation of diacylglycerol by angiotensin and phorbol ester in bovine adrenal chromaffin cells. Biochem. Biophys. Res. Commun. **190:** 181–185
136 Cook S. J., Palmer S., Plevin R. and Wakelam M. J. (1990) Mass measurement of inositol 1,4,5-trisphosphate and sn-1,2-diacylglycerol in bombesin-stimulated Swiss 3T3 mouse fibroblasts. Biochem. J. **265:** 617–620
137 Bocckino S. B., Blackmore P. F., Wilson P. B. and Exton J. H. (1987) Phosphatidate accumulation in hormone-treated hepatocytes via a phospholipase D mechanism. J. Biol. Chem. **262:** 15309–15315
138 Kennerly D. A. (1987) Diacylglycerol metabolism in mast cells. Analysis of lipid metabolic pathways using molecular species analysis of intermediates. J. Biol. Chem. **262:** 16305–16313
139 Pettitt T. R., Martin A., Horton T., Liossis C., Lord J. M. and Wakelam M. J. (1997) Diacylglycerol and phosphatidate generated by phospholipases C and D, respectively, have distinct fatty acid compositions and functions. Phospholipase D-derived diacylglycerol does not activate protein kinase C in porcine aortic endothelial cells. J. Biol. Chem. **272:** 17354–17359
140 Fleischman L. F., Chahwala S. B. and Cantley L. (1986) ras-transformed cells: altered levels of phosphatidylinositol-4,5-bisphosphate and catabolites. Science **231:** 407–410
141 Bell R. M., Hannun Y. A. and Loomis C. R. (1986) Mechanism of regulation of protein kinase C by lipid second messengers. Symp. Fund. Can. Res. **39:** 145–156
142 Wolfman A. and Macara I. G. (1987) Elevated levels of diacylglycerol and decreased phorbol ester sensitivity in ras-transformed fibroblasts. Nature **325:** 359–361
143 Carnero A., Dolfi F. and Lacal J. C. (1994) ras-p21 activates phospholipase D and A2, but not phospholipase C or PKC, in *Xenopus laevis* oocytes. J. Cell. Biochem. **54:** 478–486
144 Martin A., Duffy P. A., Liossis C., Gomez-Munoz A., O'Brien L., Stone J. C. et al. (1997) Increased concentrations of phosphatidate, diacylglycerol and ceramide in ras- and tyrosine kinase (fps)-transformed fibroblasts. Oncogene **14:** 1571–1580
145 Casamassima F., Pacini S., Dragotto A., Anichini M., Chiarugi V. and Ruggiero M. (1996) Intracellular diacylglycerol: a mitogenic second messenger proposable as marker of transformation in squamous cell carcinoma of the lung. Lung Cancer **15:** 161–170
146 Holub B. J., Kuksis A. and Thompson W. (1970) Molecular species of mono-, di- and triphosphoinositides of bovine brain. J. Lipid Res. **11:** 558–564
147 Pettitt T. R., McDermott M., Saqib K. M., Shimwell N. and Wakelam M. J. (2001) Phospholipase D1b and D2a generate structurally identical phosphatidic acid species in mammalian cells. Biochem. J. **360:** 707–715
148 Mori T., Takai Y., Yu B., Takahashi J., Nishizuka Y. and Fujikura T. (1982) Specificity of the fatty acyl moieties of diacylglycerol for the activation of calcium-activated, phospholipid-dependent protein kinase. J. Biochem. **91:** 427–431
149 Marignani P. A., Epand R. M. and Sebaldt R. J. (1996) Acyl chain dependence of diacylglycerol activation of protein kinase C activity in vitro. Biochem. Biophys. Res. Commun. **225:** 469–473
150 Schachter J. B., Lester D. S. and Alkon D. L. (1996) Synergistic activation of protein kinase C by arachidonic acid and diacylglycerols in vitro: generation of a stable membrane-bound, cofactor-independent state of protein kinase C activity. Biochim. Biophys. Acta **2:** 167–176
151 Go M., Sekiguchi K., Nomura H., Kikkawa U. and Nishizuka Y. (1987) Further studies on the specificity of diacylglycerol for protein kinase C activation. Biochem. Biophys. Res. Commun. **144:** 598–605
152 Martin T. F., Hsieh K. P. and Porter B. W. (1990) The sustained second phase of hormone-stimulated diacylglycerol accumulation does not activate protein kinase C in GH3 cells. J. Biol. Chem. **265:** 7623–7631
153 Leach K. L., Ruff V. A., Wright T. M., Pessin M. S. and Raben D. M. (1991) Dissociation of protein kinase C activation nd sn-1,2-diacylglycerol formation. Comparison of phosphatidylinositol- and phosphatidylcholine-derived diglycerides in alpha-thrombin-stimulated fibroblasts. J. Biol. Chem. **266:** 3215–3221
154 Kiley S. C., Parker P. J., Fabbro D. and Jaken S. (1991) Differential regulation of protein kinase C isozymes by thyrotropin-releasing hormone in GH4C1 cells. J. Biol. Chem. **266:** 23761–23768
155 Lin P., Fung W. J. and Gilfillan A. M. (1992) Phosphatidylcholine-specific phospholipase D-derived 1,2-diacylglycerol does not initiate protein kinase C activation in the RBL 2H3 mast-cell line. Biochem. J. **287:** 325–331

156 Ha K. S. and Exton J. H. (1993) Differential translocation of protein kinase C isozymes by thrombin and platelet-derived growth factor. A possible function for phosphatidylcholine-derived diacylglycerol. J. Biol. Chem. **268:** 10534–10539
157 Olivier A. R., Hansra G., Pettitt T. R., Wakelam M. J. and Parker P. J. (1996) The co-mitogenic combination of transforming growth factor beta 1 and bombesin protects protein kinase C-delta from late-phase down-regulation, despite synergy in diacylglycerol accumulation. Biochem. J. **318:** 519–525
158 Becker K. P. and Hannun Y. A. (2004) Isoenzyme-specific translocation of protein kinase C (PKC) betaII and not PKC betaI to a juxtanuclear subset of recycling endosomes: involvement of phospholipase D. J. Biol. Chem. **279:** 28251–28256
159 Hu T. and Exton J. H. (2004) Protein kinase C translocates to the perinuclear region to activate phospholipase D1. J. Biol. Chem. **279:** 35702–35708