Chapter 2
Regulation of Conventional and Novel Protein Kinase C Isozymes by Phosphorylation and Lipids

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Abstract The amplitude of protein kinase C signaling is precisely controlled by mechanisms that regulate the amount of protein kinase C in the cell that is available to become activated with appropriate stimuli. Two mechanisms critically control the amount and activity of protein kinase C in cells. First, a series of phosphorylation events prime conventional and novel protein kinase C isozymes into stable, signaling-competent species. Second, signals that cause phospholipid hydrolysis cause protein kinase C to bind to membrane lipids, an interaction that allosterically activates the kinase. Deregulation of either step alters the amplitude of protein kinase C signaling in the cell, resulting in pathophysiological states. This chapter focuses on the molecular mechanisms by which phosphorylation and lipid binding control protein kinase C.

Keywords Protein kinase C • Diacylglycerol • Phosphorylation • C1 domain • C2 domain

Abbreviations

AGC kinases	Protein kinases A, G and C
DG	Diacylglycerol
PI3 kinase	Phosphatidylinositol 3 kinase
PDK-1	Phosphoinositide-dependent kinase-1
PH	Pleckstrin homology
PHLPP	PH domain Leucine-rich repeat Protein Phosphatase
PS	Phosphatidylserine
PIP_2	Phosphatidylinositol-4,5-bisphosphate
PIP_3	Phosphatidylinositol-3,4,5-trisphosphate
RACK	Receptor for activated C kinase
TORC2	Target of rapamycin complex 2

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M.G. Kazanietz (ed.), *Protein Kinase C in Cancer Signaling and Therapy*,
Current Cancer Research, DOI 10.1007/978-1-60761-543-9_2,

2.1 Introduction

The ten members of the protein kinase C family are grouped into three classes that are defined by the composition of their regulatory modules (Nishizuka 1995; Newton 2001) (Fig. 2.1, left). These, in turn, dictate the cofactor requirements for activity (Fig. 2.1, right). Conventional isozymes (protein kinase C α, the alternatively spliced βI and βII, and γ) are composed of two tandem C1 domains (Hurley et al. 1997), allowing them to respond to diacylglycerol, and a C2 domain, which binds anionic membranes in a Ca^{2+}-dependent manner. The C1 domain also stereospecifically binds the anionic phospholipid, phosphatidylserine (Johnson et al. 1998, 2000). It is also the binding site for the potent tumor promoting phorbol esters, which bind the same site as diacylglycerol (Sharkey et al. 1984). The C2 domain of conventional protein kinase C isozymes binds anionic phospholipids, with a modest (but not stereospecific) preference for phosphatidylserine

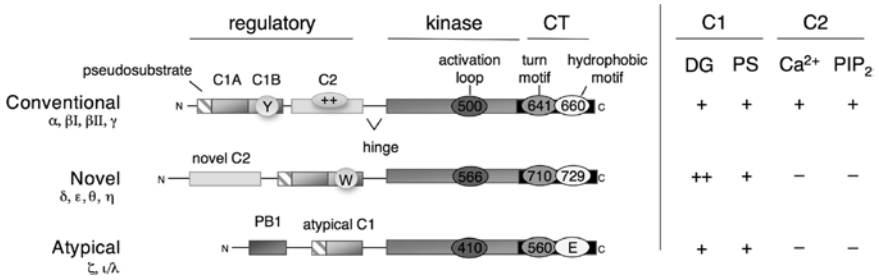

Fig. 2.1 Schematic of protein kinase C family members showing membrane-targeting modules in amino-terminal regulatory moiety and phosphorylation sites in carboxyl-terminal kinase moiety. Conventional isozymes (α, alternatively spliced βI and βII, γ) have a tandem C1 domain that confers specificity for diacylglycerol and phosphatidylserine and a C2 domain that binds anionic phospholipids via a Ca^{2+}-occupied ligand binding site and via a basic patch distal to the Ca^{2+} site (*oval* with ++), with selectivity for PIP_2. Novel isozymes (δ, ε, θ, η) also have tandem C1 domains, but Trp at position 22 (*circle* with W) in the C1B domain confers an order of magnitude higher affinity for diacylglycerol than the C1B domain of conventional protein kinase C isozymes, which have a Tyr at position 22 of domain (*circle* with Y; numbering of (Hurley et al. 1997)). Atypical isozymes have a single C1 domain whose highly basic ligand-binding pocket is unable to bind diacylglycerol but retains binding to phosphatidylserine. In addition, atypical protein kinase C isozymes have a protein–protein interaction PB1 domain. All isozymes contain an autoinhibitory pseudosubstrate sequence directly preceding the C1 domain (*stippled area*) and have a proteolytically labile hinge segment that separates the regulatory moiety from the kinase moiety. The kinase domain contains three conserved phosphorylation sites modified during the maturation of the enzyme into a catalytically competent species: the activation loop in the kinase domain (*dark gray circle*; Thr 500 in protein kinase C βII; Thr 566 in protein kinase C ε; Thr 410 in protein kinase C ζ), and the turn motif (*medium gray circle*; Thr 641 in protein kinase C βII; Thr 710 in protein kinase C ε; Thr 560 in protein kinase C ζ) and the hydrophobic motif (*light gray circle*; Ser 660 in protein kinase C βII; Ser 729 in protein kinase C ε; and the phosphomimetic Glu 579 in protein kinase C ζ) in the carboxyl tail (CT). Table on *right* summarizes second messenger (diacylglycerol (DG) or Ca^{2+}) and phospholipid (phosphatidylserine (PS) or PIP_2) binding to the two membrane-targeting modules, the C1 and C2 domains, in the three subclasses of isozymes

(Medkova and Cho 1999; Johnson et al. 2000; Conesa-Zamora et al. 2001), and a significant preference for phosphatidylinositol-4,5-bis phosphate (PIP_2) mediated by a basic patch distal to the Ca^{2+}-binding site (Fig. 2.1, ++ in C2 domain of conventional protein kinase C isozymes) (Corbalan-Garcia et al. 2007). Novel isozymes (protein kinase C δ, ε, η, and θ) also contain two tandem C1 domains, conferring diacylglycerol sensitivity, but they contain a "novel" C2 domain that does not bind Ca^{2+} and does not serve as a membrane-binding module. Atypical isozymes (ζ and ι/λ) possess an "atypical" C1 domain whose highly basic ligand-binding pocket does not allow ligand binding, so these isozymes respond to neither diacylglycerol nor Ca^{2+} (Kazanietz et al. 1994; Pu et al. 2006). These isozymes contain a PB1 protein-binding domain which poises this class of protein kinase C isozymes at discrete intracellular locations (Lamark et al. 2003). All protein kinase C isozymes have an autoinhibitory segment, the pseudosubstrate (Fig. 2.1, stippled box), that occupies the substrate-binding cavity in the absence of lipid binding, thus maintaining the kinase in an autoinhibited state. Engagement of the membrane-binding modules provides the energy to release the pseudosubstrate from the substrate-binding cavity, allowing downstream signaling. All isozymes are also regulated by a conserved segment at the carboxyl-terminal tail (Fig. 2.1, CT) that controls the stability of the kinases, serves as a docking site for key regulatory molecules, and provides a phosphorylation switch for kinase function (phosphorylation sites indicated by ovals).

2.2 Regulation of Protein Kinase C by Priming Phosphorylation

Before protein kinase C is competent to respond to lipid second messengers, it must first be processed by a series of ordered and tightly coupled phosphorylation events at three conserved positions in the carboxyl-terminal half of the protein (Fig. 2.2) (Newton 2003; Parker and Murray-Rust 2004). These phosphorylation events are required to structure protein kinase C into a catalytically competent and stable species. It is this phosphorylated species that transduces signals. Constructs of protein kinase C that cannot be phosphorylated are shunted to the detergent-insoluble fraction of cells and degraded. Thus, it is important to note that phosphorylation is not only required for the catalytic competence of protein kinase C, but also to protect the mature (but inactive) enzyme from degradation.

The first phosphorylation is catalyzed by the upstream kinase phosphoinositide-dependent kinase-1 (PDK-1) and occurs on a conserved Thr on a loop near the entrance to the active site of the kinase core (Chou et al. 1998; Dutil et al. 1998; Le Good et al. 1998). This phosphorylation triggers two ordered phosphorylations on the carboxyl-terminal tail of protein kinase C. These sites, identified by mass spectrometry in the mid-1990s, are referred to as the turn motif and the hydrophobic motif (Keranen et al. 1995). The species of conventional protein kinase C found in the detergent-soluble fraction of mammalian cells is quantitatively phosphorylated

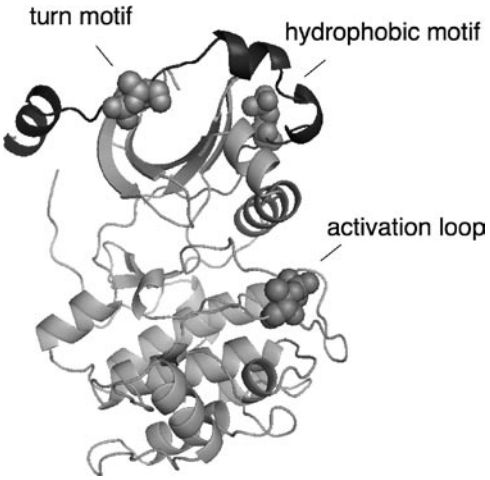

Fig. 2.2 Structure of kinase domain of conventional isozyme, protein kinase C βII, showing the position of the three priming phosphorylations. Phosphorylated residues are shown in space filling rendition. These phosphorylations are the activation loop site on a segment near the entrance to the active site and the turn motif and hydrophobic motif on the carboxyl tail (CT) (dark segment of structure). Note how the turn motif and hydrophobic motif clamp the CT around the upper lobe of the kinase core

at the two carboxyl-terminal sites but may have incomplete occupancy of the activation loop site. Species that are not phosphorylated at the carboxyl-terminal sites are targeted for degradation. Note that protein kinase C isozymes are controlled by additional phosphorylations on Ser, Thr and Tyr that fine-tune the function of specific isozymes (reviewed in (Gould and Newton 2008)); this chapter focuses on the priming phosphorylations.

2.2.1 Activation Loop Phosphorylation and PDK-1

PDK-1 serves as the upstream kinase for many members of the AGC superfamily of kinases, catalyzing the phosphorylation of a Thr on a segment near the entrance to the active site referred to as the activation loop (Taylor and Radzio-Andzelm 1994). Phosphorylation on this Thr correctly aligns residues for catalysis. Phosphorylation of PDK-1 substrates is controlled by the conformation of the substrate (Toker and Newton 2000; Mora et al. 2004); conformational changes that unmask the activation loop site promote the phosphorylation by PDK-1. In the case of protein kinase C family members, newly synthesized protein kinase C is membrane-associated in a conformation in which the pseudosubstrate sequence is expelled from the substrate-binding cavity, thus unmasking the activation loop site. Thus, newly synthesized protein kinase C is constitutively phosphorylated by PDK-1.

In striking contrast, the activation loop site of Akt (protein kinase B) is masked until agonist stimulation recruits the kinase to the membranes. Engaging its membrane-targeting module, a PH domain, to phosphatidylinositol-3,4,5,-tris phosphate (PIP_3) unmasks the PDK-1 site. Thus, the phosphoinositide-dependence of Akt derives from the phosphoinositide-dependence of unmasking the activation loop site.

PDK-1 docks on the carboxyl-terminal tail of the newly synthesized protein kinase C (Fig. 2.3, species of protein kinase C on upper left), specifically recognizing the hydrophobic phosphorylation motif, to phosphorylate the activation loop Thr (Thr500 on protein kinase C βII). This is generally considered to be the first step in the processing of protein kinase C. Phosphorylation at this site is required to continue the processing of protein kinase C by phosphorylation at the two carboxyl-terminal sites: mutation of the activation loop Thr to a nonphosphorylatable neutral residue results in an inactive protein kinase C (Cazaubon et al. 1994; Orr and Newton 1994). Because unphosphorylated protein kinase C is not stable, constructs that cannot be phosphorylated at the activation loop are degraded. Consistent with this, embryonic stem cells lacking PDK-1 have reduced levels of conventional and novel protein kinase C isozymes (Balendran et al. 2000).

2.2.2 Carboxyl-Terminal Phosphorylations and TORC2

The immediate consequence of phosphorylation at the activation loop is the phosphorylation of two conserved sites on the carboxyl-terminus: first on the turn motif, so named because the analogous position in protein kinase A is at the apex of a turn; and, secondly, on the hydrophobic motif, so named because it is flanked by hydrophobic residues (Keranen et al. 1995; Newton 2001). Phosphorylation at both sites depends on the intrinsic catalytic activity of protein kinase C, suggesting that they are catalyzed by autophosphorylation. Enzymological studies with pure conventional protein kinase C βII have shown that this is the case for the hydrophobic motif: under conditions where the enzyme is a monomer, it incorporates phosphate at this position in a concentration-independent manner, revealing intramolecular autophosphorylation (Behn-Krappa and Newton 1999). The mechanism of phosphorylation of the turn motif is not clear. However, recent reports have established that phosphorylation of the turn motif depends on the mTORC2 complex, a structure comprising the kinase mTOR, sin1, rictor, and mLST8 (Facchinetti et al. 2008; Ikenoue et al. 2008; Jacinto and Lorberg 2008). Specifically, protein kinase C cannot be processed by phosphorylation in cells lacking this complex and, because the unphosphorylated species is unstable, it is degraded (Guertin et al. 2006; Ikenoue et al. 2008). Whether this complex assists by noncatalytic mechanisms, for example chaperoning or positioning newly synthesized protein kinase C for processing by phosphorylation, or whether it directly phosphorylates protein kinase C is unclear. It is noteworthy, however, that mTORC2 is not able to phosphorylate protein kinase C in vitro (Ikenoue et al. 2008).

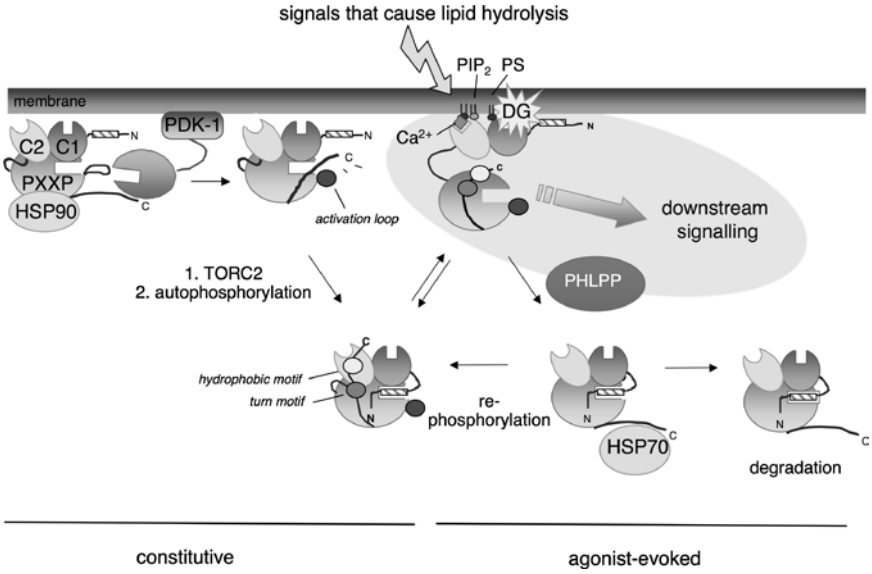

Fig. 2.3 Schematic illustrating the life cycle of conventional protein kinase C. Newly synthesized protein kinase C (species in *top left*) associates with a membrane fraction in a conformation in which the autoinhibitory pseudosubstrate (*stippled rectangle*) is removed from the substrate-binding cavity (*rectangular indentation in large circle*), thus exposing the activation loop phosphorylation site to phosphorylation by PDK-1 which is docked to carboxyl tail. Hsp90 binds this newly synthesized species on a surface that depends on an intramolecular clamp formed between a conserved PXXP motif on the carboxyl tail and a helix in the kinase domain. The structural integrity of the clamp and the interaction with Hsp90 are essential for the processing of protein kinase C PDK-1, constitutively docked to the carboxyl-terminal tail, phosphorylates the activation loop, correctly aligning residues in the active site for catalysis. One of the immediate consequences of this phosphorylation is the tightly coupled phosphorylation of the turn motif and then the hydrophobic motif. Phosphorylation of the turn motif is rate-limiting, depends on a functional mTORC2 complex, and is required to complete the processing of protein kinase C. The mechanism of this phosphorylation has yet to be elucidated. The third and last phosphorylation on the hydrophobic motif occurs by intramolecular autophosphorylation. The fully phosphorylated enzyme localizes primarily to the cytosol or is scaffolded to specific intracellular locations by protein–protein interactions (Schechtman and Mochly-Rosen 2001). This phosphorylated "mature" species adopts a conformation in which the pseudosubstrate occupies the substrate-binding cavity, thus autoinhibiting the enzyme (*bottom left species*). This processing by phosphorylation is constitutive. In response to signals that cause phospholipid hydrolysis, protein kinase C associates with cellular membranes and becomes activated. For conventional protein kinase C isozymes, activation typically requires hydrolysis of PIP_2 to generate two second messengers: Ca^{2+} and diacylglycerol (DG). Ca^{2+} binds the C2 domain, promoting the association of protein kinase C with the plasma membrane via interaction with anionic lipids, and importantly PIP_2. The enzyme then diffuses in two-dimensional space until the C1 domain finds its membrane-embedded ligand, diacylglycerol. This interaction is strengthened by stereospecific binding to phosphatidylserine (PS). The binding energy provided by engaging both the C1 and C2 domains on membranes releases the pseudosubstrate, allowing substrate phosphorylation and downstream signaling (*top*, *species on right*). This "open" conformation of protein kinase C (pseudosubstrate exposed and hinge between regulatory moiety and kinase domain unmasked) is sensitive to dephosphorylation; PHLPP initiates the dephosphorylation of the hydrophobic motif, with PP2A-type phosphatases contributing to the complete dephosphorylation of the priming sites. This dephosphorylated species associates with a detergent-insoluble fraction and is degraded. Hsp70 sustains the signaling lifetime of dephosphorylated protein kinase C by binding the dephosphorylated turn motif and promoting the rephosphorylation of protein kinase C

However, the turn motif may very well be modified by another kinase: while lack of PDK-1 prevents phosphorylation of the activation loop and the hydrophobic motif, the turn motif has recently been reported to be efficiently phosphorylated in PDK-1$^{-/-}$ cells (Ikenoue et al. 2008). Consistent with another kinase catalyzing the phosphorylation of the turn motif, but not the hydrophobic motif, a GST-fusion construct of the carboxyl-terminal tail is efficiently phosphorylated at the turn motif, but not the hydrophobic motif (Ikenoue et al. 2008). These priming phosphorylations are constitutive for conventional protein kinase C isozymes. For novel isozymes, basal phosphorylation of the priming sites is high but can increase modestly upon agonist stimulation. For atypical protein kinase C isozymes, the PDK-1 step displays the highest agonist sensitivity. Note that atypical isozymes contain a constitutive negative charge (Glu) at the phospho-acceptor position of the hydrophobic motif.

The processing of protein kinase C by phosphorylation depends on the binding of Hsp90 to a carboxyl-terminal motif conserved amongst the AGC kinases that comprises the sequence PXXP. This PXXP motif forms an intramolecular clamp with residues in the kinase domain that provides a surface for binding Hsp90. Disruption of the clamp, or inhibition of Hsp90, prevents the processing of protein kinase C by phosphorylation (Gould et al. 2009).

Once protein kinase C is phosphorylated at the two carboxyl-terminal sites, phosphorylation of the activation loop becomes dispensable. In fact, mass spectrometric analysis has revealed that about half the protein kinase C in brain extracts or in mammalian cultured cells is not phosphorylated on the activation loop despite quantitative phosphorylation on the carboxyl-terminal sites (Keranen et al. 1995). Thus, phosphorylation of the activation loop site is required to process protein kinase C, but once the mature conformation is achieved, the phosphorylation state of the activation loop site does not impact the activity of protein kinase C.

2.3 Regulation of Protein Kinase C by Lipid Second Messengers

2.3.1 Conventional Protein Kinase C

The processing of conventional protein kinase C by phosphorylation occurs at the membrane (Sonnenburg et al. 2001), but once phosphorylated, the "mature" enzyme is released to the cytosol where it adopts an autoinhibited conformation with the pseudosubstrate occupying the substrate-binding cavity (Dutil and Newton 2000). This species of protein kinase C is also poised at specific intracellular locations by protein scaffolds (Schechtman and Mochly-Rosen 2001). Phospholipase C-catalyzed hydrolysis of PIP_2 results in elevated levels of two second messengers required for agonist-evoked activation of conventional protein kinase C isozymes: diacylglycerol and Ca^{2+} (Nishizuka 1995). Biophysical and molecular imaging studies suggest that in the absence of these ligands, conventional

protein kinase C bounces on and off the membrane by diffusion-controlled mechanisms, with membrane interactions of far too low an affinity to retain protein kinase C there (Schaefer et al. 2001). However, elevation of intracellular Ca^{2+} recruits protein kinase C to the plasma membrane via a low-affinity interaction of the Ca^{2+}-bound C2 domain (Oancea and Meyer 1998; Nalefski and Newton 2001) (Fig. 2.3). The affinity of this interaction is too low to activate protein kinase C, but it has the important biological function of poising protein kinase C on the plasma membrane, where it can effectively search for its membrane-embedded ligand, diacylglycerol, in two-dimensional space (Nalefski and Newton 2001). The interaction of the C1 domain with diacylglycerol-containing membranes is strengthened by the stereo-specific interaction with phosphatidylserine (Johnson et al. 1998, 2000). The enrichment of phosphatidylserine at the plasma membrane thus likely contributes to the translocation of PKC to the plasma membrane (Yeung et al. 2008). Once the C1 domain is engaged, the binding energy of protein kinase C to membranes is sufficiently high to release the autoinhibitory pseudosubstrate from the substrate-binding cavity, allowing substrate binding and phosphorylation. Note that the affinity of each membrane-targeting module of conventional protein kinase C isozymes, the C1 and C2 domains, is too low to allow pseudosubstrate release in response to physiological levels of diacylglycerol or Ca^{2+} alone. However, the C1 domain of conventional protein kinase C isozymes binds membranes containing phorbol esters (functional analogs of diacylglycerol) with two orders of magnitude higher affinity than membranes containing diacylglycerol (Mosior and Newton 1996). Thus, phorbol ester treatment of cells recruits protein kinase C to membranes with sufficiently high affinity to promote pseudosubstrate release in the absence of Ca^{2+} binding to the C2 domain. Importantly, under physiological conditions, activation of conventional protein kinase C requires the coordinated binding of the C1 and C2 domains to membranes, with Ca^{2+} binding to the C2 domain pretargeting protein kinase C to membranes where it can efficiently engage diacylglycerol.

Conventional protein kinase C isozymes are primarily recruited to the plasma membrane, despite the relatively high levels of diacylglycerol at the Golgi, where novel isozymes are primarily recruited (Carrasco and Merida 2004; Gallegos et al. 2006; Dries et al. 2007). The molecular basis for the selective translocation of conventional protein kinase C isozymes to the plasma membrane is likely accounted for by their affinity for PIP_2, a lipid found primarily on the plasma membrane (Yeung et al. 2006). This lipid binds a basic surface on conventional C2 domains (Corbalan-Garcia et al. 2007; Marin-Vicente et al. 2008; Evans et al. 2006; Landgraf et al. 2008). Phosphatidylserine is also enriched at the plasma membrane relative to Golgi membranes (Yeung et al. 2008), an enrichment that may also contribute to the targeting of conventional protein kinase C isozymes, which are tightly regulated by phosphatidylserine, to the plasma membrane. Thus, the unique phospholipid composition of the plasma membrane, which results in the most negatively charged membrane surface in cells (Yeung et al. 2006), favors the recruitment of conventional protein kinase C isozymes.

2.3.2 *Novel Protein Kinase C*

The affinity of the C1B domain of novel protein kinase C isozymes for diacylglycerol-containing membranes is two orders higher than that of conventional protein kinase C isozymes (Giorgione et al. 2006). This low vs. high affinity binding depends on the nature of the hydrophobic residue at position 22 of the C1B domain: when present as a Trp, as it is in novel isozymes, the domain binds diacylgycerol membranes with high affinity and when present as a Tyr, as it is in conventional protein kinase C isozymes, the domain binds with low affinity (Dries et al. 2007). Thus, the isolated C1B domain of novel enzymes, but not conventional isozymes, is recruited to membranes following agonist-evoked increases in diacylglycerol. This enhanced affinity for diacylglycerol allows novel protein kinase C isozymes to translocate to membranes in response to physiological increases in diacylglycerol. Because basal levels of diacylglycerol are relatively high at Golgi, significant levels of novel isozymes are localized at this membrane (Carrasco and Merida 2004). Agonist-evoked increases in diacylglycerol increase the association of novel protein kinase C isozymes with Golgi and, to a lesser extent, plasma membrane (Gallegos et al. 2006). There are also differences in the cellular locations of individual members of the novel protein kinase C family driven by differences in lipid interactions (Stahelin et al. 2005). For example, the C2 domain of protein kinase C ε also binds phosphatidic acid, an interaction that also tunes the membrane interaction of this isozyme (Pepio and Sossin 1998).

2.4 Termination of Protein Kinase C Signaling

Signaling by protein kinase C is terminated by the removal of the activating second messengers. However, prolonged activation of protein kinase C, as occurs with phorbol esters, results in the "down-regulation" of protein kinase C (Parker et al. 1995; Leontieva and Black 2004; Gould and Newton 2008). Membrane-bound protein kinase C adopts an open (pseudosubstrate-exposed) conformation that exposes the phosphorylation sites to cellular phosphatases (Fig. 2.3). The first dephosphorylation event appears to be catalyzed by the recently discovered hydrophobic motif phosphatase, the PH domain Leucine-rich repeat Protein Phosphatase (PHLPP) (Gao et al. 2005, 2008). Its selective dephosphorylation of the hydrophobic motif shunts protein kinase C to the detergent-insoluble fraction of cells, where it is further dephosphorylated at the turn motif and activation loop by additional phosphatases, including PP2A-type phosphatases (Hansra et al. 1996; Gao et al. 2008). The dephosphorylated species is targeted for degradation. Interestingly, nature has devised a mechanism to "rescue" protein kinase C from degradation: the molecular chaperone Hsp70 specifically binds the dephosphorylated turn motif, an event that stabilizes protein kinase C. This binding is proposed to promote the rephosphorylation of the enzyme at the priming sites, thus sustaining the signaling lifetime of the enzyme (Gao and Newton 2002, 2006). In addition to agonist-stimulated

degradation, the total cellular levels of protein kinase C, independent of activation or phosphorylation state, have recently been shown to be controlled by a protein kinase C-interacting E3 ligase, RINCK, which ubiquitinates protein kinase C and targets it for proteasomal degradation (Chen et al. 2007). There are likely to be additional ligases that control the degradation of specific species of protein kinase C. Of particular interest will be the identification of ligases that control the phorbol ester-dependent down-regulation.

Protein scaffolds are essential for coordinating components of signaling pathways (Smith et al. 2006), and they play key roles in poising specific protein kinase C isozymes near regulatory molecules and substrates (Mochly-Rosen 1995; Jaken and Parker 2000; Schechtman and Mochly-Rosen 2001). It is protein scaffolds, rather than subtle changes in second messenger affinities, that confer specificity in signaling by the structurally similar protein kinase C isozymes within each subclass. Thus, for example, specific scaffolds for the conventional isozymes protein kinase C α and protein kinase C βII promote isozyme-specific signaling. One class of scaffolds termed Receptors for Activated C Kinase (RACKs) specifically recognizes sequences that are exposed in the active conformation of protein kinase C. The first RACK was in fact identified as a protein kinase C βII-specific adaptor (Ron et al. 1994). These scaffolds finely tune the location of protein kinase C isozymes within the cell via protein–protein interactions, stabilizing the active conformation. Mochly-Rosen and coworkers have taken advantage of sequences on specific isozymes that either directly bind the RACK scaffolds or sequences within the protein kinase C isozyme that intramolecularly bind and mask the RACK-binding sequence in the inactive conformation (Souroujon and Mochly-Rosen 1998) to generate peptide inhibitors and activators, respectively (Schechtman and Mochly-Rosen 2001). Additionally, the last three amino acids of protein kinase C α encode a PDZ ligand which has been shown to bind the PDZ-domain containing protein PICK1 (Staudinger et al. 1997). Furthermore, a recent proteomics approach identified several other potential partners for this PDZ ligand on protein kinase C α (Stiffler et al. 2007). Thus, whereas lipids acutely control the activation state of protein kinase C by releasing the pseudosubstrate, protein partners poise protein kinase C isozymes at precise intracellular locations to control substrate access and interactions with regulatory molecules (phosphatases, E3 ligases, chaperones, etc).

2.5 Spatiotemporal Dynamics of Protein Kinase C Signaling

The advent of genetically encoded reporters revolutionized the study of the spatiotemporal dynamics of protein kinase C signaling (Sakai et al. 1997; Oancea and Meyer 1998; Oancea et al. 1998; Violin and Newton 2003; Violin et al. 2003). The ability to simultaneously visualize protein kinase C translocation, protein kinase C activity, and the second messengers, diacylglycerol and Ca^{2+}, has revealed that protein kinase C isozymes have a unique signature of activation depending on the cellular location (Gallegos et al. 2006; Gallegos and Newton 2008). In response to

agonists such as UTP that activate G protein-coupled receptors and cause Ca^{2+} and diacylglycerol levels to rise, conventional protein kinase C isozymes are rapidly recruited to, and activated at, the plasma membrane, with the kinetics of activation mirroring the rise in Ca^{2+} (Gallegos et al. 2006). This rise in Ca^{2+} is followed by a rise in plasma membrane diacylglycerol, and it is the diacylglycerol levels that then sustain the activity of membrane-bound protein kinase C presumably through activation of novel protein kinase C isozymes. Some agonists cause oscillations in Ca^{2+} levels which in turn cause oscillations in protein kinase C activity; if diacylglycerol levels remain elevated, protein kinase C can remain membrane bound but the activity oscillates depending on whether Ca^{2+} levels are high and the C2 domain is membrane-engaged (and thus the pseudosubstrate is expelled from the substrate-binding activity), or low such that the C2 domain is not membrane-engaged (and thus the pseudosubstrate occupies the substrate-binding cavity) (Violin et al. 2003). Diacylglycerol levels at the Golgi are significantly elevated compared to the plasma membrane under basal conditions and, in addition, agonist-evoked increases of this lipid second messenger are much more sustained at the Golgi compared to the plasma membrane. The unique profile of diacylglycerol at Golgi produces, in turn, a protein kinase C signature unique to Golgi: not only is there preferential recruitment of novel protein kinase C isozymes, which have an intrinsically higher affinity for diacylglycerol because of a C1 domain tuned for tighter binding to diacylglycerol (Carrasco and Merida 2004; Giorgione et al. 2006; Dries et al. 2007), but the agonist-evoked activity at Golgi is much more prolonged than at the plasma membrane (Gallegos et al. 2006).

2.6 Summary

The amplitude of the protein kinase C signal in cells depends not only on the levels of second messengers, but also on the total level of protein kinase C. One key mechanism that precisely controls the levels of protein kinase C in the cell is the balance between phosphorylation and dephosphorylation of the enzyme: species of enzyme that are not phosphorylated are degraded. Thus, alterations in the mechanisms that drive the priming phosphorylations (PDK-1, mTORC2, Hsp90, among others) or drive the dephosphorylation reactions (PHLPP) alter the levels of protein kinase C. Protein kinase C levels are altered in many pathophysiological states, most notably cancer (Griner and Kazanietz 2007), suggesting that the mechanisms that control the phosphorylation/dephosphorylation are potential therapeutic targets.

While phosphorylation mechanisms control the amount of signaling-competent protein kinase C in the cell, binding to lipid second messengers provides spatiotemporal control of agonist-evoked signaling. Conventional protein kinase C isozymes are pretargeted to the plasma membrane following the elevation of intracellular Ca^{2+}, where their C2 domain selectively binds PIP_2. This pretargeting to membranes facilitates the binding of the C1 domain to its membrane-embedded ligand,

diacylglycerol, a membrane interaction that is increased by the specific binding of phosphatidylserine to the C1 domain. Novel isozymes translocate to membranes enriched in diacylglycerol, with selective activation at Golgi membranes. Activity at this location tends to be significantly sustained relative to the shorter-lived activation of conventional protein kinase C isozymes at the plasma membrane because of the sustained elevation of diacylglycerol at Golgi following agonist stimulation. Protein scaffolds also play a major role in fine-tuning the cellular location of specific protein kinase C isozymes. Thus, a unique signature of protein kinase C activity exists throughout the cellular terrain.

Acknowledgments This work was supported in part by National Institutes of Health R01 GM43154 (ACN). I thank Lisa Gallegos and Christine Gould for helpful comments.

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http://www.springer.com/978-1-60761-542-2

Protein Kinase C in Cancer Signaling and Therapy
(Ed.)M.G. Kazanietz
2010, XIV, 494 p. 37 illus., 11 in color., Hardcover
ISBN: 978-1-60761-542-2
A product of Humana Press