Protein Kinase C Isotypes and Their Specific Functions: Prologue

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Protein kinase C: a model for understanding cellular signaling mechanisms

In 1977, a novel protein kinase that can be activated by calcium-dependent neutral protease (now known as calpain) was identified in mammalian tissues (*1*). Proteolytic cleavage of the kinase generates two functional domains, one hydrophobic and the other showing full catalytic activity that is suppressed in the "proenzyme." Subsequently, the "proenzyme" was shown to be activated in a manner dependent on both Ca^{2+} and phospholipid and named protein kinase C (PKC). Studies on the activation mechanism of PKC led to the critical finding that diacylglycerol (DG), one of the metabolites of membrane phospholipids, can greatly lower the Ca^{2+} concentration required for enzyme activation (*2*). This led Nishizuka and colleagues to hypothesize that DG plays a role as the second messenger in the activation of PKC and the transmission of an extracellular signal. The activated PKC then triggers a cell response to the signal through the phosphorylation of critical target proteins. To confirm this hypothesis they employed platelets, in which thrombin triggers the turnover of inositolphospholipids, which generates DG, mobilizes Ca^{2+}, and leads to cell activation. A series of experiments filled the missing links among membrane phospholipid metabolism, calcium mobilization, protein phosphorylation, and cell activation, which occur simultaneously when cells are exposed to a variety of extracellular signals including growth factors. The subsequent finding that tumor-promoting phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (also known as phorbol 12-myristate 13-acetate, PMA) can directly activate PKC by substituting for DG not only confirmed the importance of PKC in cell growth and differentiation but also led to the use of the PKC activator for analyses of the roles of PKC and the DG-PKC signaling pathway (*3*). It must be noted several of these epoch-making findings came from the same laboratory in Kobe.

After this initial period until the early 1980s, studies that focused on PKC became widespread in a variety of biomedical fields including biochemistry, pharmacology, endocrinology, oncology, neuroscience, and cell and developmental biology (*4, 5*). During the past 20 years, more than ten thousand papers containing the words "protein kinase C" in their titles have been published. During this period, the study of PKC has been a model for the analysis of cellular signal transduction mechanisms and has provided essential information about the features of signal transduction molecules and pathways, the importance of cell membranes, transient and long-lasting activation of signaling proteins through multiple pathways, spatial and temporal redistribution of signaling molecules to specific cellular compartments, and molecular multiplicity of signaling proteins.

PKC isotypes: C1 and C2 domains, and the serine/threonine-specific protein kinase domain of the AGC group

There are at least 10 PKC isotypes encoded by 9 genes in mammals. Each PKC isotype is expressed in a cell type-specific manner, *i.e.* multiple PKC isotypes are expressed in a single cell. These isotypes can be subdivided into three classes based on primary structure and biochemical properties: classical or conventional PKC isotypes (cPKC), novel PKC isotypes (nPKC), and atypical PKC isotypes (aPKC). All PKC isotypes share a characteristic sequence motif C1 in addition to a serine/threonine-protein kinase domain (Fig. 1).

The cPKC isotypes include PKCα, βI, βII, and γ, and share structural motifs C1 and C2. The cPKC-C1 domains contain a repeat of a cysteine-rich sequence, C1A and C1B, each of which tightly binds two zinc ions and constitutes a binding site for DGs and phorbol esters. The cPKC-C2 domains are Ca^{2+}-dependent phospholipid binding domains that show specificity to acidic phospholipids such as phosphatidylserine. Purified cPKC isotypes can be activated by DGs or phorbol esters in the presence of Ca^{2+} and acidic phospholipids, showing typical features of the original PKC discovered in Kobe.

The nPKC isotypes include PKCδ, ε, η, and θ, and share structural motifs C1 and C2. The nPKC-C1 domains bind DG and phorbol ester as in the case of cPKCs. The nPKC isotypes also have the C2 domain whose sequence is somewhat diverged from that of cPKC-C2. Purified nPKC isotypes can be activated by DGs or phorbol esters in the presence of phospholipids, but this activation does not require Ca^{2+}, in clear contrast to cPKC isotypes.

aPKC isotypes include PKCζ and λ/ι. (human PKCι and mouse PKCλ are orthologues and referred to as PKCλ/ι hereafter). They share structural motifs C1 and OPR. The C1 sequences of the aPKC isotypes are not repeated, unlike in the cPKC and nPKC isotypes. Further, they lack critical residues required for the interaction with DGs and phorbol esters. Consistently, purified aPKC isotypes do not bind to

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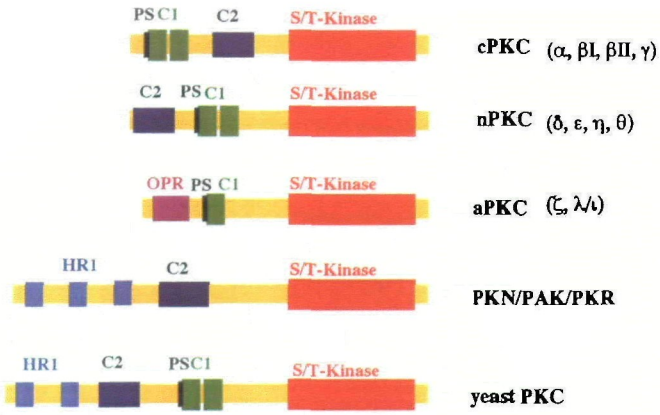

Fig. 1. **Schematic structure of protein kinase C isotypes.** The S/T-kinase domain of protein kinase C belongs to the AGC group of protein kinases that includes cyclic-nucleotide regulated protein kinases, PKB/RAC-PK/Akt kinases, PKD/PKCm, G protein–coupled receptor kinases (βARKs), S6 kinases, and myotonic dystrophy protein kinase. The C1 domains (http://pfam.wustl.edu/cgi-bin/getdesc?name=DAG_PE-bind) of cPKC and nPKC are the binding site for DG and phorbol ester and contain a repeat of a cysteine-rich sequence, whereas the C1 domain aPKC contains only cysteine-rich sequence and binds neither DG nor phorbol ester. The C2 domain (http://pfam.wustl.edu/cgi-bin/getdesc?name=C2) is the binding site for phospholipid. The OPR domain (Octicosapeptide repeat) contains a 28-residue motif present in aPKC and other proteins and is involved in protein–protein interactions. The HR1 domain (http://pfam.wustl.edu/cgi-bin/getdesc?name=HR1), which is also called ACC (antiparallel coiled coil) domain, was first described as a three times repeated homology region of the N-terminal non-catalytic part of protein kinase PKN/PAK/PKR. PS represents a pseudosubstrate sequence that masks the catalytic site to suppress kinase activity.

nor are activated by DGs or phorbol esters. The N-terminal sequences of aPKC isotypes contain the OPR motif and constitute a binding site for PAR-6 and ZIP/p62.

Conservation of cPKC, nPKC, and aPKC in multicellular organisms

Three classes of PKC isotypes, cPKC, nPKC, and aPKC, are conserved in a variety of metazoan species including two model genetic organisms, *Caenorhabditis elegans* and *Drosophila*. In *Drosophila*, there are at least two cPKC, two nPKC (δ/θ-type and ε/η-type) and one aPKC. In *C. elegans*, there are at least one cPKC, two nPKC (δ/θ-type and ε/η-type), and one aPKC. The evolutionary conservation of cPKC, nPKCδ/θ-type, nPKCε/η-type, and aPKC from warms to mammals implies independent roles for the four classes of PKC that are fundamental to multicellular organisms (Table I).

In addition to the PKC isotypes listed above, there are kinases called PKN, PAK or PRK that share protein kinase domains most closely related to the PKC isotypes. This family of enzymes also shares the C2 with cPKC and nPKC and is included as an extended member of the PKC family in this minireview series.

There are kinases more distantly related to the PKC family. A kinase called protein kinase B (PKB), rac-PK, or Akt, has a kinase domain similar to that in the PKC family but does not share C1 or C2. Kinases known as protein kinase D (PKD) or PKCμ and PKCnu share the C1 sequence with PKC but their kinase domains are more diverged from the extended PKC family.

In yeast, the three classes of PKC and PKN/PAK/PKR do not exist, but one (*Saccharomyces cerevisiae*) or two closely (*Shizosaccharomyces pombe*) related PKCs exist. For example, yeast PKC (*S. cerevisiae*) contains the sequence motifs

TABLE I. **Mammalian PKC isotypes in comparison with their counterparts in lower eukaryotes.** Mammalian PKC isotypes are classified into four groups, cPKC, nPKCδ/θ, nPKCε/η, and aPKC. Each class has counterparts, orthologues, in two model genetic organisms, *Drosophila* and *C. elegans*. Yeast has only one (or two) PKC that shares structural features with cPKC, nPKC, aPKC, and PKN/PAK/PKR. The blue boxes indicate that genetic evidence is available for the function of the isotype in the whole body. Numbers in the parentheses show amino acid residue numbers. Numbers starting with # indicate chromosome number and locus.

subclass		cPKC				nPKC				aPKC		PKN/PAK/PKR		
Eukaryota, Metazoa	Mammals	PKCα (alpha)	PKCβ I (beta)	PKCβ II (beta)	PKCγ (gamma)	PKCδ (delta)	PKCθ (theta)	PKCε (epsilon)	PKCη (eta)	PKCζ (zeta)	PKCλ/ι (lambda /iota)	PKNα/ PRK1	PKNβ	PRK2
	Human	(672)	(671)	(673)	(697)	(676)	(706)	(737)	(682)	(592)	(587)	(942)		(984)
		#17q22-q23.2	#16p11.2		#19q13.4	#3p	#10p15	#2p21	#14q22-q23	#1p36	#3q26.31	#19p13.1		
	Mouse	(672)		(673)	(697)	(674)	(707)	(737)	(683)	(592)	(586)			
		#11	#7		#7	#14	#2				#3q13.8			
	Zebrafish (*Danio rerio*)										lambda/iota (580)			
	Frog (*Xenopus*)	PKC I		PKC II							zeta (588)	PKN		
	Fruit fly (*Drosophila*)	PKC53E(BR) (639;670;679)		eyePKC/ InaC (700)		putative PKC delta type (514)		PKC98E (634)		DaPKC (606)		PKC-related Kinase (1174,1386)		
	California sea hare (*Aplysia californica*)		APL I/PKC I (protein kinase C I)(649)					APL II/PKC II (743)						
	Nematoda (*C. elegans*)	PKC-2 (680~717)				TPA-1 (A,704; B,567)		PKC-1 (A,763; B,707)		PKC-3 (597)		F46F6.2.p (1018)		
Eukaryota, Fungi	Fission yeast					Pck1p(988)		Pck2p(1016)						
	Budding yeast					Pkc1p(1151)								

HR1, C2, C1, and serine/threonine kinase. This suggests that yeast PKC retains an ancestral form that evolved into cPKC, nPKC, aPKC, and PKN/PAK/PKR in multicellular organisms (*6*).

Thus, the genetic analysis of PKC isotypes in model organisms provides important clues for understanding the roles of mammalian PKC isotypes. This is one of the aims of this series in which PKC in yeast, *C. elegans*, and *Drosophila* will be reviewed.

The time for reviews focusing on isotype-specific functions

Studies on the biochemical properties of mammalian PKC isotypes have revealed that they are very sophisticated kinases with multiple activation modes. Phosphorylation and membrane targeting, in addition to the binding to second messengers, play critical roles in the activation of cPKC and nPKC isotypes (reviewed in Refs. *7–9*). However, the activation modes of PKCs *in vivo* under specific biological contexts remain to be evaluated one by one. The translocation of a specific PKC isotype to a specific cellular compartment can be monitored by using isotype-specific antibodies. GFP-tagged PKC has also been used to visualize the translocation of the respective PKC isotypes. In some cases, the activity of PKC in cells can be monitored after immunoprecipitation of the PKC isotype. These approaches can provide supporting evidence for the role of a specific PKC isotype in a specific function.

Many of the studies on PKC function are based on the use of activators and inihibitors. Although phorbol esters have been widely used, the results of these studies must be examined carefully because they can activate many of the cPKC and nPKC isotypes in cells. In addition, phorbol esters usually cause the rapid down regulation of most cPKC and nPKC isotypes. Further, another class of proteins that can bind phorbol esters exists (reviewed in Ref. *10*). Thus, some of the effect of phorbol esters is not caused solely by PKC family members but can be caused by other cellular phorbol ester receptors. PKC inhibitors that can inhibit a subset of PKC family members have also been widely used to evaluate the involvement of PKC. Again, the results of these studies should be evaluated carefully in terms of specificity of the drug.

Overexpression studies have also been widely employed. Constitutively activated PKC isotypes with point mutations in their pseudosubstrate sequences have been used successfully to reveal the ability of a specific isotype to modify a specific cellular function. Kinase-inactive mutants with a point mutation at the ATP-binding site have also been successfully used as dominant negative mutants to evaluate the involvement of the endogenous PKC isotype in a specific cellular function. Some studies use anti-sense nucleotides for the analysis of isotype-specific function. The genetic and reverse-genetic approaches using mice and other model organisms are now starting to reveal the function of specific PKC isotypes in the whole body. Studies based on knockout mice clearly show that each PKC isotype has distinct functions *in vivo*.

As for the downstream mediators of PKC, a huge number of proteins have been identified as candidate PKC substrates, however, very few of them have been identified as physiological substrates. More recently, many PKC binding proteins, some of which show isotype-specificity, have been identified. Although the physiological meanings of most of the substrates and binding proteins remain to be clarified, this approach, in combination with other approaches based on biochemistry, cell and developmental biology, and genetics, is now opening new avenues to understanding the specific function of each PKC isotype.

The wide variety of the research fields as well as the huge number of research papers, however, makes it quite difficult to follow what is clear and what is still unclear about PKC and, especially, about specific PKC isotypes. On the contrary, the need to review the current understanding of PKC is increasing because many people in various fields are continuously entering the PKC field. Although there are some excellent reviews that focus on specific aspects of PKC, so far there are very few reviews that focus on specific PKC isotypes (*11, 12*). This is the major aim of this series in which each review will focus on the function of a specific PKC isotype.

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