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THE PROTEIN KINASE C FAMILY FOR THE REGULATION OF CELLULAR FUNCTIONS

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ABSTRACT

• *The physiological importance of protein kinase C (PKC) activation is widely appreciated and well documented. It is now clear that there is more than one species of PKC molecule, and se^>eral discrete subspecies have been defined. These proteins are derived from both multiple genes and from alternative splicing of a single mRNA transcript, yet possess a primary structure containing conserved structural motifs with a high degree of sequence homology. In mammalian tissues at least seven subspecies can be distinguished, one of which is expressed only in the central nen'ous tissues. Biochemical and immunocytochemical studies have revealed that these PKC subspecies are differently located in particular cell types, and at limited intracellular locations.' The enzyme subspecies purified from tissue show subtle differences in their mode of activation, sensitivity to* Ca^{2+}*, and catalytic activity. It is worth noting that unsaturated free fatty acids including arachidonic, oleic, and linoleic acids dramatically activate several members of the PKC family in the presence of diacylglycerol at the basal level of* Ca^{2+}*. It is possible that activation of the enzyme is an integral part of the signal-induced degradation cascade of various membrane phospholipids catalyzed by phospholipases C, A j and perhaps D as well. Evidence now accumulates that PKC plays pivotal roles in controlofa number of membrane functions, such as exocytosis, release reactions, and ion channel conductivity, as well as in cross-talks of various cell-signalling systems. It is also clear that PKC plays roles of crucial importance for regulation of gene expression and cell growth.*

INOSITOL PHOSPHOLIPID FOR SIGNAL TRANSDUCTION

The receptor-mediated hydrolysis of inositol phospholipids is now generally accepted to be a common mechanism for transducing various extracellular signals into the cell, such as those from a group of hormones, neurotransmitters, antigens, some growth factors, and many other biologically active substances. In as early as 1953, the response of inositol phospholipids to the stimulation of cell surface receptors was recognized by Hokin and Hokin (1), who first showed that, in some excretory tissues such as pancreas, acetylcholine induces a rapid incorporation of ^{32}P into phosphatidylinositol and phosphatidic acid. It soon became evident that this incorporation results from the enhanced breakdown and resynthesis of inositol phospholipids occuring in many stimulated cells. Michell (2) subsequently postulated that the phospholipid breakdown might be related to Ca^{2+} gate opening. In 1983, Berridge and his coworkers (3) demonstrated that inositol 1,4,5-trisphosphate (IP_3), one of the earliest products of phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis, serves as a mediator of Ca^{2+} mobilization from its internal store, probably in a compartment of the endoplasmic reticulum.

When we discovered protein kinase C (PKC) in 1977, as a proteolytically activated protein kinase present in many tissues, the enzyme had no obvious role in signal transduction (4). Later, it was shown to be a Ca^{2+} - activated, phospholipid-dependent enzyme, and firmly linked to signal transduction by the demonstration that 1,2-diacylglycerol, the other product of the inositol phospholipid hydrolysis, is essential for the activation of the enzyme, that we call PKC presently (5). The cellular responses elicited by PKC activation are separate from and synergistic to those activated via an increase in intracellular Ca^{2+} as schematically shown in Fig. 1.

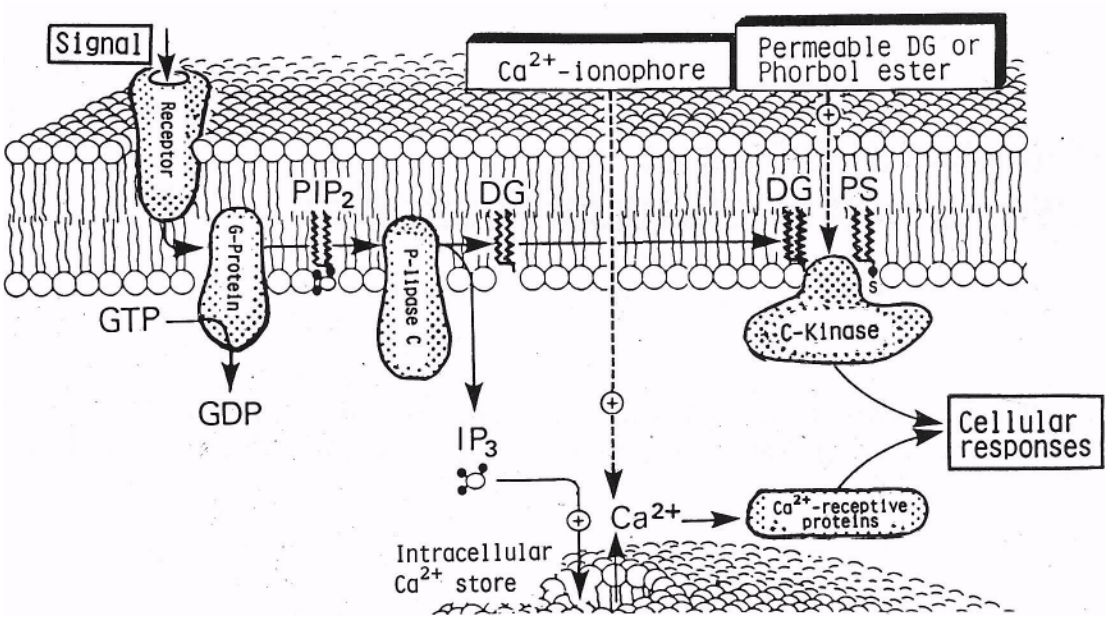

FIGURE 1. Schematic representation of signal transduction pathway initiated inositol phospholipid hydrolysis. DG, diacylglycerol; PS, phosphoatidylserine; C-kinase, protein kinase C; P-lipase C, phospholipase C.

SYHERGISTIC ACTION OF PROTEIN KINASE C AND CALCIUM ION

• Under appropriate conditions, the signal bifurcating pathway, PKC activation and Ca^{2+} mobilization, can be stimulated selectively and independently by the application of a permeable diacylglycerol for the former and a Ca^{2+} ionophore for the latter.

Diacylglycerols possessing two long fatty acyl moieties are normally insoluble, and cannot be intercalated into cell membranes. When one fatty acyl moiety is replaced by a short chain, such as an acetyl group, then the resulting diacylglycerol, for instance, l-oleoly-2-acetylglycerol, obtains detergent-like properties, and is easily dispersed into membranes to activate PKC without damage of the cell. By using this procedure the pivotal role of PKC in signal transduction was first demonstrated in the release of serotonin from platelets (6). Since then, the importance of this enzyme has been unequivocally shown for the release and exocytosis of cellular constituents from a variety of endocrine, exocrine, and neuronal tissues, as well as for the modulation of several membrane functions, cell proliferation and differentiation, as shown in Table 1

Table 1. Examples of the proposed role of protein kinase C in cellular responses.

Tissues and cells	Cellular responses
Endocrine systems	
Adrenal medulla	Catecholamine secretion
Adrenal cortex	Aldosterone secretion
	Steroidogenesis
Pancreatic islets	Insulin release
Hypothalamus	Releasing hormone release
Pituitary cells	Growth hormone release Luteinizing hormone release
	Prolactin release
	Thyrotropin release
Parathyroid cells	Parathyroid hormone release
Thyroid C cells	Calcitonin release
Leydig cells	Steroidogenesis
Exocrine systems	
Pancreas acinar cells	Amalyse secretion
Parotid gland	Amalyse and mucin secretion
Submandibular gland	Mucin secretion
Gastric gland	Pepsinogen secretion
	Gastric acid secretion
Alveolar cells	Surfactant secretion
Nervous systems	
Neuronal synapses	Transmitter release
Neuromuscular junction	Transmitter release
PC 12 cells	Dopamine release
Neurons	Membrane conductance
Muscular systems	
Cardiac uscle	Muscle contraction
Inflammation and immune systems	
Platelets	Serotonin release
	Lysosomal enzyme release
	Arachidonate release
Neutrophils	Thromboxane synthesis
	Superoxide generation
	Lysosomal enzme release
	Hexose transport
Basophils	Histamine release
Mast cells	Histamine release
Lymphocytes	T-lymphocyte activation
	B-lymphocyte activation

Tissues and cells	Cellular responses
Metabolic and other cell systems	
Adipocytes	Lipogenesis
	Glucose transport
Hepatocytes	Gluconeogenesis
	Glucose transport
Epidermal cells	Inhibition of gap junction

References are given elsewhere (Ref. 7). This table is adapted from Ref. 7.

(for a review, see Ref. 7).

During the course of these studies it was noticed that a powerful tumour promoter, 12-0-tetradecanoylphorbol-13-acetate (TPA) mimics the action of diacylglycerol and activated PKC directly as given in Fig. 2 (8). Fig. 3 shows the structure of TPA, together with the permeable diacylglycerol which we employed to activate PKC in intact cells. It is worth noting that this tumour promoter has a diacylglycerol-like structure in its molecule.

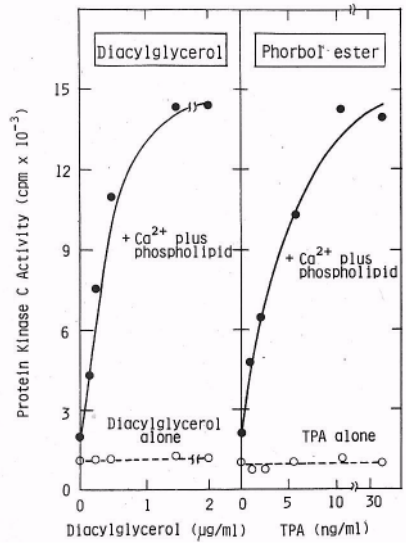

FIGURE 2. Direct activation of protein kinase C by diacylglycerol and phorbol ester. Detailed experimental conditions are described elesewhere (8).

SIGNAL ROUTES FOR PROTEIN KIHASE C ACTIVATION

• Although the hydrolysis of inositol phospholipids was initially though to be only one mechanism leading to the activation of PKC, recent studies suggest that there are several additional routes to provide the diacylglycerol that is needed for enzyme activation as shown in Fig. 4 (for a review, see Ref. 9).

For instance, phosphatidylcholine also may be hydrolized to produce diacylglycerol at a relatively later phase of cellular responses, particularly of those to long-acting signals such as some growth factors (for a review, see Ref. 10). In addition, both voltage-dependent and receptor-mediated Ca^{2+}-gate opening may cause phospholipid breakdown due to the activation of Ca^{2+}-dependent phospholipase C, and in addition probably phospholipase A^2 andphospholipase D as well. The exact biochemical basis initiating this phospholipid breakdown, however, has not been fully understood. As discussed below, unsaturated free fatty acids are also involved to activate PKC under certain conditions. It is thus possible that the signal routes leading to the activation of PKC may greatly vary with cell types, extracellular signals, and perhaps with the time after stimulation.

MOLECULAR HETEROGENEITY

• PKC is most prominent in the brain in both quantity and variation. Molecular cloning and enzymological analysis has revealed the existence of multiple subspecies of PKC. Initially, four cDNA clones that encode cc-,pl-, pTI- and y-subspecies were found. Subsequently, another group of cDNA clones, encoding at least three further subspecies having 5-, e- and ^-sequence, have been isolated. These

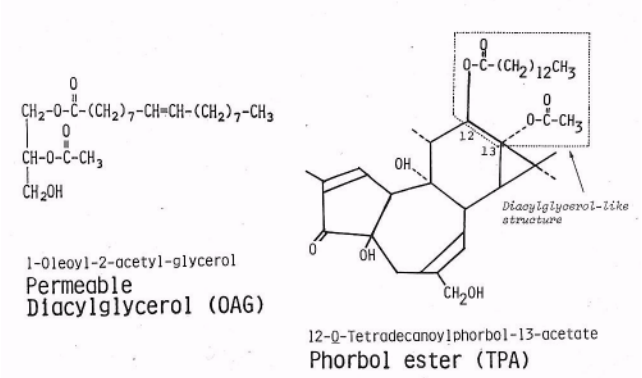

FIGURE 3.. Chemical structure of permeable diacylglycerol and tumour-promoting phorbol ester.

subspecies are all composed of a single polypeptide chain, with the group of a-, pi-, pll- and y- subspecies each having four conserved (C_t-C_4) and five variable (Vj-V_5) regions. The second group of 8-, e- and ^-subspecies lack the region C_2. The common structure of these subspecies is schemati-

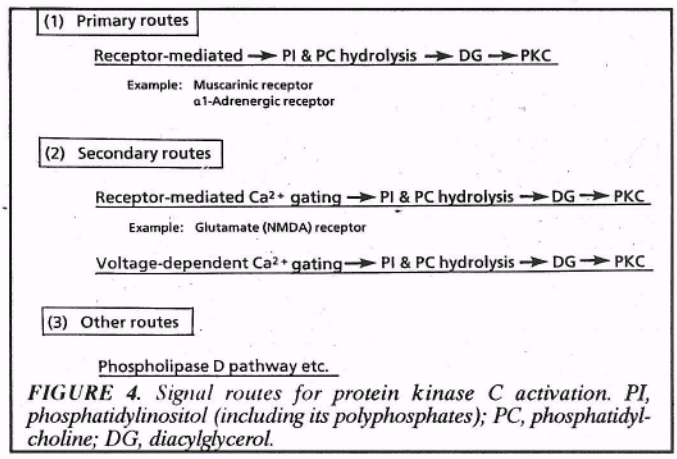

FIGURE 4. Signal routes for protein kinase C activation. PI, phosphatidylinositol (including its polyphosphates); PC, phosphatidylcholine; DG, diacylglycerol.

cally given in Fig. 5 (for reviews, see Refs. 11, 12).

The amino-terminal half of the molecule is the regulatory domain. The region Cj of each subspecies contains a tandem repeat of a cysteine-rich sequence,

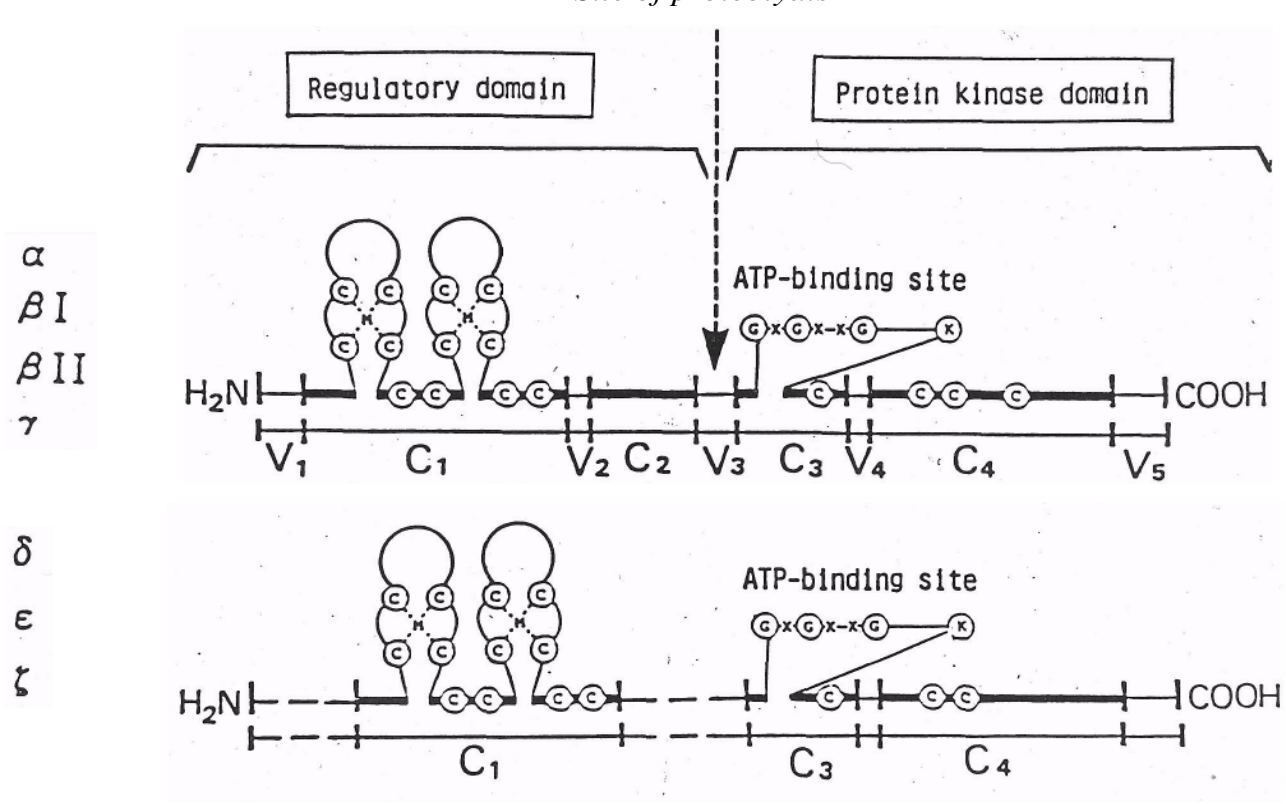

***FIG** URE 5. Common structure of the protein k inase C family.* V_r- V_y *variable regions; Cj-*C_f *conserved regions; C, cysteine; G, glycine, K, lysine; X, any amino acids; M, metal.*

similar to a zinc-finger like structure, except that £-PKC contains only one cysteine-rich sequence,and consequently has a relatively smaller molecular mass. Recent analysis with several polypeptide fragments of PKC expressed in Escherichia coli suggests that this cysteine-rich sequence is essential for the binding of phorbol ester, and possibly by analogy diacylglycerol, implying its involvement in the membrane-PKC interaction. The region C_2 is apparently needed for the Ca^{2+} sensitivity of the enzyme. On the other hand, the carboxy-terminal half of the molecule is the protein kinase domain. The conserved region C_3 has an ATP-binding sequence. The regulatory and protein kinase domains are cleaved by limited proteolysis, catalyzed by the Ca^{2+}-dependent neutral protease, calpain, at one or two specific sites in the variable region V_3. This proteolysis may take part in the down-regulation of the PKC molecule itself (for a review, see Ref. 11).

DIFFERENTIAL TISSUE EXPRESSION

• Using a combination of immunohistochemical, biochemical, biochemical and in situ hydridization procedures, the relative activity and individual pattern of expression of multiple PKC subspecies in several tissues and cell types has been examined extensively and clarified in detail. The results obtained thus far are summarized in Table 2 (for a review, see Ref. 11).

PKC with ^-sequence is expressed only in specific cells of the central nervous system. On the other hand, PKC's with pi- and fill-sequence are expressed in the brain, as well as in other tissues, in different ratios. In contrast, PKC with a-sequence is widely distributed in many tissues and cell types. In general, one cell type co-expresses more than one subspecies of PKC. These subspecies apparently show a distinct intracellular location. At present, the distribution and biochemical properties of the enzymes encoded by 8-, e- and ^-sequence have not been clarified.

Table 2. Subspecies of protein kinase C from mammalian tissues.

Subspecies	α	βI	βII	γ	δ	ε	ζ
Amino acid residues	672	671	673	697	673	737	672
Calculated molecular weight	76,799	76,790	76,933	78,366	77,517	83,474	76,799
Chromatographic sub-fraction	type III	type II	type II	type I	not identified	not identified	not identified
Activators	PS+DG+Ca^{2+} AA+Ca^{2+}	PS+DG+Ca^{2+}	PS+DG+Ca^{2+}	PS+DG+Ca^{2+} AA	PS+DG+(Ca^{2+})	PS+DG+(Ca^{2+})	PS+DG+(Ca^{2+})
Tissue expression	Universal	Some tissues & cells	Some tissues & cells	Brain & spinal cord only	Many tissues	Brain only	many tissues
Chromosome location (human)	17	16	16	19	?	?	?

More detailed explanations and references of each PKC subspecies are given elsewhere (ref.ll). This table is adapted from ref.ll. PSq, phosphatidylserine; DG, diacylglycerol; and AA, arachidonic acid.

ENZYMOLOGICAL CHARACTERISTICS

• The diversity of the sequence in the variable region allows separation of this enzyme into several subfractions upon chromatography on an hydroxyapatite column (13). To date, three subfractions, type I, II and III, have been shown to correspond to y-, (3 (pi and PII) - and cc-subspecies, respectively. The pi- and pll-subspecies are derived by alternative splicing of a single mRNA transcript, and show nearly identical kinetics and catalytic properties (for reviews, see Refs. 11, 12).

The PKC subfractions thus far obtained from various tissues exhibit subtle differences in their mode of activation and enzymological properties. PKC with y-sequence (type I) shows less activation by diacylglycerol. PKC's with pi- and pit-sequence (type II) show substantial activity without added Ca^{2+}. PKC with a-sequence (type III) is most sensitive to diacylglycerol for activation.

Although responses of PKC enzyme subspecies to these lipids significantly differed from one another, these subspecies were greatly activated by synergistic action of cis-unsaturated free fatty acid and diacylglycerol in the presence of phosphatidylserine. Arachidonic, oleic and linolenic acids were active in this role, whereas saturated free fatty acids such as palmitic and stearic acids were inert. In the presence of both phosphatidylserine and diacylglycerol the cis-unsaturated free fatty acids increased further an apparent affinity of PKC to Ca^{2+}, and allowed the enzyme to exhibit almost full activation at nearly basal level of Ca^{2+} concentration. Thus, the receptor-mediated release of unsaturated free fatty acids probably may take part, in synergy with the diacylglycerol produced, in the activation of PKC. It is possible that activation of the PKC family is an integral part of the signal-induced degradation cascade of various membrane phospholipids which are initiated by the action of phospholipase C and phospholipase A_2.

In addition to these well defined PKC subspecies, structurally undefined enzymes having slightly different properties are obtained from some tissues and cell types (11). Presumably, the members of the PKC family each show distinctly different preferences for substrate proteins that are located in specific intracellular compartments of the cell in which they are expressed.

PHYSIOLOGICAL FUNCTIONS

• PKC appears to modulate a number of membrane functions such as ion channel conductivity and the cross-talk between various receptors. In addition, PKC appears to show a dual action, providing both positive forward, as well as negative feedback, control over various steps of cell signalling processes. In short-term responses, for instance, PKC appears to play a role in decreasing the Ca^{2+} concentration within the cell. A number of reports have suggested that in various cell types PKC is able to activate the Ca^{2+}-transport ATPase and the $NaVCa^{2+}$ exchanger, both of which remove Ca^{2+} from the cytosol. PKC may also inhibit the receptor-mediated hydrolysis of inositol phospholipids, thereby blocking the activation of the Ca^{2+}-signalling pathway (for reviews, see Refs. 7, 11,12, 14).

Although it sounds paradoxical, such a feedback role of PKC is not confined to short-term responses such as the release reaction, but may be extended to the receptor functions of some growth factors, including epidermal growth factor and insulin. It is becoming clear, on the other hand, that PKC may play major roles of crucial importance for regulation of gene expression and growth control. Apparently, sustained activation of PKC is needed to cause gene activation, eventually leading to cell proliferation (for reviews, see Refs. 15,16). Our recent results with purified T-cells, for instance, show that multiple and

repeated additions of permeable diacylglycerol and Ca^{2+} ionophore result in both interleukin-2 receptor expression and cell proliferation (17). A single dose of the diacylglycerol, which causes only a transient activation of PKC, can not elicit sufficient T-cell activation.

Studies on the precise molecular basis of the role of the individual PKC subspecies are at their outset, but the members of this enzyme family play presumably each specific and distinct functions in the processing and modulation of a variety of physiological and pathological responses to external signals.

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