# Protein Kinase Cη (PKCη): Its Involvement in Keratinocyte Differentiation

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**The η isoform of protein kinase C (PKCη) is classified into the $Ca^{2+}$-independent novel PKC subfamily and assigned to human chromosome 14 (14q22-23) and mouse chromosome 12 (12C3-D2). It is highly expressed in epithelial tissues especially in squamous epithelia. PKCη is unique in that it is specifically activated by cholesterol sulfate and sulfatide, sulfated metabolites of cholesterol and cerebroside, respectively. PKCη overexpression induces G1 arrest and differentiation in keratinocytes. PKCη-induced differentiation is accompanied by the transcriptional activation of transglutaminase I, a key enzyme in squamous differentiation, and involucrin, a precursor of cornified envelopes. In keratinocytes, PKCη associates with the cyclin E/cdk2/p21 complex and inhibits the cdk2-kinase activity, leading to G1 arrest. Cholesterol sulfate inhibits the promotional phase of skin carcinogenesis. Moreover, PKCη-knockout mice show a much higher sensitivity to carcinogenesis, suggesting that PKCη is negatively involved in tumor promotion through stimulation of keratinocyte differentiation. In addition to epithelial cells, recent studies revealed that PKCη acts as a key regulator in early B-cell development. Although the functions of PKCη in other cell types are not yet fully elucidated, available evidence indicates that this particular isoform plays crucial roles in the signaling of cell differentiation in a cell-type-specific manner.**

**Key words: B-lymphocytes, cell cycle control, cell differentiation, cholesterol sulfate, squamous epithelia.**

Protein kinase C (PKC) is known as a key enzyme in signal transduction, which is involved in the regulation of numerous cellular functions (*1, 2*). PKC isoforms comprise a family of serine/threonine kinases the activity of which is typically initiated by lipid second messengers on the plasma membrane. The phosphorylation of PKC molecules by upstream kinases contributes to the fully active state of PKCs (*3*). To elucidate the physiological functions of PKCs, it is essential to study the individual features of each PKC isoform, such as expression, post-translational modification, substrate specificity, subcellular localization and cross talk with other signaling pathways.

The η isoform of PKC (PKCη) was isolated from a cDNA library of mouse skin in 1990 (*4*) and assigned to human chromosome 14 (14q22–23) and mouse chromosome 12 (12C3–D2) (*5, 6*). It contains an open reading frame encoding 683 amino acid residues. PKCη corresponds to PKC98E in *Drosophila*, APLII/PKCII in *Aplysia californica* and PKC-1 in *Caenorhabditis elegans* (*7, 8*). It is classified into the $Ca^{2+}$-independent novel PKC subfamily and has the highest sequence similarity to PKCε. Similar to PKCδ, PKCε, and PKCθ, it has a zinc-finger-like cysteine-rich sequence (C1 region), an ATP-binding lobe (C3), and a substrate-binding lobe (C4) but lacks a putative $Ca^{2+}$-binding region (C2) (*4, 9*). This article deals with cellular functions of PKCη, highlighting its involvement in keratinocyte differentiation.

## Expression

**Tissue distribution.** PKCη can be cited as a good example of a protein with a tissue-specific expression. It is predominantly expressed in squamous epithelia or epithelia from which squamous cell carcinomas arise. These include skin, tongue, esophagus, forestomach, trachea and bronchus. Of particular interest is that PKCη is expressed in close association with the differentiation of squamous epithelia. It is stained predominantly in differentiating or differentiated suprabasal layers (*10*). In mouse primary keratinocytes, the expression level of PKCη increases with $Ca^{2+}$-induced differentiation (*11*). In squamous cell carcinoma, PKCη is stained in keratotic cells around horny pearls, whereas basal cell epithelioma is not stained. No expression of PKCη is detected in mesenchymal cells at the mRNA or protein level (*4, 10*). These data imply that PKCη plays an essential role(s) in the differentiation of squamous epithelia.

**Subcellular localization of PKCη.** In primary human keratinocytes, PKCη is located in the cytoplasm particularly in the perinuclear region, but not in the nucleus. Immunoelectron microscopy revealed that PKCη is located on the rough endoplasmic reticulum and on the outer nuclear membrane, which is continuous with the endoplasmic reticulum membrane (*12*).

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### Promoter and enhancer sequences

The 5′-flanking sequence of the PKCη gene contains regulatory elements sufficient to regulate cell-type-specific transcription; the PKCη gene reporter construct displays significant activity in primary human keratinocytes and HaCaT human keratinocyte cell line but is inactive in human skin fibroblasts, consistent with endogenous PKCη-mRNA expression in these cells (*5*). The proximal promoter contains several potential Sp1 and Ets motifs. In conjunction with other transcription factors, Sp1 and Ets contribute to the transcription of several differentiation-related genes in keratinocytes, *i.e.*, SPRR2A and involucrin genes. Sp1 and Ets members may coordinately regulate PKCη expression.

### PKCη activation

Similar to other members of the novel PKC family, the activity of PKCη primarily depends on two membrane lipids, *i.e.*, phosphatidylserine (PS) and diacylglycerol (DAG), the latter of which can be substituted by phorbol ester, a tumor promoter, *e.g.*, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (*13*). When activated, PKC is translocated from the cytoplasm to the plasma membrane, followed by its down regulation. However, PKCη is unique in that it is not translocated to the plasma membrane upon TPA stimulation, or down regulated in keratinocytes (*14*). Our recent study demonstrated that the C-terminal V5 region is critical for PKCη subcellular distribution when stimulated by TPA (unpublished data).

The skin epidermis contains several unique lipids that may function as a physical barrier as well as a component of tissue architecture (*15*). Among these skin-specific lipids, we demonstrated that cholesterol sulfate (CS) and sulfatide activate PKCη, while their unsulfated parental lipids, cholesterol and cerebroside, fail to activate it. We found that CS activates PKCη only when assayed with the enzyme partially purified from cells or tissues, although other isoforms are also activated when recombinant PKC preparations are used (*13, 16, 17*). CS is formed in the upper layers of the epidermis *in vivo* and is formed during squamous differentiation in cultures of bronchial, tracheal, and epidermal keratinocytes (*18, 19*). CS induces the differentiation of mouse keratinocytes *in vivo* and *in vitro* and activates the transcription of transglutaminase I (TGase I), a key enzyme in the formation of cornified envelop, in human keratinocytes (*20*). TGase I catalyzes the ε-(γ-glutamyl) lysine cross-linking reaction of proteins to form cornified cell envelopes on the inner surface of the differentiated keratinocytes. These lines of evidence indicate the possibility that CS acts as a second messenger in PKCη activation and subsequent induction of squamous differentiation.

### Induction of squamous differentiation

A series of studies conducted by our laboratory demonstrated that PKCη overexpression causes terminal differentiation of normal human keratinocytes. When PKC genes are introduced by an adenovirus vector, differentiation is induced by PKCη and PKCγ, not by the other PKC. Induction of the differentiation is accompanied by growth arrest. These effects are cell-type specific, being observed in keratinocytes but not in fibroblasts (*21*).

At the onset of keratinocyte differentiation, multiple tyrosine kinase activities are induced in response to calcium and TPA. One of these activities was identified as Fyn, a tyrosine kinase of the Src family. Cabodi *et al.* reported that PKCη is a direct upstream activator of Fyn; PKCη associates with, and activates Fyn, leading to keratinocyte growth arrest and differentiation (Fig. 1) (*22*). These findings reveal a direct cross talk between PKCη and Fyn, which presides over the decision of whether to activate keratinocyte cell growth or differentiation.

The differentiation process of keratinocytes proceeds

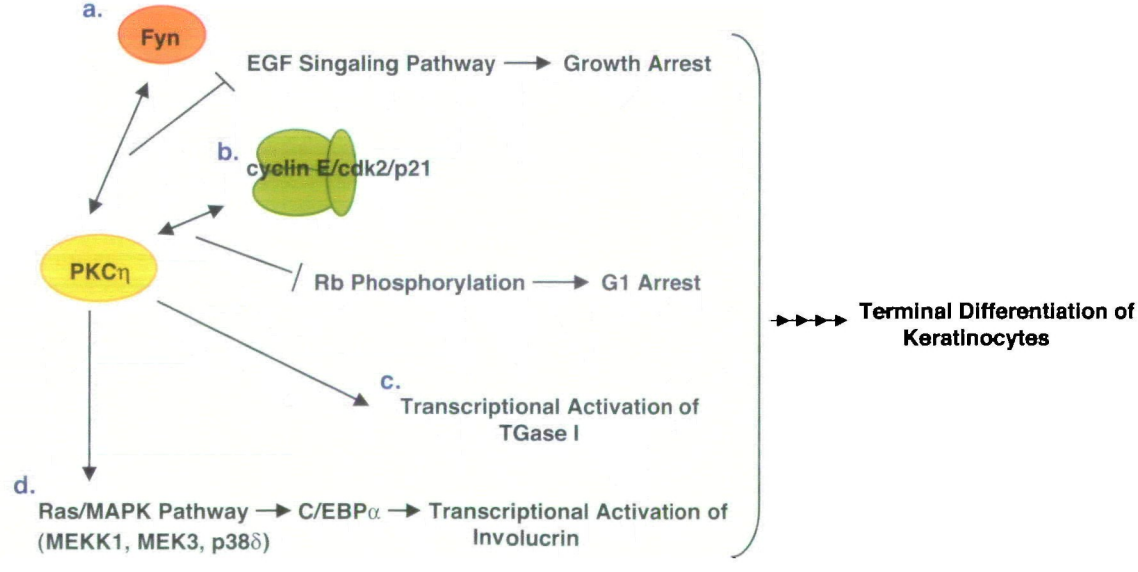


Fig. 1. **Cross talk of PKCη-pathway with related signaling pathways in differentiation of keratinocytes.** a: PKCη associates and activates Fyn that negatively regulates the EGF signaling pathway, leading to keratinocyte growth arrest. b: PKCη exists in the cyclin E/cdk2/p21 complex and inhibits cdk2 kinase activity, leading to G1 arrest. c: PKCη activates TGase I at the transcriptional level, leading to differentiation through the formation of cornified envelopes. d: PKCη induces the expression of C/EBPα and DNA binding to the involucrin promoter through a cascade including Ras, MEKK, MEK3, and p38δ, leading to differentiation through involucrin gene expression.

with the ordered expression of the related genes. PKCη-induced terminal differentiaton is accompanied by the transcriptional activation of TGase I and involucrin, a precursor of cornified envelopes (Fig. 1) (*21, 23, 24*). The activation pathway of the involucrin gene by PKCη appears to operate through a cascade including Ras, MEKK, and MEK3. PKCη inhibits ERK1/2 expression and activates p38δ *via* the Ras, MEKK1, MEK3 pathway. These changes in MAPK activity are associated with the increased expression of the C/EBPα transcription factor and DNA binding to the involucrin promoter, leading to involucrin gene expression (Fig. 1) (*24, 25*).

The induction of differentiation by PKCη is also demonstrated using an organ-culture system of a developing-mouse skin, in which the skin tissue isolated from 12.5- or 13.5-d postcoitus embryos develop in a manner that is histologically and temporally similar to the process *in vivo*. When infected with PKCη-adenovirus, thicker cornified and granular layers are observed in comparison with the lacZ-infected control (*26*).

The essential role of PKCη in squamous differentiation is further demonstrated in transgenic mice, in which PKCη is transduced under the control of the involucrin promoter in the suprabasal layer of squamous epithelia. The mice exhibit hyperdifferentiation in squamous epithelia, *i.e.*, skin, esophagus and cornea. The hyperdifferentiation is accompanied by stimulation of epithelial growth and aberrant expression of keratin genes (unpublished data).

### Induction of G1 arrest

During the process of terminal differentiation, the precise coordination between cell cycle arrest and differentiation is required. However, molecular and cellular mechanisms by which cell cycle is negatively regulated in terminally differentiating cells have not been elucidated so far. Progression of cell cycle is controlled by the activation of a series of cyclin-dependent kinases (CDKs). In addition to positive regulation by cyclins, CDK activity is regulated by phosphorylation or dephosphorylation at specific residues, as well as by association with a number of inhibitory proteins.

There are a number of studies demonstrating that PKCs are involved in the progression of cell cycle in cell-type- and isoform-specific manner. As for PKCη, G1 arrest is induced in keratinocytes, but not in fibroblasts (*21*). PKCη is highly expressed in cells at the G1/S boundary in synchronously growing BALB/MK2 mouse keratinocytes (*27*). The molecular mechanism by which PKCη induces G1 arrest was demonstrated in our recent study. PKCη associates with the cyclin E/cdk2/p21 complex in the cytoplasm of normal human keratinocytes. This association results in the inhibition of cdk2 kinase activity, leading to the dephosphorylation of Rb proteins and thereby G1 arrest (Fig. 1). Under these conditions, T160 of cdk2, which is necessary for cdk2 activation, is dephosphorylated (*28*).

PKCη associates with the cyclin E/cdk2/p21 complex through its catalytic domain. This association is isoform-specific: PKCη associates with the cyclin E/cdk2/p21 complex to a lesser extent than PKCη, but PKCα, PKCε, and PKCζ do not (Table I). The kinase-defective mutant of PKCη also associates with cdk2 complex, but slightly inhibits cdk2 kinase activity. An *in vitro* reconstitution assay suggests the possible requirement of an endogenous factor(s). In the PKCη-cyclin E/cdk2/p21 complex, p21 is identified as a substrate of PKCη (Table I) (*28*). Our on going experiments indicate that S146 of the p21 protein is the site of phosphorylation by PKCη.

A series of evidence provide new insights into the regulatory mechanisms of cell cycle progression and the physiological functions of PKC. Further studies are needed to identify of possible adaptor proteins and the role(s) of phosphorylated p21 in the regulation of keratinocyte growth and differentiation.

### Implication in carcinogenesis

Carcinogenesis in skin and other epithelia can be divided into three distinct stages termed initiation, tumor promotion and tumor progression. The best-known and most potent tumor promoter is TPA, which activates PKC most strongly in replace of DG, a physiological activator of PKC. However, it is not well established which of the 10 PKC isoforms is involved in tumor promotion.

Two lines of evidence suggest that PKCη is negatively involved in tumor promotion. We demonstrated that CS, an activator of PKCη, inhibits skin tumor formation when applied prior to application of tumor promoters (*29*). Chida established PKCη-null mice that show much higher sensitivity to tumor promotion than wild type mice (unpublished data). These data suggest the possibility that PKCη inhibits this process by inducing terminal differentiation of keratinocytes, consistent with our differentiation studies.

### Functions of PKCη in B cells and other cell systems

In addition to epithelial cells, recent studies revealed that PKCη acts as a key regulator in early B-cell development. PKCη mRNA is expressed at high levels in purified pro-B cells and early-stage thymocytes, while at low levels in purified pre-B cells and mature B cells. In apoptotic lymphocytes, PKCη is proteolyzed by caspase-3, generating a kinase-active fragment, which in turn induces apoptosis when expressed in a pro-B cell line (*30*). That report revealed that the expression and proteolytic activation of PKCη play an important role in the regulation of cell division and cell death during early B-cell development.

PKCη was also reported to be involved in triggering secretory events in oocytes and mast cells aside from epi-

TABLE I. **PKCη interacting proteins.**

| Name | Function | Interaction | Interacting domain | | Substrate | Other PKCs | Reference |
|---|---|---|---|---|---|---|---|
| | | | in binding proteins | in PKCη | | | |
| Fyn | Tyrosine kinase | Direct | ? | ? | Yes | PKCθ | *22* |
| cyclin E/cdk2/p21 complex | Serine/Threonine kinase | Indirect | ? | Catalytic region | Yes (p21; S146) | PKCδ | *28* |
| PKCμ/PKD | Serine/Threonine kinase | Direct | Preckstrin homology (PH) domain | ? | Yes (S738/742) | PKCε | *34* |

thelial cells and B cells (*31, 32*).

Although the functions of PKCη in other cell types are not yet fully elucidated, available evidence indicates that this particular isoform plays a crucial role in the signaling of distinct cell functions in a cell-type-specific manner.

## Interaction with PKCμ/PKD

PKCμ/PKD is a subgroup of PKC-related kinases and contains a pleckstrin homology (PH) domain, but lacks a pseudosubstrate site. PKCμ/PKD is highly expressed in thymus and hematopoietic cells, suggesting its potential role in immune functions (*33*).

Waldron *et al.* demonstrated that PKCμ/PKD binds to PKCη selectively through its PH domain, while it forms complexes only very weakly with PKCε, but not with PKCζ (Table I) (*34*). PKCη acts as a direct activator for PKCμ/PKD to trigger downstream cellular processes (*35*).

## Conclusion

Although much has been gleaned from studies on PKCη, we are still in the early stages of understanding how it contributes to cellular functions. Recent studies suggest that PKCη participates in various signal transduction cascades by cross talk with complex signal-transduction networks, such as the Ras/MAPK pathway, tyrosine kinase pathways, PKCμ/PKD pathway, and cyclin/cdk pathways. In seeking deeper insight into the biological functions of PKCη, it will be important to fully understand the regulatory inputs and the downstream events that occur as a consequence of PKCη activation, and to identify target proteins that are directly or indirectly regulated *in vivo*.

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