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SPECIAL REPORTS AND REVIEWS

The Role of Protein Kinase C in Gastrointestinal Function and Disease

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Externally triggered signaling pathways allow cells to adapt to their environment through diverse cellular responses. In a given milieu, depending on cell and stimulus specificity, the activation of these pathways may induce survival, proliferation, differentiation, or death. These responses may be triggered by receptor-mediated or independent mechanisms and frequently involve alterations in protein phosphorylation. The activation state of many intracellular second messengers is dependent on their phosphorylation state, and thus kinases and phosphatases are often key regulators of cell response. The phosphorylation state of a protein can control cellular location, enzymatic activity, susceptibility to protease degradation, and the ability of the protein to interact with substrates and other proteins.[1] The kinases involved in this signaling are divided into 2 categories, those that phosphorylate tyrosine residues and those that phosphorylate serine and threonine residues. The serine/threonine kinases include protein kinase A (PKA), protein kinase B (more often referred to as Akt), protein kinase C (PKC), and the protein kinase D (PKD) family members. This review will focus on the regulation of PKCs and their role in the development, function, and pathology of the gastrointestinal tract.

The Protein Kinase C Family

Classification and Structure

PKC is a multigene family whose members are involved in a plethora of cellular signaling cascades and divergent biologic functions. A role for PKCs has been demonstrated in development, differentiation, homeostasis, migration, contraction, secretion, and immunity.[2–5] PKCs are activated via tyrosine kinase and G-protein-coupled receptors, as well as by nonreceptor-mediated signaling cascades. At least 11 mammalian PKC isozymes have been isolated, and these are divided into 3 subfamilies consisting of the classical, novel, and atypical PKC isozymes (Table 1) based on their specific requirements for Ca^{+2}, phosphatidyl-L-serine (PS), and 1,2-sn-diacyglycerol (DAG) for activity.[2,4,6,7]

PKCs are a single polypeptide chain consisting of several conserved regions (C1–C4) interrupted by variable regions (Figure 1). Similar to other inducible enzymes, the PKCs contain both a regulatory (20–70 kilodaltons) and catalytic (45 kilodaltons) domain.[8] The regulatory domain spans the amino terminus up to the adenosine triphosphate (ATP)-binding site (C3). It is responsible for the dependence on cofactors and includes an amino proximal pseudosubstrate (autoinhibitory) motif in all of the PKC isozymes. The regulatory regions of the classical PKCs (cPKC) contain a C1 domain that binds phosphatidyl-L-serine, DAG, and phorbol esters; a C2 domain that mediates Ca^{+2} dependency; and a C3 domain that binds ATP that is required for substrate phosphorylation (Figure 1). The novel PKCs (nPKC) have a similar structure, but they lack the calcium-dependent C2 domain. The regulatory domain of the atypical PKCs (aPKC) is even further truncated and only contains phosphatidylserine and ATP-binding sites. Thus, the dependence of each subfamily on cofactors is due to the presence or absence of these regulatory domains.

Regulation

For simplification, the activation of the cPKCs will be used as a model for PKC regulation in general (Figure 2). De novo-translated PKC associates with the cytoskeleton in an "immature" form[2,3,8,9] that is phosphorylated into its "mature" form.[9] In 1989, approximately 10 years after the discovery that PKCs are lipid-regulated enzymes, Fabbro et al demonstrated that PKCs are phosphorylated in vivo.[10] After synthesis, the immature PKCs are then phosphorylated at 3 sites.[6,11] The

Abbreviations used in this paper: PDK-1, phosphoinositide-dependent kinase-1; PK, protein kinase.

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Table 1. Subfamilies of Protein Kinase C

Subfamily Name	Included isoforms	Required cofactors
Conventional PKC (cPKC)	α	Calcium
	βI, βII	Diacyglycerol
	γ	Phosphatidylserine
Novel PKC (nPKC)	δ	Diacyglycerol
	ε	Phosphatidylserine
	η	
	θ	
Atypical PKC (aPKC)	ζ	Phosphatidylserine
	ι/λ	

mechanism of phosphorylation during maturation has best been described for cPKCβII, and the series of events seems fairly well conserved among all PKCs. During maturation, as shown in Figure 2, an initial phosphorylation of the active loop is performed by phosphoinositide-dependent kinase-1 (PDK-1).[2,7,12–14] In cPKCβII, this rate-limiting phosphorylation occurs in the C4 domain at Thr500, which is highly conserved among PKCs. Modeling studies suggest that this amino acid is blocked by binding of the pseudosubstrate and that binding of DAG or other lipids may be required to make the activation loop accessible to PDK-1.[6] Once PDK-1-dependent phosphorylation occurs, 2 autophosphorylations then occur in the catalytic domain at Thr641 and Ser660 in cPKCβII, and these 2 residues are also highly conserved in most, but not all, mammalian PKCs. For example, aPKCζ and λ have a Glu residue at this position, which, because of its negative charge, may confer the same effect as phosphorylation.[7]

The mature (phosphorylated) PKC is still inactive and binds via low-affinity interactions to both plasma and cytosolic membranes containing a negatively charged phospholipid head group.[8] The association with these membranes is short-lived, and an equilibrium establishes between cytosolic and membrane bound PKC. In its unstimulated state, the kinase is rendered inactive by association of the pseudosubstrate of the regulatory domain with the kinase core of the catalytic domain, which is structurally highly similar to that of the PKA domain, via electrostatic interaction with acidic residues.[7,15,16] In quiescent cells, the majority of PKCs (90%) are believed to be in this inactive state in equilibrium between being weakly membrane bound and unbound in the cytosol.[9]

Activation of the kinase involves a series of conformational changes in the regulatory domain induced by binding to 1,2-sn-diacylglycerol (DAG) and phosphatidyl-L-serine (PS).[2,6–8,17] In the classical model, agonists bind at the cell surface via receptors or membrane interaction and activate phospholipase type C (Figure 2). This leads to the generation of DAG and soluble inositol phosphates through the hydrolysis of membrane inositol phospholipids as well as the release of Ca^{+2} from intracellular stores. Ca^{+2} binds to the C2 domain of cytosolic PKC, allowing the C2 domain to tether to the plasma membrane upon subsequent binding to negative head groups.[9] This tethered PKC then diffuses in the plane of the membrane at which plasma membrane-embedded DAG binds to the C1 domain. The energy of DAG binding is used to release the pseudosubstrate, relieving autoinhibition and thus activating the PKC, which binds and phosphorylates its substrate using the C3 bound ATP. This DAG-induced conformational change is mimicked by the addition of phorbol ester (PMA). Both

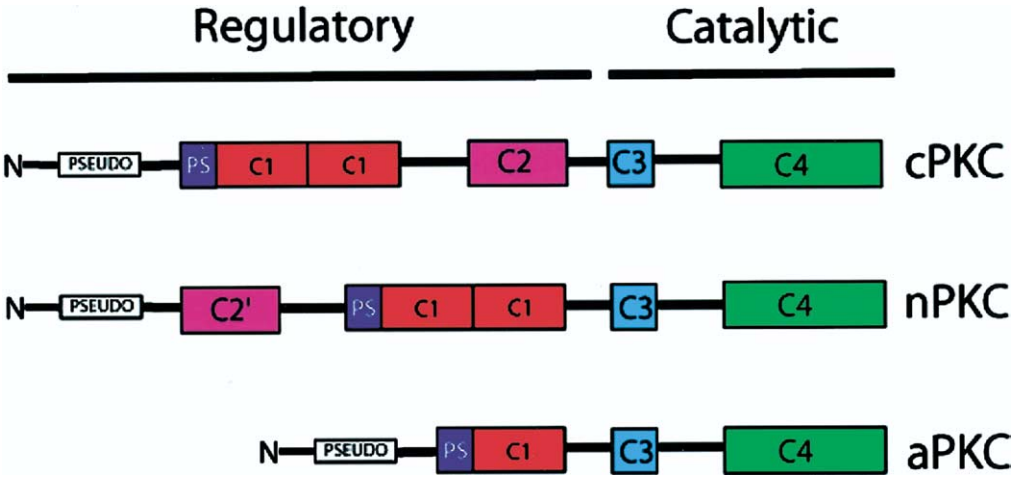

Figure 1. Structure of PKC isoforms. The structure for classical (cPKC), novel (nPKC), and atypical (aPKC) isoforms is shown schematically. They are divided into their regulatory and catalytic domains as shown. The N-terminal pseudosubstrate (*PSEUDO*), the phophatidyl-L-serine (*PS*), and the ATP (*C3*) binding domains and the C4 catalytic domain are common to all 3 subfamilies.[70] The cPKC and the nPKCs both contain functional 1,2-sn-diacylglycerol (DAG) binding domains (*C1*) and the *cPKC* contains a Ca^{+2}-binding domain that is nonfunctional and more amino terminal in the nPKCs and completely absent in the aPKCs.