Protein Kinase C-ε (PKC-ε): Its Unique Structure and Function

Yoshiko Akita[1]

Department of Laboratory Animal Science, The Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613

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Protein kinase C (PKC)-ε was first discovered among novel PKC isotypes by cDNA cloning, and characterized as a calcium-independent but phorbol ester/diacylglycerol-sensitive serine/threonine kinase. PKC-ε is targeted to a specific cellular compartment in a manner dependent on second messengers and on specific adapter proteins in response to extracellular signals that activate G-protein-coupled receptors, tyrosine kinase receptors, or tyrosine kinase-coupled receptors. PKC-ε then regulates various physiological functions including the activation of nervous, endocrine, exocrine, inflammatory, and immune systems. The controlled activation of PKC-ε plays a protective role in the development of cardiac ischemia and Alzheimer's disease, whereas its uncontrolled chronic activation results in severe diseases such as malignant tumors and diabetes. This review summarizes recent progress in our understanding of the unique structure and physiological and pathological roles of PKC-ε with a focus mainly on knockout, transgenic, and mutational studies.

Key words: ischemia, knockout, protein kinase C-ε, transgenic, tumor.

Protein kinase C (PKC)-ε, a novel PKC isotype characterized as a calcium-independent and phorbol ester/diacylglycerol-sensitive serine/threonine kinase (Fig. 1), is expressed in many tissues and cells, but abundantly in neuronal, hormonal, and immune cells (*1, 2*). To date, the essential roles of PKC-ε have been established in many signaling systems including proliferation (*3*), differentiation (*4*), gene expression (*5*), muscle contraction (*6*), mechanical force adaptation (*7*), metabolism (*8*), transport (*9*), exocytosis (*10*), and endocytosis (*11*) systems, and also have nervous, inflammatory, immune, and circular functions (Table I and Fig. 2). Moreover, evidence suggesting critical roles for PKC-ε in various diseases such as tumors, ischemia, and diabetes is accumulating.

I. Unique structure, characteristic function, and subcellular targeting

PKC-ε, as in the case of other members of the PKC family, requires phosphorylation at three converved sites in order to become responsive to second messengers: Thr-566 in the activation loop, Ser-729 in C-terminal hydrophobic site, and Thr-710 at an autophosphorylation site (Figs. 1 and 2) (*12*). Non- or hypo-phosphorylated PKC-ε (immature form) appears to associate directly with anchoring proteins such as CG-NAP (centrosome and Golgi localized PKN-associated protein) *via* its catalytic domain, and the phosphorylation of Thr-566 and Ser-729 seems to be conferred at the Golgi/centrosome area.

Mature PKC-ε is activated by several different second messengers, DAG, PIP3 (phosphatidylinositol 3,4,5-triphosphate), and fatty acids produced by physiological stimuli such as PDGF (platelet-derived growth factor) and bradykinin (*13, 14*). The subcellular localization behavior of the kinase partially depends on which second messenger is bound to the C1 domain. PKC-ε translocates to the plasma membrane and/or cytoskeleton in response to DAG and tridecanoic acids, whereas it translocates to Golgi-networks in response to arachidonic (AA) and linoleic acids (*15*).

The adapter proteins of PKC-ε also determine its localization. The coatomer protein beta'-COP (beta'-COP or RACK2), a Golgi membrane protein involved in vesicular trafficking, has been reported to be a selective adaptor protein for activated PKC-ε (*16*). The association with beta'-COP takes place *via* the C2 (-like) domain (previously termed as the D1 or V1 region) of PKC-ε (Fig. 1). PKC-ε also binds to RACK1, which is reported to be a selective binding protein for activated PKC-βII, although the affinity for RACK1 is one-tenth that for RACK2.

Uniquely, PKC-ε has an actin-binding motif (a.a. 223–228) located between the first and second cysteine-rich regions of the C1 domain, and associates with actin filaments in response to extra stimuli in a manner independent of phosphatidylserine (Fig. 1) (*17, 18*). AA and DAG synergistically stimulate the association of PKC-ε and actin. Filamentous actin not only serves as the unique anchoring protein of PKC-ε but also activates the kinase by maintaining it in a catalytically active conformation. Ultimately, both the C1 domain and C2 domain of PKC-ε function as subcellular localization signals (*19*).

Although some specific substrates of PKC-ε, such as calsequestrin and the capsaicin receptor (VR1), have been reported (*20, 21*), most substrates, including MARCKS, are ubiquitously phosphorylated by nearly all conventional and novel PKCs (*22, 23*). Substrate specificity in regulatory signaling is achieved through the spatial- and temporal-targeting of PKC-ε to subcellular compartments.

[1]For correspondence. Tel: +81-3-5449-5661, Fax: +81-3-5449-5424, E-mail: akita@rinshoken.or.jp

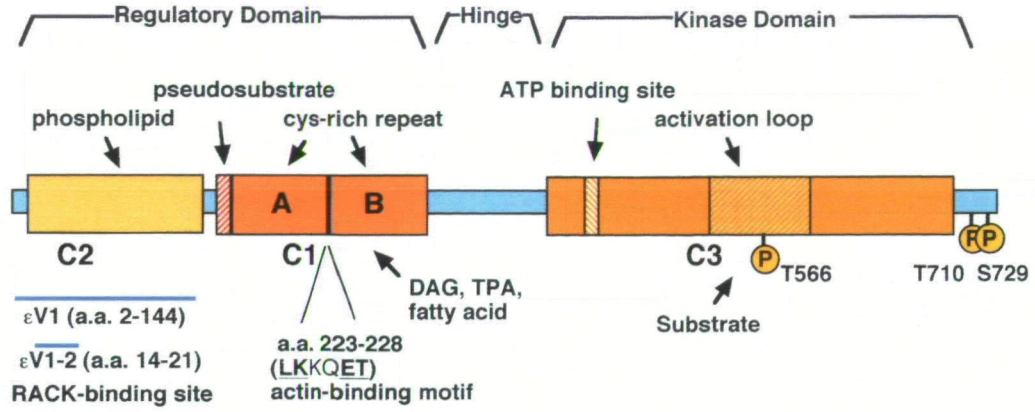

Fig. 1. **Structure of PKC-ε.** The εV1 (or εV1-2) peptide derived from the Rack-binding site located in the C2 region activates the cellular membrane translocation of PKC-ε. DAG and phorbol esters might have different C1A *versus* C1B selectivity. C1B seems to be important for fatty acid-induced targeting of the isotype. The phosphorylation of Thr-566 in the activation loop is essential for enzymatic activity. The phosphorylations of The-710 and Ser-729 are also necessary stability and competency to second messengers. Read text for actin-binding motif and more detail.

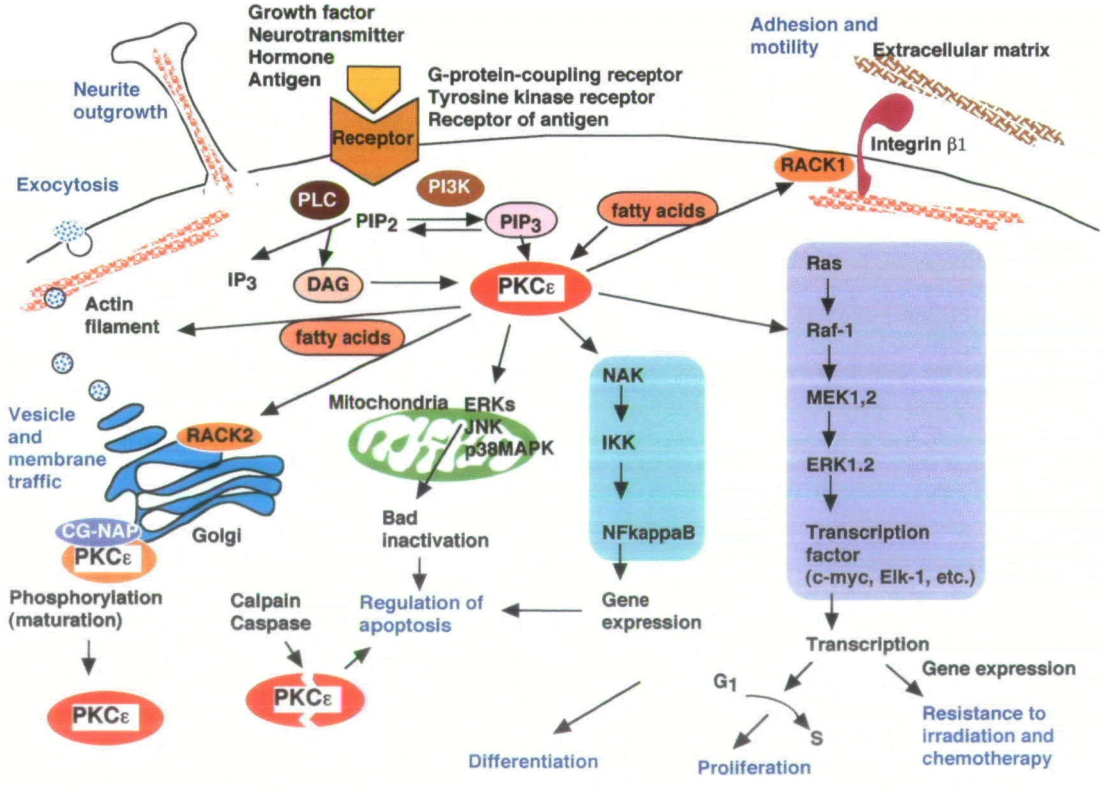

Fig. 2. **PKC-ε signaling in various cells.** PKC-ε is activated by DAG, PIP3, and fatty acid generated by various cell stimuli, and mediates corresponding responses such as neurite outgrowth, apoptosis, adhesion and motility. See text for details and references.

II. Physiological and pathological functions

1. Nervous system. Among novel PKCs, PKC-ε is the most abundant species in the central nervous system and there is the increasing evidence that PKC-ε mediates various effects in neurons. PKC-ε induces neurite outgrowth during neuronal differentiation activated by various stimuli (*24, 25*) through interaction with actin filaments, and the C1 domain, which contains the actin-binding motif, is essential in this process (*18*). The significance of the actin-binding site in the interaction with filaments has also been implicated in neurotransmitter exocytosis (*17*).

PKC-ε also seems to mediate synaptic function. In sensory neurons, PKC-ε has been implicated in the regulation of nociceptor function that participates in various types of pain (*26, 27*). Interestingly, several studies using PKC-ε–knockout mice suggest that activation of the isotype plays a critical role in alcohol dependency by regulating the sensitivity of $GABA_A$ receptors (*28*). Moreover, neuronal hyper-

excitability during alcohol administration may be mediated by PKC-ε–induced upregulation of N-type channels (*29*).

2. Inflammatory, immune, and hematopoietic circular systems. PKC-ε appears to play critical roles in activated macrophages (*30*). The macrophages from PKC-ε–knockout mice have severe deficiencies and, in the absence of PKC-ε, host defense against bacterial infection is severely compromised, resulting in increased mortality. PKC-ε and/or -δ are also necessary for IgG-stimulated phagocytosis (*31*).

IkappaB kinase (IKK) phosphorylation is essential for the activation of transcriptional factor NF-kappaB. PKC-ε has been demonstrated to mediate IKK phosphorylation *via* the activation of NAK (NF-kappaB–activating kinase) in response to growth factors (*32*). Since PKC-ε activation decreases in TNFalpha-induced apoptosis (*33*) and NF-kappaB is well established as an anti-apoptotic factor, the protective role of PKC-ε against apoptosis may be mediated through NAK activation.

PKC-ε activates the MEK-ERK1/2 cascade and mediates the induction of gene expression in erythropoietin (Epo)-stimulated erythroid cells (*34*), thrombin-stimulated jurkat T cells (*35*), and many other cell systems. On the other hand, PKC-ε seems to control MAPK cascades negatively through the induction of MAPK phosphatase 1 (MKP-1) in lipopolysaccharide-stimulated macrophages (*36*).

3. Tumorigenesis. The roles of PKC isotypes in tumorigenesis are tissue- and cell-specific. For example, PKC-α has no effect on skin tumor promotion and PKC-δ reduces papilloma development (*37*). PKC-β has a partially oncogenic effect on fibroblasts (*38*). PKC-ε seems to be involved in tumor development and tumor cell invasion and metastasis in several tissues.

When overexpressed in several fibroblast and colonic and prostatic epithelial cell lines, PKC-ε has full oncogenic effects (*39–41*). Its oncogenic activity in these cells seems to be exerted through affects on the ras-signaling cascade at the level of Raf-1 activation (*42, 43*). Consistently, PKC-ε has been shown to mediate cyclin D1 induction in Ha-ras-transformed fibroblasts (*44*). In contrast, inhibiting the high levels of PKC-ε activation may suppress tumor promotion (*45*).

Epidermis-specific transgenic overexpression of PKC-ε causes mice to develop highly malignant/metastatic carcinomas (*37*). The evidence that PKC-ε contributes to tumor metastasis is accumulating. PKC-ε is required for the cell spreading mediated by integrin β1, and Rac1 acts downstream of PI3-kinase and PKC-ε (*46, 47*). PKC-ε is reported to be linked to integrin β1 through interaction with RACK1, and to associate with F-actin *via* its actin-binding site, thereby mediating increased adhesion and mobility (*48, 49*). Moreover, PKC-ε appears to contribute to motility by regulating β1 integrin traffic that permits its recycling in cells (*50*). These are concomitant with the observation that PKC-ε levels are consistent with the degree of invasion and metastasis of human glioma. In human breast carcinoma cells, cis-polyunsatulated fatty acids stimulate β1 integrin-mediated adhesion to type IV collagen by activating PKC-ε and PKC-μ (*51*).

Chronic hypoxia in tumors can promote malignant progression and confer resistance to irradiation and chemotherapy by altering gene expression. PKC-ε also seems to play a role in these processes by activating the Raf-1/MEK/ERK cascade (*52*).

Since PKC-ε is implicated in apoptosis signaling in various cells, the possibility also arises that a loss of expression or function of PKC-ε may participate in tumorigenesis by the inhibition of programmed cell death. In thyroid tumors, rearranged amplification of the PKC-ε gene or post-transcriptional changes have been reported (*53*). In addition, PKC-ε has been implicated in ultraviolet-induced apoptosis and tumor promotion (*54, 55*).

The constitutive expression of the catalytic fragment *via de-novo* synthesis has been reported in lung carcinoma (NC1-N417) cells (*56*). On the other hand, during apoptosis or oncogenic-transforming processes, PKC-ε appears to be subjected to restrictive proteolysis by caspase (3 and 7) (*57, 58*) or selective cleavage by calpain (*59, 60*).

Taken together, these data suggest that PKC-ε plays important role in the signaling cascade accompanying apoptosis and tumorigenesis.

4. Heart disease. The function of PKC-ε in cardiomyo-

TABLE I. **Function of PKC-ε in the knockout and transgenic mice.**

A. Knockout mice

Phenotype of PKC-ε knockout mice	Reference
Dysfunction of macrophages including decreased activation of NF-kappaB, and decreased survival after bacterial infection.	(*30*)
Super-sensitivity of $GABA_A$ receptors in the cortex, and reduction of alcohol self-administration.	(*28*)
Attenuation of pain *via* nociceptor function in sensory neurons.	(27)
Reduction of the cardioprotective effect of early ischemic preconditioning.	(*62*)

B. Transgenic mice

(Expression tissue) Sequence	Phenotype of PKC-ε transgenic mice	Reference
(Cardiomyocyte)		
PsiepsilonRACK; activator of the translocation	Normal contraction in the neonatal heart, Improvement of cardiac contractile function, Protection against cell death by cardiac ischemia.	(*16*)
EpsilonV1; inhibitor of the translocation	Severe cardiomyopathy with reduced contraction in the neonatal, Attenuated protection from ischemia-induced cell death.	(*16*)
Wild type, Dominant negative, inactive, or constitutive active mutant	Cardiotrophic effect, Activation of PKC-βII through the expression of PKC-β specific RACK1 mediated by PKC-ε, contributing pathological hypertrophy, Formation of PKC-ε-Lck modules confers cardioprotection from ischemia, Colocalization of PKC-ε with three MAPKs in the mitochondria of cardiomyocytes, and protection from cell death *via* the activation of mitochondrial ERKs and inactivation of Bad.	(*69*) (*67, 68*) (*66*) (*65*)
(Epidermis in skin)		
Wild type	Formation of metastatic squamous cell carcinoma without development of papilloma.	(*37*)

cytes has been actively studied (*61*).

Studies based on the cardio-specific transgenic expression of PKC-ε–selective translocation inhibitor (epsilonV1) or activator (psiepsilonRACK) peptides clearly show that PKC-ε plays critical roles in protecting against ischemic damage as well as during normal postnatal myocardial development (*16*). Targeted disruption of the PKC-ε gene has been reported to prevent infarct size reduction following ischaemic preconditioning (*62*). Physiologically moderate ethanol consumption in the development of ischemia appears to have beneficial cardioprotective effects by directly activating PKC-ε.

The signaling pathways responsible for cardiacprotection involve the activation of PI3-K upstream (*63*) and the MEK1/2-ERK1/2 cascade downstream of PKC-ε, and, in turn, the activation of anti-apoptosis transcriptional factors such as NF-kappaB (*64*). In addition, stress-activated kinases, p46/p54 JNKs, seem to be activated by PKC-ε. PKC-ε and three MAPKs, ERKs, JNKs, and p38MAPK, are reported to colocalize with mitochondria, which are thought to be key mediators of the cardioprotective signal (*65*). The transgenic activation of PKC-ε increases in these interactions, and during the phosphorylations of ERKs and Bad, leading to cell survival. Src and Lck tyrosin kinases also appear to be involved in PKC-ε signaling (*66*).

The contribution of PKC-βII has been established in the pathogenesis of myocardial hypertrophy and dysfunction. The role of PKC-ε in hypertrophy is somewhat complex. Analysis by the transgenic expression of PKC-translocation modifiers (epsilonV1 and psiepsilonRACK) have shown that the activation of PKC-ε improves contractile dysfunction in pathological myocardial hypertrophy, whereas inhibition of PKC-ε leads to lethally dilated cardiomyopathy (*67*). However, high levels of PKC-ε activation in transgenic mice seem to lead to impaired function and significant myocardial hypertrophy (*68*), probably due to the enhancement of translocation and activation of PKC-βII through PKC-ε–mediated RACK1 expression (*69*).

5. Diabetes, Alzheimer's disease, and others. The chronic activation of PKC-ε is involved in the development of diabetes and in the progression of various diseases involving a hyperglycemic state (*70*). In the muscle, increased expression and activation of PKC-ε seem to be causally related to the development of diabetes, at least part, *via* the down-regulation of insulin receptor and lipid accumulation, resulting in impaired glycogen synthesis and insulin resistance. Enhanced activity of PKC-ε in hyperglycemic states also contributes to cardiomyopathy (*71*) and nephropathy (*72*), and to the accelerated development of vascular disease.

The deposition of plaques containing Abeta (beta-secretase–derived C-terminal fragment) is thought to be important in the pathogenesis of Alzheimer's disease. PKC-ε suppresses the production of Abeta by promoting alpha-secretase–mediated processing of APP (the beta amyloid precursor protein) (*73*). The level of PKC-ε is substantially lower in the brains of Alzheimer's disease patients compared to age-matched controls (*74*). Thus, reduced PKC-ε activity may contribute to the development of Alzheimer's disease.

Furthermore, a PKC-ε–dependent pathway is proposed in the development of interstitial lung fibrosis in systemic sclerosis (SSc) patients (*75*).

III. Conclusions and perspectives

Recent structural and functional studies of PKC-ε are briefly reviewed. Knockout, transgenic, and mutagenic experiments are greatly facilitating progress in understanding PKC-ε functions. The development of a specific activator and inhibitor, psiepsilonRACK and epsilonV1 peptides, respectively, has provided not only useful probes for experimental research but also a model for pharmacological therapy. However, little is known about the tertiary structure of PKC-ε, although the structure of the C2 domain has been reported recently (*76*). Further fundamental studies on the structure as well as the biological and pathological functions are important for total understanding and for the development of novel therapies specifically directed against PKC-ε.

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