J. Biochem. **133**, 1–7 (2003)
DOI: 10.1093/jb/mvg017

Protein Kinase Cζ (PKCζ): Activation Mechanisms and Cellular Functions

Takaaki Hirai and Kazuhiro Chida*

Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657

Received November 1, 2002; accepted December 11, 2002

The ζ isotype of protein kinase C (PKCζ) is a member of the atypical PKC subfamily and has been widely implicated in the regulation of cellular functions. Increasing evidence from studies using *in vitro* and *in vivo* systems points to PKCζ as a key regulator of critical intracellular signaling pathways induced by various extracellular stimuli. The major activation pathway of PKCζ depends on phosphatidylinositol (PI)-3,4,5-trisphosphate (PIP_3), which is mainly produced by PI-3 kinase. 3′-PI-dependent protein kinase 1, which binds with high affinity to PIP_3, phosphorylates and activates PKCζ. Many studies demonstrated the involvement of PKCζ in the mitogen-activated protein kinase cascade, transcriptional factor NFκB activation, ribosomal S6-protein kinase signaling, and cell polarity. An important molecular event in a cell is the association of PKCζ with other signaling molecules, as well as scaffold proteins, to form large complexes that regulate their pathways. The understanding of the mechanisms underlying PKCζ-mediated control of intracellular signaling is beginning to provide important insights into the roles of PKCζ in various cells.

Key words: PDK1, PI3K, PIP_3, PKCζ, ZIP/p62.

Structure of PKCζ

Protein kinase Cζ (PKCζ) was originally discovered as a unique PKC isotype (*1*). To date in mammals, it is classified into the atypical PKC (aPKC) subfamily, based on its structural similarity to PKCλ/ι [human PKCι (*2*) and mouse PKCλ (*3*) are orthologs]. The aPKCs and other PKC isotypes, namely, conventional PKC (cPKC) α, βI, βII, and γ, and novel PKC (nPKC) δ, ε, η, and θ, form the PKC family belonging to an extended group of Ser/Thr protein kinases, AGC (cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), and PKC) (*4*).

PKCζ, as well as PKCλ/ι, consists of four functional domains and motifs, including a PB1 domain in the N-terminus, a pseudosubstrate (PS) sequence, a C1 domain of a single Cys-rich zinc-finger motif, and a kinase domain in the C-terminus (Fig. 1). The PB1 domain recognizes OPCA (OPR/PC/AID) motifs of other proteins, such as PAR-6, ZIP/p62 and MEK5 (*5*) (see below). The PS is a short stretch of amino acids which resembles a substrate sequence except for Ala occupying the position of Ser or Thr as a phospho-group acceptor, and is assumed to block the substrate-binding cavity of the kinase domain as an autoinhibition mechanism. The C1 domains of aPKC isotypes are different in terms of a repeat structure from those of cPKCs and nPKCs that contain two repeated zinc-finger motifs, C1A and C1B, both of which are essential for interaction with and activation by a second messenger diacylglycerol (DG) and phorbol-diester tumor promoters. Although the C1 domains of aPKCs are similar to those of C1A, aPKCs do not respond to DG and phorbol-diesters (*6*). The kinase domain of PKCζ, as well as other members of the AGC group, includes an ATP-binding region, an activation loop, a turn motif, and a hydrophobic motif. The ATP-binding region contains a Lys residue, Lys-281, which is crucial for its kinase activity. A mutant whose Lys-281 is substituted for other amino acids is usually used as a kinase-defective dominant-negative form of PKCζ (PKCζ-kn). The activation loop and turn motif contain important Thr residues, namely Thr-410 and Thr-560, respectively, which are phosphorylated upon activation. Recent studies reveal dynamic interactions of PKCζ with other proteins.

Activation mechanisms

The mechanisms of PKC activation mainly consist of two events, release of the PS from the substrate-binding cavity and phosphorylations of the kinase domain (*7*). Upon liberation from the PS-dependent autoinhibition, some lipids play important roles. cPKCs and nPKCs interact with membrane lipids, such as phosphatidylserine, and lipid metabolites such as DG, which presumably induce the release of PSs from active sites resulting in phosphorylation of their substrate proteins.

PKCζ is also activated by lipid components, such as phosphatidylinositols (PIs) (*8*), phosphatidic acid (*9*), arachidonic acid (*10*), and ceramide (*10*). Among these lipids, PI-3,4,5-trisphosphate (PIP_3) has been the focus of much interest with regards to its regulation of aPKCs in various cells. Nakanishi *et al.* reported that enzymatically synthesized PIP_3 stimulates autophosphorylation of PKCζ purified from bovine kidney, whose phosphorylation is one of the requirements for protein kinase activation, suggesting that aPKCs can be regulated by PI-3 kinase (PI3K), which produces PIP_3 from PI-4,5-bisphos-

*To whom correspondence should be addressed. Tel/Fax: +81-3-5841-8152, E-mail: acchida@mail.ecc.u-tokyo.ac.jp

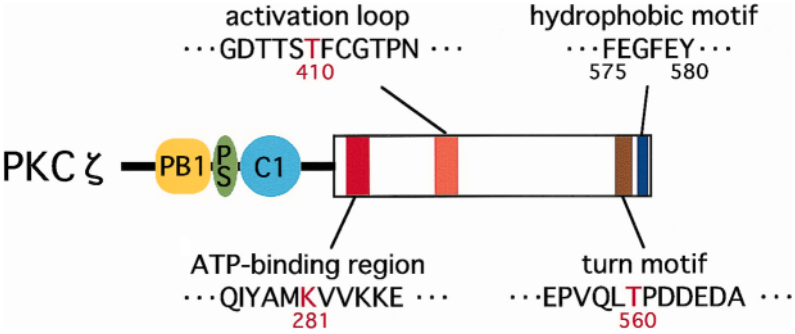

Fig. 1. **Schematic representation of domain structure of PKCζ.** PKCζ consists of a PB1 domain in the N-terminus, a pseudosubstrate (PS), a C1 domain, and a Ser/Thr kinase domain in the C-terminus. The kinase domain includes an ATP-binding region, an activation loop, a turn motif, and a hydrophobic motif. In the ATP-binding region, Lys-281 is essential for kinase activity. Thr-410 in the activation loop is phosphorylated by PDK1 which binds to the hydrophobic motif. Thr-560 in the turn motif is the autophosphorylation site and its phosphorylation is also crucial for the activation.

phate in response to various growth factors (*8*). In rat 3Y1 cells stimulated by epidermal growth factor (EGF) or platelet-derived growth factor, overexpression of p110, a catalytic subunit of PI3K, enhances PKCλ/ι activity (*11*). To date, there are many lines of evidence on activations of PKCζ and PKCλ/ι by PI3K in living cells, *e.g.*, adipocytes (*12*) and monocytes (*13*). How does PIP_3 interact with aPKCs?

PIP_3 directly binds to pleckstrin homology (PH) domain–containing protein kinases, *e.g.*, protein kinase B (PKB; also known as Akt) and 3′-PI–dependent protein kinase 1 (PDK1). The PH domain of PDK1 has a higher affinity for PIP_3 than that of PKB/Akt (*14*). PDK1 is activated by binding PIP_3 through its PH domain, and attaches to the hydrophobic motifs of AGC kinases, so that they are phosphorylated at a Thr residue in each activation loop (*15*) (Fig. 2). The hydrophobic motifs of aPKCs include a short sequence, Phe-Glu-Gly-Phe-Glu-Tyr, which is very similar to that of PDK1-binding sites of PKC-related protein kinases, Phe-X (any amino acid)-X-Phe-Asp-Tyr (*16*) (Fig. 1). In the activation loop of PKCζ, Thr-410 is phosphorylated by PDK1 (*17*, *18*). In embryonic stem cells lacking PDK1 as a result of genetic manipulation, PKCζ is not phosphorylated at Thr-410, markedly suggesting that PKCζ is a physiological substrate of PDK1 (*19*). Although a T410A mutant of PKCζ, whose Thr-410 is substituted for Ala, loses enzymatic activity, a Glu-mutant T410E, probably mimicking a phosphorylated Thr, retains its activity (*17*, *20*). These findings suggest that Thr-410 phosphorylation is essential for PKCζ activation.

Following the Thr-410 phosphorylation, PKCζ presumably exposes the kinase domain for further phosphorylation. Thr-560 in the turn motif of PKCζ is a key residue for activation (*18*), since in PKCα and PKCβII, phosphorylations of Thr-638 and Thr-641, respectively, corresponding to Thr-560 in PKCζ are required for their catalytic functions and for locking these kinases in a catalytically competent state (*21*, *22*). The T410E active mutant of PKCζ shows autophosphorylation but two Thr-560 mutants, T560A and T560E, do not in labeling experiments *in vitro*, indicating that Thr-560 is the sole autophosphorylation site in PKCζ (*23*). In living cells, whether Thr-560 of PKCζ is phosphorylated by itself, by another PKCζ intermolecularly, or by other protein kinases including other PKC isotypes, remains to be resolved.

Does PIP_3 activate aPKCs only through PDK1 activation? There are no discernible effects of PIP_3 on truncated PKCζ and PKCλ/ι, both of which lack the PS sequence, whereas PIP_3 induces dose-dependent increases in the activity of a T410E/T560E PKCζ double mutant; therefore, this mutant cannot phosphorylate the kinase domain (*23*). This suggests that PIP_3-induced activations of aPKCs are at least partly dependent on the presence of the PS sequences in their N-termini, and that these

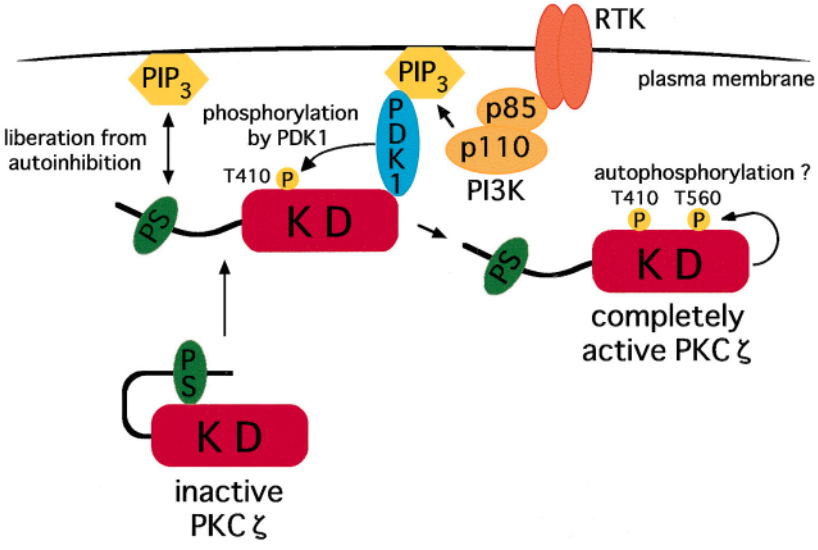

Fig. 2. **Schematic representation of PIP_3 and PDK1 in PKCζ activation.** The p85 subunit of PI3K interacts with the phosphorylated Tyr residues of receptor-Tyr kinases (RTKs) in responses to their ligands, and activates the p110 catalytic subunit, thereby producing PIP_3. PDK1 binds to PIP_3 *via* its PH domain, and becomes activated. The PDK1 interacts with PKCζ and phosphorylates the kinase domain (KD) at Thr-410, which induces Thr-560 phosphorylation. The PKCζ simultaneously and directly interacts with PIP_3, which releases PS-dependent autoinhibition. Both contributions of PIP_3 and PDK1 are necessary for the complete and stable activation of PKCζ.

apparent dependencies are most readily explained by their liberation from PS-dependent autoinhibition. Although no PIP_3-binding region in PKCζ has been identified, collectively, PIP_3 contributes to PKCζ activation in two ways: direct modulation by the PS-dependent autoinhibition and indirect modulation by phosphorylation of the kinase domain through PDK1. Both contributions may be necessary for the complete and stable activation of PKCζ.

Furthermore, specific protein–protein interactions affect PKC activities. Several proteins interact with and inhibit aPKCs. Prostate apoptosis response–4 (Par-4) interacts with the C1 motifs of aPKCs and inhibits their activities (*24*). A product of *Caenorhabditis elegans* partitioning defective gene-3 (PAR-3) (also known as ASIP, aPKC-specific interacting-protein, a mammalian homolog of PAR-3) binds the kinase domains of aPKCs and inhibits their activities (*25*, *26*). An OPCA motif–containing protein, PAR-6 (a product of partitioning defective gene–6) binds to the PB1 domains of aPKCs (*27*). Furthermore, PAR-6, PAR-3, and aPKC form a ternary complex (*25*, *27*). In the complex, PAR-6 suppresses PKCλ/ι activity, which is released by the further association of an active form of Cdc42 (*28*). In addition, several interacting proteins provide specificities of functional timing and location of aPKCs (see below).

Signaling and functions

Mitogen-activated protein kinase (MAPK) cascade. Many studies have shown that PKCζ is involved in the MAPK cascade in various cells (*29–32*). In monkey COS cells stimulated by serum or tumor necrosis factor α (TNFα), an active mutant of rat PKCζ or *Xenopus* PKCλ (previously known as an amphibian PKCζ) activates MAPK-kinase MEK1 and a MAPK ERK1, but a PKCζ-kn mutant does not (*29*). In thyroid cells, overexpression of wild-type PKCζ activates ERK1 and ERK2, and increases transcriptional activity of Elk-1, a well-established target of ERK1 and ERK2, whereas thyroid-stimulating hormone does not (*31*). What is the target of PKCζ in the MAPK cascade? Interestingly, overexpression of an active form of PKCζ, which lacks PS, also activates MEK1 but not Raf1 in COS cells (*31*). In human alveolar macrophages, lipopolysaccharide (LPS) activates MEK1, ERK1 and ERK2 but not Raf1 (*32*). At this point, endogenous PKCζ is activated and induced to associate with MEK1. Moreover, the myristoylated PKCζ-PS peptide, which inhibits PKCζ, blocks these LPS effects (*32*). These findings suggest that PKCζ functions as a MEK1 kinase, independent of the Raf1 pathway. However, it is still unclear whether PKCζ phosphorylates MEK1 directly or indirectly (*31*, *32*). Thus, to understand the functions of PKCζ in the MAPK cascade, it is important to clarify this point.

On the other hand, PKCζ was reported to function as an adapter in the MEK5-ERK5 pathway, which is another MAPK cascade critically involved in mitogenic activation by EGF (*33*). In response to EGF, endogenous PKCζ binds to MEK5 at its OPCA motif and increases ERK5 activity in the human transformed-cell line HEK293 (*33*). Conversely, overexpression of the MEK5-OPCA peptide or the PKCζ-PB1-domain peptide, both of which interfere with PKCζ-MEK5 interaction, inhibits the ERK5 activity (*33*). Importantly, overexpression of PKCζ-kn can also increase this EGF-induced ERK5 activity, suggesting that PKCζ functions only as an adapter (*33*). Furthermore, PKCζ binds to and activates PKBγ/Akt3 by phosphorylation at the C-terminal Ser of PKBγ/Akt3 (*34*). Interestingly, the phosphorylation does not depend on the PKCζ activity, but probably depends on an as yet unidentified type of PDK, PDK2 (*34*). Although a novel and interesting function of PKCζ as an adapter independent of its enzymatic activity is proposed in the above studies, further studies focusing on this issue in various signaling molecules are required.

From receptor signaling complexes to activation of NFκB transcriptional factor. In signaling for cell growth and survival, extracellular ligands, *e.g.*, TNFα, interleukin-1 (IL-1), and nerve growth factor (NGF), play their roles most likely through regulations of signaling pathways from their receptor complexes to their target gene expressions mediated by transcription factors, such as nuclear factor κB (NFκB) (Fig. 3). PKCζ–kn blocks responses of NFκB to these stimuli, indicating that PKCζ is critically involved in NFκB activation in signal transductions of TNFα and IL-1 (*35*, *36*). An important issue concerning the involvement of PKCζ in these signaling pathways is its interactions with those receptor-signaling complexes. In this regard, OPCA motif–containing ZIP (zeta-interacting protein) homologs provide a mechanistic clue (*37–39*). Puls *et al.* first showed that PKCζ binds to rat ZIP in a yeast two-hybrid system (*37*). Since ZIP homologs including human 62-kDa Lck-binding protein (p62) (*40*) have been cloned using different methods (*39*), we use the term ZIP/p62 in this article. Furthermore, two alternative-spliced forms, ZIP2 and ZIPβ, were reported (*41*, *42*). ZIP/p62 contains a TRAF6 (TNFα receptor-associated factor 6)-binding site (*43*), whereas ZIP2 deletes this site.

ZIP/p62 links PKCζ or PKCλ/ι to the TNFα receptor signaling complex including TRAF2 and receptor-interacting protein (RIP) (*35*). In human carcinoma HeLa cells and HEK293 cells, ZIP/p62 selectively interacts with RIP, but not with TRAF2 (*35*). Furthermore, ZIP/p62 links PKCζ to IL-1 and NGF receptor complexes including TRAF6 (*36*, *43*). In these cases, ZIP/p62 only functions as an adapter or a scaffolding. However, ZIP/p62 antagonizes Par-4-induced PKCζ inhibition and apoptosis of human osteosarcoma U2OS cells induced by TNFα (*44*). This suggests that ZIP/p62 also plays a critical role in the regulation of PKCζ activity in addition to its adapter function (*44*). Although the activation mechanisms of PKCζ containing ZIP/p62 are not understood, conformational changes may render PKCζ accessible to their substrates and thereby inducing NFκB activation.

Functions of NFκB including DNA binding, transactivation, and nuclear translocation are blocked by its cellular inhibitor protein IκB. An essential component of the NFκB pathway is the IκB kinase (IKK) complex, which phosphorylates IκB and triggers its degradation to release NFκB from its cytosolic state and then to translocate it into the nucleus (*45*). PKCζ phosphorylates the IKKβ subunit *in vitro*, possibly through their direct interaction (*46*). In HEK293 cells, PKCζ interacts with IKKβ through each catalytic domain in a TNFα-stimulation-

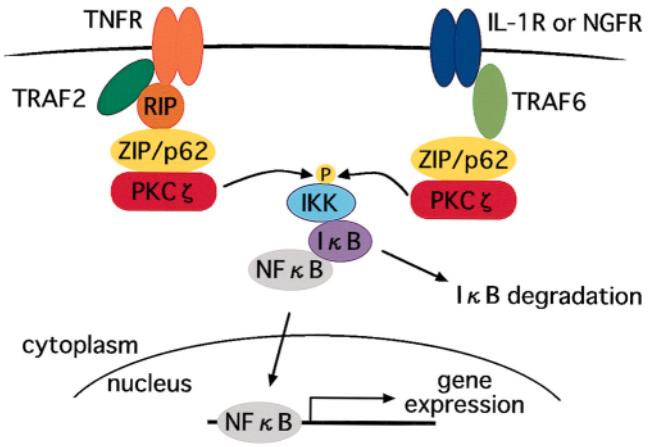

Fig. 3. **Schematic representation of involvement of PKCζ in signaling pathways from receptor complexes of TNFα, IL-1, and NGF to NFκB activation.** ZIP/p62 links PKCζ to RIP in TNFα receptor (TNFR) complex and to TRAF6 in complexes of IL-1 receptor (IL-1R) and NGF receptor (NGFR). The PKCζ phosphorylates and activates IKK, which induces IκB degradation, thereby inducing nuclear translocation, DNA-binding, and transactivation of NFκB.

dependent manner, thereby activating IKKβ (*46*). In the lungs of PKCζ-deficient mice, TNFα-induced IKK activation is depressed (*47*). These findings indicate that PKCζ is involved in the IKK signaling complex.

Collectively, aPKCs transduce signals from the receptors of TNFα, IL-1, and NGF to the activation sites of NFκB *in vivo*. Furthermore, they may be involved in a variety of signaling pathways from receptor complexes to expressions of their target genes by activation of transcription factors.

p70S6 kinase signaling cascade. p70 ribosomal S6 protein kinase (p70S6K), which is phosphorylated and activated in response to mitogenic stimuli, modulates translation of a subset of mRNAs that encode ribosomal proteins and translation elongation factors. Akimoto *et al.* first reported that PKCλ/ι directly associates with p70S6K, and that dominant negative forms of this kinase suppress the serum-induced activation of p70S6K in living cells (*48*). In coexpression experiments, PKCζ-kn antagonizes p70S6K activation by EGF, PDK1 and PI3K, and p70S6K associates with PDK1 as well as with PKCζ *in vivo* in a growth-factor-independent manner (*49*). This suggests the existence of a multimeric PI3K-p70S6K signaling complex. Then, does PKCζ modulate p70S6K activity in this complex? p70S6K is one of the AGC-kinases, and phosphorylations of its Thr-229 and Thr-389 in the activation loop and the hydrophobic motif, respectively, are important for activity. Myristoylated PKCζ, a constitutive active form of PKCζ, synergistically enhances PDK1-induced phosphorylations, as well as simply increases phosphorylations, of these residues, which induces prolonged activation of p70S6K (*50*). Thus collectively, aPKCs apparently play crucial roles in p70S6K activation. However, recombinant aPKCs do not directly phosphorylate p70S6K *in vitro* (*48*, *50*). The myristoylated PKCζ does not occlude EGF-dependent activation of p70S6K (*49*). Moreover, the myristoylated PKCζ can only enhance Thr-389 phosphorylation in the catalytically competent p70S6K (*50*). These would suggest that aPKCs alone are not sufficient for the complete activation of p70S6K. Multiple signals, such as mammalian target of rapamycin (mTOR) and PKB/Akt, are required for p70S6K activation. Thus, aPKCs may play a tuning role in translation together with other regulators.

Cell polarity. Cell polarity is fundamental not only for cell functions but also for development and tissue maintenance. Recent studies have revealed the importance of the ternary complex of PAR-3, PAR-6, and PKCζ or PKCλ in cell polarity (*25*, *27*, *51*). The PAR-3/ASIP-PAR-6-PKCλ complex controls formation of tight junctions in MDCK cells, an epithelial cell line derived from the dog kidney (*52*). PKCζ-kn, as well as PKCλ-kn, disrupts localization of ZO-1, a component of the tight junction, and probably interferes with the establishment of cell polarity (*52*). Overexpression of the regulatory domain (amino acids 1–126) of PKCζ causes a similar defect in the tight junction assembly, whereas a mutant containing two Asp residues (Asp-62 and Asp-66) within the PB1 domain does not (*53*). These findings suggest that both of the kinase activity and interactions *via* the PB1 domain are necessary for PKCζ to control cell polarity.

In addition to epithelial cells, PKCζ also controls polarity in migrating astrocytes. Scratching a confluent monolayer of rat primary astrocytes leads to their polarization at the leading edge, so that the microtubule organization center (MTOC) and the Golgi apparatus reorganize to face the new free space, and directed cell protrusion and migration specifically occur perpendicular to the scratch (*54*). Overexpressions of PKCζ, PKCζ-kn and PAR-6 inhibit MTOC and Golgi apparatus polarization without affecting the direction of protrusion (*54*). Collectively, the ternary complex of PAR-3/ASIP-PAR-6-aPKC plays an essential role in polarization of some cell types.

A neuron is a typical polarized cell. A Leu-zipper motif–containing protein FEZ1 (fasciculation and elongation protein; zygin/zeta-1) interacts with PKCζ at the N-terminal region including the PB1 domain, but does not contain any OPCA motif (*55*). FEZ1 is a human homolog of the *C. elegans* locomotory-defect gene product UNC-76 that is necessary for axonal bundling and elongation within axonal bundles (*56*). Coexpression of FEZ1 and an active form of PKCζ stimulates dendritic neurite elongation of rat pheochromocytoma PC12 cells, whereas expression of FEZ1 alone does not affect the cells (*55*). FEZ1 is phosphorylated by PKCζ and then translocated from the cytoplasmic membrane to the cytoplasm (*55*). Furthermore, a protein localized in the postsynaptic density and dendritic raft, PSD-Zip70, is a closely related homolog of FEZ1 (*57*). Although evidence on cellular functions and phosphorylation is limited, interaction of PKCζ with FEZ1/PSD-Zip70 may play an important role in neurite elongation and maintenance of the postsynaptic structure.

Furthermore, a tumor suppressor gene at 8p22 (*LZTS1*), a frequently altered chromosome region in many malignant tumors, including esophageal, prostate, and breast cancer, encodes FEZ1 (*58*, *59*). FEZ1 is associated with microtubule components in human epithelial cells (*59*). Combined with these findings, PKCζ through its

interaction with FEZ1/PSD-Zip70 might be also involved in mechanisms underlying human carcinogenesis.

Long-term potentiation (LTP). In the brain, not only a 75-kDa protein of the native PKCζ, but also a smaller 51-kDa protein is detected by immunoblotting (*60*). The amount of this 51-kDa protein referred to as PKMζ increases in hippocampal CA1 pyramidal cells during LTP maintenance (*60*, *61*). PKM is a catalytic domain released from PKC by proteolysis. Injection of a predicted recombinant PKMζ increases the AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptor–mediated excitatory postsynaptic current amplitude of neurons, and this PKMζ-mediated increase in the current completely occludes LTP (*62*). Low concentrations of chelerythrine and a myristoylated PKCζ-PS peptide, both of which inhibit PKCζ, also impair LTP maintenance (*62*). Furthermore, a transgenic fly that overexpresses PKMζ enhances memory after massed training (*63*). Chelerythrine and the induction of a dominant-negative form of PKMζ inhibit memory without affecting learning in wild-type flies (*63*). These findings interestingly imply that PKMζ is both necessary and sufficient for maintenance of LTP and memory (*62*, *63*). However, the importance of PKMζ in LTP is not clear because the inhibition of memory by the kinase-negative form of PKMζ may reflect its dominant-negative effects. Although chelerythrine also inhibits memory, its specificity to PKMζ remains unclear. Thus, the loss of function of PKMζ needs to be examined to confirm its importance in LTP maintenance.

Another possibility of production of a kinase-domain protein of PKCζ has emerged. A PKCζ-related (PKCζ′) cDNA includes a short alternative sequence upstream of the region encoding PS (*64*, *65*). Although PKCζ′ cDNA was previously assumed to be derived from a splicing intermediate of PKCζ mRNA, the alternative sequence of rat PKCζ′ cDNA presents as a single exon (exon 1′) at about 50 kb upstream of exon 5 encoding PS in the PKCζ gene, *Prkcz* (*66*). The PKCζ′ mRNA is expressed in a human prostate cancer cell line, and the sequence upstream of exon 1′ has promoter activity in the cells (*66*). From the mRNA sequence, a protein of the kinase domain with a molecular weight of 46,600 is deduced (*65*), whose size is interestingly similar to that of PKMζ. From the viewpoint of brain-specific expression, PKMζ might be a variant form of PKCζ alternatively transcribed from exon 1′ of the PKCζ gene, rather than that produced by proteolysis. Further studies are needed to clarify this point and to elucidate the molecular mechanisms underlying LTP maintenance by PKMζ.

Perspectives

What are the differences in physiological functions between PKCζ and PKCλ/ι? Although there is a report on differences in nucleocytoplasmic translocation between PKCζ and PKCλ/ι (*67*), obvious functional differences have not been revealed in *in vitro* experiments. However, clear differences may be observed *in vivo*. PKCζ-deficient mice normally develop and are apparently normal, but exhibit phenotypic features of mildly impaired maturation of B cells and a reduced number of Peyer's patches (*47*, *68*), whereas PKCλ/ι-deficient mice die at the embryonic stage (see accompanying article on PKCλ/ι by Suzuki *et al.*). Fly DaPKC (*Drosophila* aPKC) and nematode PKC-3 (aPKC of *C. elegans*) are key molecules at each stage of development, and their deficiencies cause death at the embryonic stage (*69*, *70*). On the basis of primary structures, DaPKC and PKC-3 are more related to PKCλ/ι than to PKCζ. Thus, in mammals, the developmental control must be dependent on PKCλ/ι rather than on PKCζ. What then is the main role of PKCζ? Does it only compensate the roles of PKCλ/ι? Many questions remain unanswered.

Although not discussed in this article, there are other important aspects on cellular functions of PKCζ, such as regulation of glucose-transporter vesicles (*71*). Including these aspects, many lines of evidence documenting the role of PKCζ in various cellular functions are accumulating. We can approximately account for the diverse physiological functions of PKCζ at the cellular and biological levels independently. However, it is largely unclear how the functions of PKCζ at the cellular level contribute to the maintenance of homeostasis *in vivo*. Therefore, future studies should be aimed at integration of functions of PKCζ *in vitro* and *in vivo*.

REFERENCES

1. Ono, Y., Fujii, T., Ogita, K., Kikkawa, U., Igarashi, K., and Nishizuka, Y. (1989) Protein kinase C-ζ subspecies from brain: its structure, expression and properties. *Proc. Natl. Acad. Sci. USA* **86**, 3099–3103
2. Selbie, L.A., Schmitz-Peiffer, C., Sheng, Y., and Biden, T.J. (1993) Molecular cloning and characterization of PKCι, an atypical isoform of protein kinase C derived from insulin-secreting cells. *J. Biol. Chem.* **268**, 24296–24302
3. Akimoto, K., Mizuno, K., Osada, S., Hirai, S., Tanuma, S., Suzuki, K., and Ohno, S. (1994) A new member of the third class in the protein kinase C family , PKCλ, expressed dominantly in an undifferentiated mouse embryonal carcinoma cell line and also in many tissues and cells. *J. Biol. Chem.* **269**, 12677–12683
4. Ohno, S. and Nishizuka, Y. (2002) Protein kinase C isotypes and their specific functions: prologue. *J. Biochem.* **132**, 509–511
5. Ponting, C.P., Ito, T., Moscat, J., Diaz-Meco, M.T., Inagaki, F., and Sumimoto, H. (2002) OPR, PC and AID: all in the PB1 family. *Trends Biochem. Sci.* **27**, 10
6. Ways, D.K., Cook, P.P., Webster, C., and Parker, P.J. (1992) Effect of phorbol esters on protein kinase C-ζ. *J. Biol. Chem.* **267**, 4799–4805
7. Newton, A.C. (2001) Protein kinase C: structural and spatial regulation by phosphorylation, cofactors, and macromolecular interactions. *Chem. Rev.* **101**, 2353–2364
8. Nakanishi, H., Brewer, K.A., and Exton, J.H. (1993) Activation of the ζ isozyme of protein kinase C by phosphatidylinositol 3, 4, 5-trisphosphate. *J. Biol. Chem.* **268**, 13–16
9. Limatola, C., Schaap, D., Moolenaar, W.H., and van Blitterswijk, W.J. (1994) Phosphatidic acid activation of protein kinase C-ζ overexpressed in COS cells: comparison with other protein kinase C isotypes and other acidic lipids. *Biochem. J.* **304**, 1001–1008
10. Muller, G., Ayoub, M., Storz, P., Rennecke, J., Fabbro, D., and Pfizenmaier, K. (1995) PKCζ is a molecular switch in signal transduction of TNF-α, bifunctionally regulated by ceramide and arachidonic acid. *EMBO J.* **14**, 1961–1969
11. Akimoto, K., Takahashi, R., Moriya, S., Nishioka, N., Takayanagi, J., Kimura, K., Fukui, Y., Osada, S., Mizuno, K., Hirai, S., Kazlauskas, A., and Ohno, S. (1996) EGF or PDGF receptors activate atypical PKCλ through phosphatidylinositol 3-kinase. *EMBO J.* **15**, 788–798

12. Standaert, M.L., Galloway, L., Karnam, P., Bandyopadhyay, G., Moscat, J., and Farese, R.V. (1997) Protein kinase C-ζ as a downstream effector of phosphatidylinositol 3-kinase during insulin stimulation in rat adipocytes. Potential role in glucose transport. *J. Biol. Chem.* **272**, 30075–30082
13. Harrera-Velit, P., Knutson, K.L., and Reiner, N.E. (1997) Phosphatidylinositol 3-kinase-dependent activation of protein kinase C-ζ in bacterial lipopolysaccharide-treated human monocytes. *J. Biol. Chem.* **272**, 16445–16452
14. Stokoe, D., Stephens, L.R., Copeland, T., Gaffney, P.R., Reese, C.B., Painter, G.F., Holmes, A.B., McCormick, F., and Hawkins, P.T. (1997) Dual role of phosphatidylinositol-3, 4, 5-trisphosphate in the activation of protein kinase B. *Science* **277**, 567–570
15. Parker, P.J. and Parkinson, S.J. (2001) AGC protein kinase phosphorylation and protein kinase C. *Biochem. Soc. Trans*. **29**, 860–863
16. Parekh, D.B., Ziegler, W., and Parker, P.J. (2000) Multiple pathways control protein kinase C phosphorylation. *EMBO J.* **19**, 496–503
17. Chou, M.M., Hou, W., Johnson, J., Graham, L.K., Lee, M.H., Chen, C.-S., Newton, A.C., Schaffhausen, B.S., and Toker, A. (1998) Regulation of protein kinase C ζ by PI 3-kinase and PDK-1. *Curr. Biol.* **8**, 1069–1077
18. Le Good, J.A., Ziegler, W.H., Parekh, D.B., Alessi, D.R., Cohen, P., and Parker, P.J. (1998) Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. *Science* **281**, 2042–2045
19. Balendran, A., Hare, G.R., Kieloch, A., Williams, M.R., and Alessi, D.R. (2000) Further evidence that 3-phosphoinositide-dependent protein kinase-1 (PDK1) is required for the stability and phosphorylation of protein kinase C (PKC) isoforms. *FEBS Lett*. **484**, 217–223
20. Standaert, M.L., Bandyopadhyay, G., Perez, L., Price, D., Galloway, L., Poklepovic, A., Sajan, M.P., Cenni, V., Sirri, A., Moscat, J., Toker, A., and Farese, R.V. (1999) Insulin activates protein kinases C-ζ and C-λ by an autophosphorylation-dependent mechanism and stimulates their translocation to GLUT4 vesicles and other membrane fractions in rat adipocytes. *J. Biol. Chem.* **274**, 25308–25316
21. Bornancin, F. and Parker P.J. (1996) Phosphorylation of threonine 638 critically controls the dephosphorylation and inactivation of protein kinase Cα. *Curr. Biol.* **6**, 1114–1123
22. Edwards, A.S., Faux, M.C., Scott, J.D., and Newton, A.C. (1999) Carboxyl-terminal phosphorylation regulates the function and subcellular localization of protein kinase C βII. *J. Biol. Chem.* **274**, 6461–6468
23. Standaert, M.L., Bandyopadhyay, G., Kanoh, Y., Sajan, M.P., and Farese, R.V. (2001) Insulin and PIP_3 activate PKC-ζ by mechanisms that are both dependent and independent of phosphorylation of activation loop (T410) and autophosphorylation (T560) sites. *Biochemistry* **40**, 249–255
24. Diaz-Meco, M.T., Municio, M.M., Frutos, S., Sanchez, P., Lozano, J., Sanz, L., and Moscat, J. (1996) The product of *par-4*, a gene induced during apoptosis, interacts selectively with the atypical isoforms of protein kinase C. *Cell* **86**, 777–786
25. Lin, D., Edwards, A.S., Fawcett, J.P., Mbamalu, G., Scott, J.D., and Pawson, T. (2000) A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signaling and cell polarity. *Nat. Cell Biol.* **2**, 540–547
26. Kotani, K., Ogawa, W., Hashiramoto, M., Onishi, T., Ohno, S., and Kasuga, M. (2000) Inhibition of insulin-induced glucose uptake by atypical protein kinase C isotype-specific interacting protein in 3T3-L1 adipocytes. *J. Biol. Chem.* **275**, 26390–26395
27. Joberty, G., Petersen, C., Gao, L., and Macara, I.G. (2000) The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nat. Cell Biol.* **2**, 531–539
28. Yamanaka, T., Horikoshi, Y., Suzuki, A., Sugiyama, Y., Kitamura, K., Maniwa, R., Nagai, Y., Yamashita, A., Hirose, T., Ishikawa, H., and Ohno, S. (2001) PAR-6 regulates aPKC activity in a novel way and mediates cell-cell contact-induced formation of the epithelial junctional complex. *Genes Cells* **6**, 721–731
29. Berra, E., Diaz-Meco, M.T., Lozano, J., Frutos, S., Municio, M.M., Sanchez, P., Sanz, L., and Moscat, J. (1995) Evidence for a role of MEK and MAPK during signal transduction by protein kinase C ζ. *EMBO J.* **14**, 6157–6163
30. Fernandez, N., Caloca, M.J., Prendergast, G.V., Meinkoth, J.L., and Kazanietz, M.G. (2000) Atypical protein kinase C-ζ stimulates thyrotropin-independent proliferation in rat thyroid cells. *Endocrinology* **141**, 146–152
31. Schönwasser, D.C., Marais, R.M., Marshall, C.J., and Parker, P.J. (1998) Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. *Mol. Cell. Biol.* **18**, 790–798
32. Monick, M.M., Carter, A.B., Flaherty, D.M., Peterson, M.W., and Hunninghake, G.W. (2000) Protein kinase C ζ plays a central role in activation of the p42/44 mitogen-activated protein kinase by endotoxin in alveolar macrophages. *J. Immunol.* **165**, 4632–4639
33. Diaz-Meco, M.T. and Moscat, J. (2001) MEK5, a new target of the atypical protein kinase C isoforms in mitogenic signaling. *Mol. Cell. Biol.* **21**, 1218–1227
34. Hodgkinson, C.P., Sale, E.M., and Sale, G.J. (2002) Characterization of PDK2 activity against protein kinase B γ. *Biochemistry* **41**, 10351–10359
35. Sanz, L., Sanchez, P., Lallena, M.-J., Diaz-Meco, M.T., and Moscat, J. (1999) The interaction of p62 with RIP links the atypical PKCs to NF-κB activation. *EMBO J.* **18**, 3044–3053
36. Sanz, L., Diaz-Meco, M.T., Nakano, H., and Moscat, J. (2000) The atypical PKC-interacting protein p62 channels NF-κB activation by the IL-1-TRAF6 pathway. *EMBO J.* **19**, 1576–1586
37. Puls, A., Schmidt, S., Grawe, F., and Stabel, S. (1997) Interaction of protein kinase Cζ with ZIP, a novel protein kinase C-binding protein. *Proc. Natl. Acad. Sci. USA* **94**, 6191–6196
38. Sanchez, P., De Carcer, G., Sandoval, I.V., Moscat, J., and Diaz-Meco, M.T. (1998) Localization of atypical protein kinase C isoforms into lysosome-targeted endosomes through interaction with p62. *Mol. Cell. Biol.* **18**, 3069–3080
39. Geetha, T. and Wooten, M.W. (2002) Structure and functional properties of the ubiquitin binding protein p62. *FEBS Lett.* **512** 19–24
40. Joung, I., Strominger, J.L., and Shin, J. (1996) Molecular cloning of a phosphotyrosine-independent ligand of the $p56^{lck}$ SH2 domain. *Proc. Natl. Acad. Sci. USA* **93**, 5991–5995
41. Gong, J., Xu, J., Bezanilla, M., van Huizen, R., Derin, R., and Li, M. (1999) Differential stimulation of PKC phosphorylation of potassium channels by ZIP1 and ZIP2. *Science* **285**, 1565–1569
42. Cariou, B., Perdereau, D., Cailliau, K., Browaeys-Poly, E., Bériziat, V., Vasseur-Cognet, M., Girard, J., and Burnol, A.-F. (2002) The adapter protein ZIP binds Grb14 and regulates its inhibitory action on insulin signaling by recruiting protein kinase Cζ. *Mol. Cell. Biol.* **22**, 6959–6970
43. Wooten, M.W., Seibenhener, M.L., Mamidipudi, V., Diaz-Meco, M.T., Barker, P.A., and Moscat, J. (2001) The atypical protein kinase C-interacting protein p62 is a scaffold for NF-κB activation by nerve growth factor. *J. Biol. Chem.* **276**, 7709–7712
44. Chang, S., Kim, J.H., and Shin J. (2002) p62 forms a ternary complex with PKCζ and PAR-4 and antagonizes PAR-4-induced PKCζ inhibition. *FEBS Lett.* **510**, 57–61
45. Karin, M. (1999) The beginning of the end: IκB kinase (IKK) and NF-κB activation. *J. Biol. Chem.* **274**, 27339–27342
46. Lallena, M.-J., Diaz-Meco, M.T., Bren, G., Payá, C.V., and Moscat, J. (1999) Activation of IκB kinase β by protein kinase C isoforms. *Mol. Cell. Biol.* **19**, 2180–2188
47. Leitges, M., Sanz, L., Martin, P., Duran, A., Braun, U., Garcia, J.F., Camacho, F., Diaz-Meco, M.T., Rennert, P.D., and Moscat J. (2001) Targeted disruption of the ζPKC gene results in the impairment of the NF-κB pathway. *Mol. Cell* **8**, 771–780

48. Akimoto, K., Nakaya, M., Yamanaka, T., Tanaka, J., Matsuda, S., Weng, Q.P., Avruch, J., and Ohno, S. (1998) Atypical protein kinase Cλ binds and regulates p70 S6 kinase. *Biochem. J.* **335**, 417–424
49. Romanelli, A., Martin, K.A., Toker, A., and Blenis, J. (1999) p70 S6 kinase is regulated by protein kinase Cζ and participates in a phosphoinositide 3-kinase-regulated signaling complex. *Mol. Cell. Biol.* **19**, 2921–2928
50. Romanelli, A., Dreisbachn, V.C., and Blenis, J. (2002) Characterization of phosphatidylinositol 3-kinase-dependent phosphorylation of the hydrophobic motif site Thr^{389} in p70 S6 kinase 1. *J. Biol. Chem.* **277**, 40281–40289
51. Ohno, S. (2001) Intercellular junctions and cellular polarity: the PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. *Curr. Opin. Cell Biol.* **13**, 641–648
52. Suzuki, A., Yamanaka, T., Hirose, T., Manabe, N., Mizuno, K., Shimizu, M., Akimoto, K., Izumi, Y., Ohnishi, T., and Ohno, S. (2001) Atypical protein kinase C is involved in the evolutionarily conserved PAR protein complex and plays a critical role in establishing epithelia-specific junctional structures. *J. Cell Biol.* **152**, 1183–1196
53. Gao, L., Joberty, G., and Macara, I.G. (2002) Assembly of epithelial tight junctions is negatively regulated by Par6. *Curr. Biol.* **12**, 221–225
54. Etienne-Manneville, S. and Hall, A. (2001) Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCζ. *Cell* **106**, 489–498
55. Kuroda, S., Nakagawa, N., Tokunaga, C., Tatematsu, K., and Tanizawa, K. (1999) Mammalian homologue of the *Caenorhabditis elegans* UNC-76 protein involved in axonal outgrowth is a protein kinase C ζ-interacting protein. *J. Cell Biol.* **144**, 403–411
56. Bloom, L. and Horvitz, H.R. (1997) The *Caenorhabditis elegans* gene *unc-76* and its human homologs define a new gene family involved in axonal outgrowth and fasciculation. *Proc. Natl. Acad. Sci. USA* **94**, 3414–3419
57. Konno, D., Ko, J.-A., Usui, S., Hori, K., Maruoka, H., Inui, M., Fujikado, T., Tano, Y., Suzuki, T., Tohyama, K., and Sobue, K. (2002) The postsynaptic density and dendritic raft localization of PSD-Zip70, which contains an N-myristoylation sequence and leucine-zipper motifs. *J. Cell Sci.* **115**, 4695–4706
58. Ishii, H., Baffa, R., Numata, S., Murakumo, Y., Rattan, S., Inoue, H., Mori, M., Fidanza, V., Alder, H., and Croce, C.M. (1999) The *FEZ1* gene at chromosome 8p22 encodes a leucine-zipper protein, and its expression is altered in multiple human tumors. *Proc. Natl. Acad. Sci. USA.* **96**, 3928–3933
59. Ishii, H., Vecchione, A., Murakumo, Y., Baldassarre, G., Numata, S., Trapasso, F., Alder, H., Baffa, R., and Croce, C.M. (2001) *FEZ1/LZTS1* gene at 8p22 suppresses cancer cell growth and regulates mitosis. *Proc. Natl. Acad. Sci. USA* **98**, 10374–10379
60. Sacktor, T.C., Osten, P., Valsamis, H., Jiang, X., Naik, M.U., and Sublette, E. (1993) Persistent activation of the ζ isoform of protein kinase C in the maintenance of long-term potentiation. *Proc. Natl. Acad. Sci. USA* **90**, 8342–8346
61. Hrabetova, S. and Sacktor, T.C. (1996) Bidirectional regulation of protein kinase Mζ in the maintenance of long-term potentiation and long-term depression. *J. Neurosci.* **16**, 5324–5333
62. Ling, D.S.F., Benardo, L.S., Serrano, P.A., Blace, N., Kelly, M.T., Crary, J.F., and Sacktor, T.C. (2002) Protein kinase Mζ is necessary and sufficient for LTP maintenance. *Nat. Neurosci.* **5**, 295–296
63. Drier, E.A., Tello, M.K., Cowan, M., Wu, P., Blace, N., Sacktor, T.C., and Yin, J.C.P. (2002) Memory enhancement and formation by atypical PKM activity in *Drosophila melanogaster*. *Nat. Neurosci.* **5**, 316–324
64. Ono, Y., Fujii, T., Ogita, K., Kikkawa, U., Igarashi, K., and Nishizuka, Y. (1988) The structure, expression, and properties of additional members of the protein kinase C family. *J. Biol. Chem.* **263**, 6927–6932
65. Powell, C.T., Fair, W.R., and Heston, W.D.W. (1994) Differential expression of protein kinase C isozyme messenger RNAs in dunning R-3327 rat prostatic tumors. *Cell Growth Differ.* **5**, 143–149
66. Marshall, B.S., Price, G., and Powell, C.T. (2000) Rat protein kinase C zeta gene contains alternative promoters for generation of dual transcripts with 5′-end heterogeneity. *DNA Cell Biol.* **19**, 707–719
67. Perander, M., Bjørkøy, G., and Johansen, T. (2001) Nuclear import and export signals enable rapid nucleocytoplasmic shuttling of the atypical protein kinase C λ. *J. Biol. Chem.* **276**, 13015–13024
68. Martin, P., Duran, A., Minguet, S., Gaspar, M.L., Diaz-Meco, M.T., Rennert, P., Leitges, M., and Moscat, J. (2002) Role of ζPKC in B-cell signaling and function. *EMBO J.* **21**, 4049–4057
69. Tabuse, Y., Izumi, Y., Piano, F., Kemphues, K.J., Miwa, J., and Ohno, S. (1998) Atypical protein kinase C co-operates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. *Development* **125**, 3607–3614
70. Wodarz, A., Ramrath, A., Grimm, A., and Knust, E. (2000) *Drosophila* atypical protein kinase C associates with bazooka and controls polarity of epithelia and neuroblasts. *J. Cell Biol.* **150**, 1361–1374
71. Farese, R.V. (2002) Function and dysfunction of aPKC isoforms for glucose transport in insulin-sensitive and insulin-resistant states. *Am. J. Physiol. Endocrinol. Metab.* **283**, E1–E11