Tuning the Signaling Output of Protein Kinase C

Corina E. Antal[*,#] and **Alexandra C. Newton**[*,1]

[*]Department of Pharmacology, University of California at San Diego, La Jolla, CA, 92093, USA

[#]Biomedical Sciences Graduate Program, University of California at San Diego, La Jolla, CA, 92093, USA

Abstract

Precise control of the amplitude of protein kinase C signaling is essential for maintaining cellular homeostasis, and disruption of this control leads to pathophysiological states such as cancer and neurodegeneration. This amplitude is precisely tuned by multiple inputs that regulate the amount of protein kinase C in the cell, its ability to sense its allosteric activator diacylglycerol, and protein scaffolds that coordinate access to substrates. Key to regulation of the signaling output of most protein kinase C isozymes is the ability of cytosolic enzyme to respond to the membrane-embedded lipid second messenger, diacylglycerol, in a dynamic range that prevents signaling in the absence of agonists but allows efficient signaling in response to small changes in diacylglycerol. This review discusses the regulatory inputs that control the spatiotemporal dynamics of protein kinase C signaling, focusing on conventional and novel PKC isozymes.

Keywords

Introduction

The protein kinase C (PKC) family transduces a multitude of signals that control diverse cellular processes such as proliferation, migration, invasion, differentiation, apoptosis, transcription, and translation. Therefore, aberrant PKC activity or localization has been linked to numerous diseases, most notable of which are cancer, neurodegeneration, and diabetes [1]. This serine/threonine kinase family belongs on the AGC kinase branch of the kinome and comprises 9 genes that share a similar architecture with an N-terminal regulatory moeity and a C-terminal kinase domain (Figure 1). PKCs are classified according to their regulatory domains, and thus the 2nd messengers that regulate their activity. Conventional PKCs (cPKCs: α, β and γ) contain tandem C1 domains that bind diacylglycerol (DAG) and phosphatidylserine (PS) and a C2 domain that binds anionic phospholipids, including phosphatidylinositol 4,5-bisphosphate (PIP$_2$), in a Ca^{2+}-dependent manner. Novel PKCs (nPKCs: δ, ε, η, θ) contain tandem C1 domains that bind diacylglycerol and a novel C2 domain that is Ca^{2+}-unresponsive and does not assist in

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[1]To whom correspondence should be addressed (anewton@ucsd.edu).

membrane binding. Atypical PKCs (aPKCs: ι and ζ) have an atypical C1 domain that does not bind diacylglycerol and lack a C2 domain all together, but instead contain a PB1 domain that mediates protein-protein interactions. Atypical PKCs and PKCα contain a C-terminal PDZ ligand.

Regulation by priming phosphorylation

Phosphorylation is absolutely critical 1] to render PKC in a catalytically competent conformation and 2] to protect PKC from degradation [2]. Because, unlike many other kinases, the phosphorylation of PKC is constiututive, its activity is not acutely regulated by phosphorylation. Rather, cellular levels of PKC are directly regulated by its phosphorylation. Conventional and novel PKCs are constitutively phosphorylated at 3 conserved residues: the activation loop, the turn motif, and the hydrophobic motif [3] (Figure 1). Atypical PKCs are also phosphorylated at the activation loop and turn motif, but contain a phosphomimetic glutamic acid at the hydrophobic motif. The first priming phosphorylation occurs at the activation loop within the kinase domain and is catalyzed by the phosphoinositide-dependent kinase, PDK-1 [4, 5]. Phosphorylation at this site properly aligns residues within the active site for catalysis, an event that induces two tightly-coupled and ordered phosphorylations on the C-terminal tail: phosphorylation at the turn motif and intramolecular autophosphorylation at the hydrophobic motif. For conventional and novel PKCs such as PKCε, but not PKCδ, these phosphorylation events require mTORC2; however, whether mTOR is the direct kinase for this site in cells remains controversial [6–9]. Phosphorylation at the hydrophobic motif controls the stability of the enzyme. Indeed, dephosphorylation of this site is the first step in the degradation of PKC as it destabilizes PKC and promotes its degradation. The PH domain leucine-rich repeat protein phosphatases (PHLPP) directly dephosphorylates the hydrophobic motif of PKCs, an event that requires the PH domain of PHLPP [10]. Thus, loss of PHLPP in the cell leads to an increase in steady state PKC levels. Conversely, PKC levels are low in cells in which any of the phosphorylation steps have been perburbed, including loss of TORC2 or loss of PDK-1 [7, 11]. Thus, priming phosphorylations regulate the steady state levels of PKC but not activity in an acute, agonist-dependent manner; instead, the spatial and temporal dynamics of PKC signaling are regulated by second messengers.

Regulation by second messengers

Although phosphorylated PKC is catalytically competent, an autoinhibitory pseudosubstrate the binds the substrate binding cavity maintains PKC inactive until the appropriate second messengers bind (Figure 2). Conventional PKCs are allosterically activated by binding to two second messengers: Ca^{2+} and diacylglycerol. Binding of the C2 domain to Ca^{2+} targets the kinase to the plasma membrane through hydrophobic interactions that drive binding to anionic phospholipids and through electrostatic interactions that contribute to retention of the C2 domain to membranes [12]. Once at the membrane, one of PKC's C1 domains can then find and bind DAG, and event that provides the necessary energy to expel the pseudosubstrate and activate PKC [13]. Novel PKCs are activated solely by DAG, whereas atypical PKCs do not respond to either of these second messengers and are instead activated by protein-protein interactions.

Differential binding of 2nd messengers between conventional and novel PKCs leads to substantial differences in their spatiotemporal dynamics of signaling. Firstly, conventional and novel PKCs predominantly translocate to different membranes. Conventional PKCs translocate to and are active at the plasma membrane because their Ca^{2+}-binding C2 domain targets them there via PIP_2, where they can then find diacylglycerol [12]. Novel PKCs do not have a functional C2 domain but have a C1A domain that has a 100-fold higher affinity for DAG [14] and, therefore, they translocate to DAG-rich endomembranes such as the Golgi. Indeed, impairing the ability of the C2 domain of PKCα to bind membranes forces it to the Golgi instead, as the C1 domain interaction becomes the dominant force [12]. Secondly, the kinetics of activation differ between conventional and novel PKCs. Conventional PKCs display rapid but transient activity at the plasma membrane that tracks with the initial Ca^{2+} release [15]. In fact, the kinetics of cPKC activation directly correlate with Ca^{2+} levels [16]. PKCβII exhibits oscillatory translocation to the plasma membrane in response to Ca^{2+} oscillations produced by histamine stimulation of HeLa cells. This in turn leads to oscillatory phosphorylation of membrane-localized substrates that tracks Ca^{2+} levels with a slight lag. Novel PKCs, on the other hand, are activated with slower kinetics because they do not respond to this fast Ca^{2+} release [15]. Thirdly, the duration of PKC activity, which is ultimately controlled by the persistence of the second messengers at a particular subcellular location, also differs among the PKCs. For example, DAG levels are more sustained at the Golgi than the plasma membrane, leading to prolonged novel PKC activity at the Golgi compared to the short-lived conventional PKC activity at the plasma membrane [15]. Thus, second messengers precisely dictate the kinetics, manganite, duration, and location of PKC activity and are responsible for the apparent differences between the three classes of PKCs.

PKC activity is exquisitely controlled by various mechanisms both under basal conditions and after agonist stimulation. For example, under basal conditions the E3 ligase for PKC, RING-finger protein that interacts with C kinase (RINCK), controls the amplitude of PKC signaling by regulating its levels [17]. RINCK interacts with the C1A domain of PKC and induces its ubiquitination and thus its degradation. Moreover, under basal conditions with limited DAG levels, PKCα interacts with diacylglycerol kinase ζ, which prevents its activation by locally metabolizing DAG [18]. Only under stimulated conditions in which enough DAG is locally produced to activate PKCα, allows PKCα to phosphorylate diacylglycerol kinase ζ leading to disassociation of the two proteins. However, agonist stimulation also ultimately leads to termination of PKC signaling through various mechanisms. PKC signaling is quickly terminated by the clearance of the respective 2nd messengers, but also by agonist-induced downregulation of the enzyme. Active PKC can be dephosphorylated by PHLPP or protein phosphatase 2A, shunting PKC to the detergent-insoluble fraction and inducing its ubiquitination and ultimately its proteasome-mediated degradation [19]. Agonist induced proteasome-mediated degradation of PKCα can, however, also occur via ubiquitination of plasma membrane-localized, fully primed PKC [20, 21]. Additionally, phosphorylated PKCα can also be internalized through lipid raft-mediated endocytic pathways and degraded by the lysosome [21, 22]. These mechanisms lead to desensitization of PKC signaling, thus maintaining its activity under exquisite control.

Regulation independent of 2nd messengers

Particular conventional and novel PKCs can also be activated independently of second messengers, adding to the complexity of PKC signaling. For example, certain PKCs can be activated by the accumulation of reactive oxygen species, which are often elevated in diseases such as cancer, cardiovascular disease, and neurodegeneration [23]. Specifically, H_2O_2 causes oxidation of cysteine residues within the C1B domain of PKCγ, inducing conformational changes that release PKCγ from its scaffold, leading to its translocation to the plasma membrane and subsequent DAG-independent activation [24, 25]. PKCδ is phosphorylated at multiple tyrosine residues by Src family kinases in response to acute stimulation of cells by H_2O_2, PMA, epidermal growth factors, or platelet-derived growth factor. Tyrosine phosphorylation can induce PKCδ's DAG-independent activation without requiring membrane translocation and can alter its subcellular localization [26, 27]. For example, tyrosine phosphorylation of PKCδ at Tyr64 and Tyr155 in response to apoptotic stimuli induces a conformational change that exposes its nuclear localization sequence and Hsp90 binding site, allowing importin-α and Hsp90 to bind and import PKCδ into the nucleus where it can induce apoptosis [28]. H_2O_2-induced tyrosine phosphorylation at Tyr311 has also been proposed to activate PKCδ by inducing caspase-3 cleavage between its regulatory and catalytic domains [29]. Therefore, these agonist-induced phosphorylations and conformational changes can activate PKCs independently of 2nd messengers by either releasing PKCs from scaffolds or exposing binding sites for scaffolds to interact with.

Regulation by conformational changes

Another level of regulation of PKC activity is imparted by conformational changes, as PKCs are under precise conformation control, particularly during maturation. When first synthesized, PKC is in an open conformation that has both the C1A and C1B domains exposed (Figure 2). Upon phosphorylation at its 3 priming sites, PKC adapts a closed conformation that masks its C1 domains such that the lower affinity C1B domain is the predominant DAG binder. This conformational change optimizes PKC's dynamic range of signaling such that it is not activated by basal diacylglycerol but can readily detect and be activated by a small, local increase in DAG [30]. Binding to their respective 2nd messengers leads to another conformational change that results in expulsion of the autoinhibitory pseudosubstrate and subsequent activation of PKC. Similarly to conventional PKCs, the pseudosubstrate and C1 domain of the atypical PKCζ also inhibit its activity through intramolecular interactions with the substrate-binding site and the αC helix, respectively [31, 32].

A recent study proposed that PKC is not only regulated by intramolecular interactions, but also by intermolecular interactions. Swanson et al. [33] showed evidence that PKCβII forms homo-dimers upon activation and that the dimers maintain PKC in an active conformation. Both C1 domains and the C2 domain contribute to this interaction, but the C-terminal tail is absolutely necessary for dimer formation. In fact, phosphorylation of the C-terminal tail is critical in maintaining PKC in a closed, inactive conformation, as lack of phosphorylation at these sites leads to PKC remaining in an open, exposed conformation [30] and increases

basal dimerization [33]. Therefore, PKC undergoes conformational changes both during its maturation and during its activation in order to finely tune its activity.

Regulation by scaffolding

Scaffolding also plays an integral part in determining the precise location, duration, and amplitude of PKC activity, as well as in establishing substrate specificity (reviewed in [34, 35]). Considering that there are multiple PKC isozymes expressed in the same cell that are activated by the same stimuli, scaffolds provide a level of functional selectivity. Among the PKC scaffolds are receptors for activated C kinase (RACK), 14-3-3 proteins, and A-kinase anchoring proteins (AKAPs).

Particular scaffolds augment PKC signaling whereas others inhibit it. RACKs were the first scaffolds found to associate with active PKC. For PKCβII, this interaction occurs via its C2 domain and C-terminal tail and it stabilizes PKC's active conformation, thus enhancing its activity towards co-scaffolded substrates [36–38]. The phosphoserine/threonine binding protein 14-3-3 binds to a pair of phosphoserines within the hinge region of PKCε, leading to its activation [39]. However, scaffolds can also be inhibitory towards PKC by sequestering it away from its substrates or maintaining it in an inactive conformation. For example, in lens epithelial cells, 14-3-3ε binds to PKCγ via its C1B domain and controls both its activity and localization, thus regulating gap junction activity [40]. AKAPs are another set of PKC scaffolds and were first identified to bind protein kinase A. AKAP12 binds to and attenuates PKCα and PKCδ signaling, thus preventing senescence and oncogenic transformation [41]. Similarly, AKAP5 inhibits PKC activity by binding to its catalytic pocket [42].

Scaffolds can also regulate the duration of PKC activity towards a substrate by co-scaffolding a phosphatase of the substrate. The phosphatase can thus rapidly dephosphorylate and attenuate signaling downstream of PKC substrates. Such an example is the coordination of PKC and protein phosphatase 2B/calcineurin on AKAP79 at the postsynaptic density in neurons [43]. Another AKAP (AKAP350/AKAP450/centrosome and Golgi localized PKN-associated protein or CG-NAP) was proposed to act as a scaffold for the maturation of PKC [44]. This AKAP was found to only associate with nascent PKCε at the Golgi/centrosome and this complex disassembled after PKCε maturation by phosphorylation. Interestingly, this complex also contains protein phosphatase 2A [45], which dephosphorylates PKC leading to its degradation. Perhaps PKC levels can be dynamically controlled on this scaffold through regulation of its phosphorylation. Scaffolding of PKC also has some clinical relevance because scaffolds have been shown to change the pharmacological prolife of PKC. Specifically, ATP-competitive inhibitors are ineffective against scaffolded PKC [42]. To explain how scaffolds amplify, accelerate, and insulate PKC signaling, Greenwald et al. [46] proposed a stochastic state-switching model. In this model, the complex containing PKC, its substrate, and its scaffold alters between inactive and active intermediate states, thus allowing phosphorylation of the substrate, even in the presence of active-site PKC inhibitors. Thus scaffolds are able to precisely control PKC activity and to confer functional selectivity.

Concluding Remarks

As PKC activity has to be precisely balanced at every subcellular location, its regulation is under intricate control. This control of the spatial and temporal dynamics of PKC signaling comes from regulation through various mechanisms, such as phosphorylation, binding to 2nd messengers, conformational changes, and binding to scaffolds. Perturbation of any of these mechanisms of control can lead to pathophysiological states. In fact, dysregulation of PKC activity has been observed in many diseases including cancer [47], diabetes [48], and neurodegenerative diseases such as Alzheimer's [49]. Changes in expression of PKC scaffolds, such as RACK1, as opposed to PKC itself, have also been found to dysregulate PKC signaling in Alzhemier's disease [50]. Therefore, understanding all mechanisms through which PKC is regulated is key to developing novel therapeutics to restore PKC signaling to physiological levels and subcellular locations.

Acknowledgments

Abbreviations used

PKC	protein kinase C
cPKC	conventional PKC
DAG	diacylglycerol
PS	phosphatidylserine
PIP$_2$	phosphatidylinositol 4,5-bisphosphate
nPKC	novel PKC
aPKC	atypical PKC
PHLPP	PH domain leucine-rich repeat protein phosphatase
RINCK	RING-finger protein that interacts with C kinase
RACK	receptor for activated C kinase
AKAP	A-kinase anchoring protein

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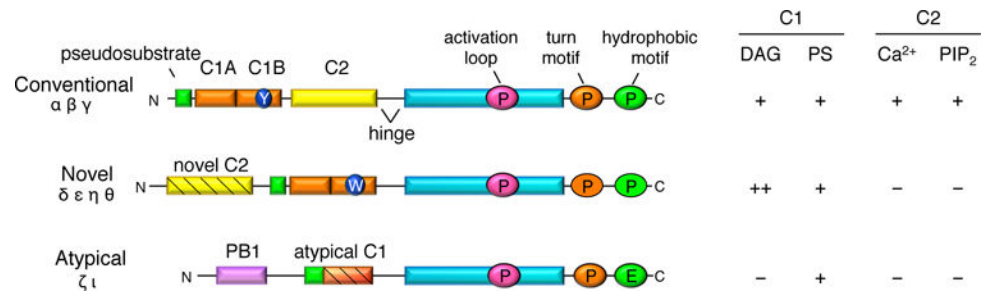
Figure 1. Schematic showing domain composition of the three classes of protein kinase C
Conventional PKCs (α, β, and γ) contain an N-terminal pseudosubstrate (green), tandem C1 domains (orange) that bind diacylglycerol (DAG) and phosphatidylserine (PS), a C2 domain (yellow) that binds phosphatidylinositol 4,5-bisphosphate (PIP_2) in a Ca^{2+}-dependent manner, and a C-terminal kinase domain (cyan). Novel PKCs (δ, ε, η, and θ) have a similar domain composition except that their C2 domain cannot bind Ca^{2+} or PIP_2 and their C1B domain has ~100-fold higher affinity DAG as it contains a Trp (W) at position 22 in the domain, as opposed to a Tyr (Y) as the C1B domain of conventional PKCs has. Atypical PKCs (ζ and ι) have a protein-binding module, PB1 domain, and an atypical C1 domain that cannot bind DAG. All PKCs are phosphorylated at 3 conserved sites: the activation loop within the kinase domain and the turn and hydrophobic motifs within the C-terminal tail (except for atypical PKCs which have a phosphomimetic at the hydrophobic motif).
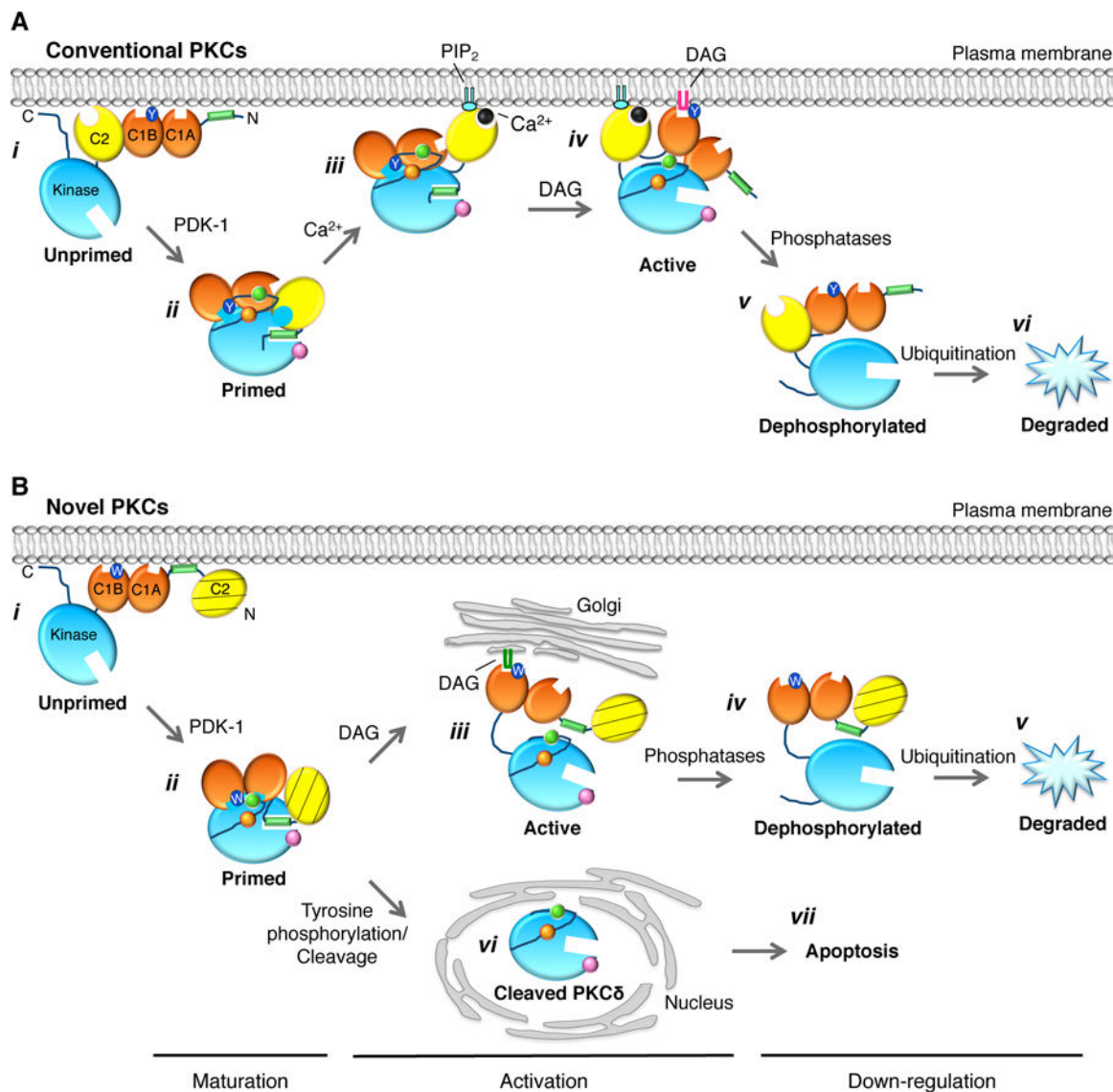
Figure 2. Regulation of protein kinase C

Model showing how maturation of cPKC masks C1 domains to increase the dynamic range of DAG sensing and thus PKC output. (**A**) Unprimed PKC is in an open conformation that associates with membranes via weak interactions from the C2 domain, both C1A and C1B domains, the exposed pseudosubstrate, and the C-terminal tail. In this conformation, both C1A and C1B domains are fully exposed. (**B**) Upon ordered phosphorylation of PKC at its activation loop (pink), turn motif (orange), and hydrophobic motif (green) sites, PKC matures into its closed conformation, in which both the C1A and C1B domains become masked, the pseudosubstrate binds the substrate binding site, and the enzyme localizes to the cytosol. This masking of the C1 domains prevents pretargeting of PKC to membranes in the absence of agonist-evoked increases in DAG, thus decreasing basal signaling. (**C**) In response to agonists that promote PIP_2 hydrolysis, Ca^{2+}-dependent binding of the C2 domain of cPKCs to the plasma membrane allows the low-affinity DAG sensor to find its membrane-embedded ligand, DAG. (**D**) Binding of DAG, predominantly to the C1B domain

of PKCβII, expels the pseudosubstrate from the substrate-binding cavity and activates PKC. Use primarily of the lower-affinity C1B domain increases the dynamic range of PKC output as the signal does not saturate as readily using the lower affinity module and allows cPKCs to signal at the plasma membrane as opposed to the Golgi. (**E**) Dephosphorylation of activated PKC allows it to regain the exposed (open) conformation of unprimed PKC.