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55. I thank my many colleagues at the National Cancer Center, Tokyo, especially K. Wakabayashi for his assistance in the preparation of this manuscript. Many studies cited here were supported by grants-in-aid from the Ministry of Health and Welfare, the Ministry of Education, Science and Culture, the 10-Year Strategy for Cancer Control Program of the Japanese Government, the Princess Takamatsu Cancer Research Fund, and the Foundation for Promotion of Cancer Research.

Intracellular Signaling by Hydrolysis of Phospholipids and Activation of Protein Kinase C

Yasutomi Nishizuka

Hydrolysis of inositol phospholipids by phospholipase C is initiated by either receptor stimulation or opening of Ca^{2+} channels. This was once thought to be the sole mechanism to produce the diacylglycerol that links extracellular signals to intracellular events through activation of protein kinase C. It is becoming clear that agonist-induced hydrolysis of other membrane phospholipids, particularly choline phospholipids, by phospholipase D and phospholipase A_2 may also take part in cell signaling. The products of hydrolysis of these phospholipids may enhance and prolong the activation of protein kinase C. Such prolonged activation of protein kinase C is essential for long-term cellular responses such as cell proliferation and differentiation.

Protein kinase C (PKC) takes part in cellular responses to various agonists including hormones, neurotransmitters, and some growth factors. The enzyme is activated by increased amounts of diacylglycerol in membranes that result from agonist-induced hydrolysis of inositol phospholipids (*1*). However, hydrolysis of other phospholipids, particularly phosphatidylcholine, produces diacylglycerol at a relatively later phase in cellular responses, and a possible function of phospholipase D in cell signaling has been suggested (*2*). In fact, sustained activation of PKC is essential for subsequent responses such as cell proliferation and differentiation (*3*, *4*). It is also plausible that phospholipase A_2 is activated by most of the agonists that induce inositol phospho-

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lipid hydrolysis (5–7). Although arachidonic acid regulates many physiological processes through its conversion to various eicosanoids, several cis unsaturated fatty acids and lysophosphatidylcholine, which are the primary products of phosphatidylcholine hydrolysis catalyzed by phospholipase A_2, potentiate PKC activation and thereby contribute to signal transduction through the PKC pathway (8–11). The biochemical mechanism of interaction between various phospholipases remains largely to be explored, but in this article evidence is presented that an agonist-induced cascade of degradation of various membrane phospholipids is important for transmitting information from extracellular signals across the membrane. Most results appear to indicate that PKC participates in this process. The heterogeneity of the PKC family and its possible implications in cellular regulation are also summarized.

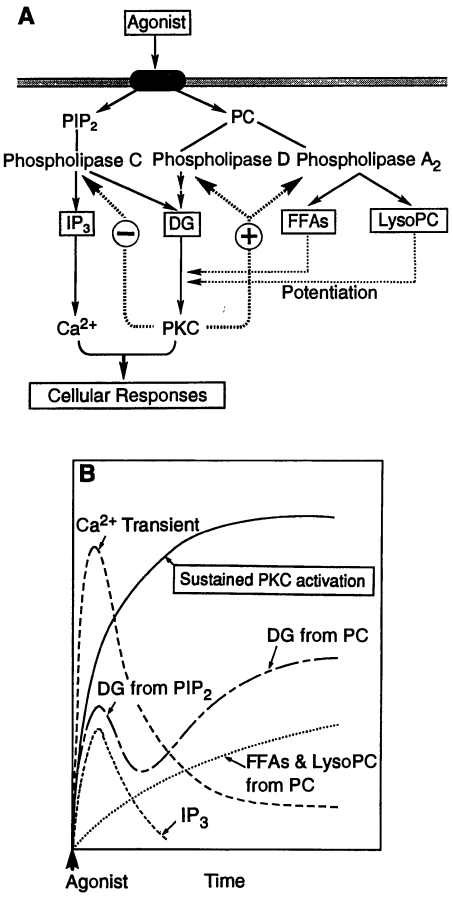

Fig. 1. Schematic representation of agonist-induced membrane phospholipid degradation for sustained PKC activation (**A**). Time course of generation of various signalling molecules (**B**). PIP_2, phosphatidylinositol 4,5-bisphosphate; PC, phosphatidylcholine; IP_3, inositol 1,4,5-trisphosphate; DG, diacylglycerol; FFAs, free cis unsaturated fatty acids; LysoPC, lysophosphatidylcholine.

Diacylglycerol and Sustained Activation of PKC

Upon cell stimulation, diacylglycerol is detected in various intracellular compartments at different times during the cellular response. In response to agonists, diacylglycerol is initially produced as a result of hydrolysis of inositol phospholipids, particularly phosphatidylinositol 4,5-bisphosphate (*1, 12*). This diacylglycerol production is normally transient and temporally corresponds to the formation of inositol 1,4,5-trisphosphate (IP_3). It is frequently followed by a more sustained increase in the amount of diacylglycerol (Fig. 1) (*13*). Extensive analysis of fatty acid compositions has indicated that this second phase of diacylglycerol formation probably results from hydrolysis of phosphatidylcholine in various stimulated cells including neutrophils, hepatocytes, fibroblasts, and mast cells (*14–16*). Phosphatidylethanolamine appears to be a minor source of this diacylglycerol. The sustained increase in the amount of diacylglycerol occurs in response to some mitogens and growth factors (*17*), phorbol esters (*15*), and oncogenic Ras (*18*), all of which induce activation of PKC. Activation of PKC itself may be responsible for causing the sustained elevation of diacylglycerol.

Isotope-labeling studies indicate that diacylglycerol is metabolized very rapidly both when it is produced endogenously (*19*) or when it is added exogenously to intact cells (*3, 20*). In platelets, diacylglycerol is largely converted to phosphatidic acid by the action of diacylglycerol kinase and then to inositol phospholipids (*20*), whereas in T lymphocytes, diacylglycerol exogenously added is hydrolyzed quickly by diacylglycerol lipase and presumably by nonspecific esterases (*3*). Tumor-promoting phorbol esters, which mimic diacylglycerol and activate PKC, are metabolically stable. Thus, the cellular responses caused by phorbol esters differ somewhat from those caused by membrane-permeant diacylglycerols (*21*). Experiments with multiple additions of a membrane-permeant diacylglycerol have confirmed that sustained activation of PKC is a prerequisite for long-term cellular responses such as activation of T lymphocytes (*3*) and differentiation of HL-60 cells into macrophages (*4*). In contrast, only a single dose of phorbol ester is required to produce similar cellular responses (*22*).

Role of Phospholipase D in Activation of PKC

Several mechanisms have been postulated for the agonist-induced formation of diacylglycerol from phosphatidylcholine (Fig. 2). Cell-free preparations from various mammalian tissues contain enzymatic activities that hydrolyze phosphatidylcholine, and a phospholipase C that utilizes this phospholipid as a substrate has been proposed to occur in dog heart cytosol (*23*), bull seminal plasma (*24*), and promonocytic cells (*25*). This type of phospholipase C may require tyrosine phosphorylation for its activation (*26*). However, phospholipase C reactive with phosphatidylcholine has not been purified extensively. The occurrence of this enzyme in tissues is expected because an enzymatic activity exists that produces choline phosphate from phosphatidylcholine (*27*).

On the other hand, several lines of evidence suggest that phosphatidylcholine is hydrolyzed by phospholipase D in agonist-dependent manner, resulting in the formation of phosphatidic acid, which is then converted to diacylglycerol by the action of phosphatidic acid phosphomonoesterase (*2, 15*) (Fig. 2). Mammalian phospholipase D was detected in rat brain (*28*), and subsequently in homogenates and membranes from various tissues and cell types

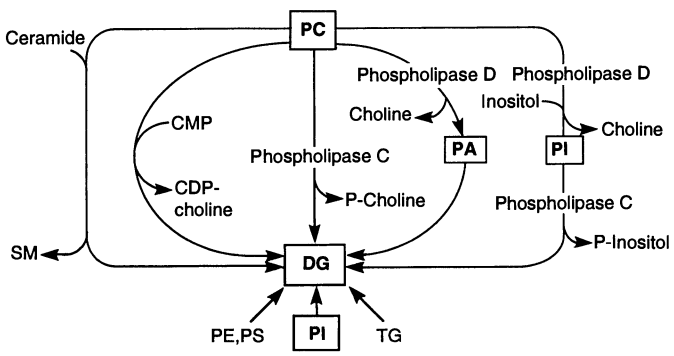

Fig. 2. Potential pathways for diacylglycerol formation. Three pathways to produce diacylglycerol from phosphatidylcholine that involve phospholipase C and phospholipase D are discussed in the text. The reverse reaction of phosphatidylcholine synthesis (*105*) to produce diacylglycerol may take place under ischemic conditions (*106*) but not in an agonist-dependent manner. Diacylglycerol may also be produced in association with sphingomyelin synthesis in the Golgi apparatus but its physiological function is unclear. PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PS, phosphatidylserine; PA, phosphatidic acid; SM, sphingomyelin; TG, triacylglycerol; P-Choline, choline phosphate; P-Inositol, inositol phosphate; CMP, cytidine 5′-monophosphate; CDP, cytidine 5′-diphosphate.

(29–32). The enzyme is found primarily in particulate fractions, presumably membranes, and has been purified partially from rat brain (30), human eosinophils (31), and rat neutrophils (32). However, a soluble phospholipase D has been detected in bovine tissues (33). Phospholipases D from the plasma membranes of rat brain synaptosomes (30) and hepatocytes (34) require Ca^{2+} but exhibit considerable activity in the presence of Ca^{2+} concentrations less than 1 μM. However, kinetic properties of phospholipase D vary from tissue to tissue (29), suggesting its extensive heterogeneity. The enzyme reacts preferentially with phosphatidylcholine and to a lesser extent with phosphatidylethanolamine.

Phospholipases D from mammalian tissues also catalyze a transphosphatidylation reaction (base exchange reaction) and incorporate choline, ethanolamine, and serine into phospholipids (35). The base exchange reaction appears to represent a minor pathway for the synthesis of most phospholipids (36), except for phosphatidylserine, which is produced in mammalian tissues mainly from phosphatidylcholine and serine (37). Apparently, multiple enzymes with different kinetics and distinct substrate specificities for base exchange reactions are present in various tissues with different intracellular localizations.

In several intact cell systems, phorbol esters stimulate breakdown of phosphatidylcholine to produce choline and choline phosphate (2, 27). In fact, phospholipase D is activated by a phorbol ester or a membrane-permeant diacylglycerol, sometimes synergistically with Ca^{2+} ionophore (15).

In various membrane fractions and permeabilized cell preparations, both base exchange reactions and phospholipase D activities are stimulated by guanosine triphosphate (GTP) analogs (2, 34, 38). Phorbol esters also cause activation of phospholipase D in membrane preparations obtained from hepatocytes (34), platelets (39), and brain synaptosomes (40), and in crude homogenates from HL-60 cells (41).

An assay to measure the phospholipase D and base exchange reactions has been developed. Cells, such as human neutrophils and HL-60 cells, are incubated with ^{3}H- or ^{32}P-labeled 1-O-alkyl-2-lyso-*sn*-glycero-3-phosphorylcholine, which is incorporated selectively into phosphatidylcholine in the form of alkyl-phospholipid (32, 41–43). In membrane preparations endogenously labeled by this procedure, both phosphatidic acid generation and the transphosphatidylation reaction are activated by GTP analogs and phorbol esters (32, 41–43). This activation by phorbol esters requires adenosine triphosphate (ATP) and is inhibited by staurosporine and peptides that inhibit PKC. Studies with fibroblasts overexpressing PKC suggest that this enzyme may participate in agonist-dependent activation of phospholipase D (44). However, it is not known whether PKC directly phosphorylates phospholipase D (45).

The choline moiety of phosphatidylcholine appears to be exchanged with free inositol to produce phosphatidylinositol, and this exchange reaction is stimulated by phorbol esters or a membrane-permeant diacylglycerol in HL-60 cells (46). Thus, it is possible that, upon stimulation of the cell, phosphatidylcholine is first converted to phosphatidylinositol by an exchange reaction catalyzed by phospholipase D, and the resulting phosphatidylinositol is subsequently hydrolyzed by phospholipase C to produce diacylglycerol and inositol phosphate in agonist-dependent manner (Fig. 2). The concentration of free *myo*-inositol within the cell is about 1 mM. If this mechanism of diacylglycerol formation from phosphatidylcholine operates in many tissues and cell types, it would be consistent, at least in part, with the prediction (47) that, after breakdown, inositol phospholipid is not re-synthesized simply from the corresponding phosphatidic acid.

However, inositol phospholipid hydrolysis may not always be essential or sufficient for agonist-induced phosphatidylcholine hydrolysis. Several mechanisms may be responsible for agonist-induced phospholipase D activation. Protein tyrosine phosphorylation is proposed to be necessary for phospholipase D activation (48). PKC, which is activated by inositol phospholipid hydrolysis, may take part in activation of phospholipase D, which would then enhance phosphatidylcholine hydrolysis. This may result in continuous production of diacylglycerol and prolonged activation of PKC (Fig. 1).

Role of Phospholipase A_2 in Activation of PKC

Phospholipase A_2, which hydrolyzes phospholipids to liberate free fatty acids and lysophospholipids, is ubiquitously present in mammalian tissues (7, 49) and receptor-mediated activation of this enzyme has been proposed (5). Agonists that provoke hydrolysis of inositol phospholipids usually also cause release of arachidonic acid. Although arachidonic acid can be produced from diacylglycerol by the action of diacylglycerol lipase, it is mainly derived from phospholipids by the activation of phospholipase A_2.

Diacylglycerol produced in membranes increases the apparent affinity of PKC for Ca^{2+}, and thereby renders the enzyme active in the presence of micromolar concentrations of Ca^{2+} (1, 50). Several cis unsaturated fatty acids activate PKC to various degrees, and a potential role of free fatty acids as second messengers has been suggested (51). Cis unsaturated fatty acids including oleic, linoleic, linolenic, arachidonic, and docosahexaenoic acids, which are all produced from phospholipids by the action of a nonselective type of phospholipase A_2, enhance the diacylglycerol-dependent activation of PKC (8, 9), and allow PKC to exhibit nearly full activity in the presence of Ca^{2+} concentrations less than 1 μM (8). Kinetic analysis with intact human platelets revealed that these fatty acids enhance activation of PKC if a membrane-permeant diacylglycerol is also present (10). Measurements of concentrations of intracellular Ca^{2+} with the Ca^{2+}-sensitive fluorescent dye fura-2 indicate that platelets are activated by the simultaneous addition of diacylglycerol and cis unsaturated fatty acid without an measurable increase in the intracellular Ca^{2+} concentration (10). Saturated fatty acids and trans unsaturated fatty acids do not have this effect.

Potentiation of cellular responses by cis unsaturated fatty acids has also been reported for diacylglycerol-induced interleukin-2 synthesis by human T lymphocytes (52) and for diacylglycerol-induced reduction of K^{+} channel conductance in *Hermissenda* photoreceptor cells (53). It is possible that in stimulated cells, the activity of PKC is increased initially by the increase in the concentrations of intracellular Ca^{2+} and diacylglycerol that result from inositol phospholipid hydrolysis. The activity of PKC may then be sustained, even after the concentration of intracellular Ca^{2+} is no longer increased, if diacylglycerol and cis unsaturated fatty acids both become available (Fig. 1).

Lysophosphatidylcholine, the other product of phosphatidylcholine hydrolysis, has membrane-lytic activity and is toxic to cells (54). When added to a cell, lysophosphatidylcholine is either converted rapidly to phosphatidylcholine by acyltransferases or metabolized further by lysophospholipase. However, lysophosphatidylcholine has several biological actions including chemotaxis (55) and relaxation of smooth muscle (56). In the presence of diacylglycerol, lysophosphatidylcholine potentiates cellular responses, especially long-term responses such as cell proliferation and differentiation. For instance, lysophosphatidylcholine enhances the activation of human T lymphocytes induced by a membrane-permeant diacylglycerol and ionomycin, as determined by interleukin-2 receptor-α expression and thymidine incorporation (Fig. 3) (11, 57). This action of lysophosphatidylcholine can also be observed when diacylglycerol is replaced by phorbol esters. Similarly, lysophosphatidylcholine stimulates HL-60 cell differentiation into macro-

phages as measured by expression of a specific cell surface marker, CD11b (the α subunit of the complement receptor type 3) (58). In either case, lysophosphatidylcholine has an effect only when both diacylglycerol and ionomycin are present, indicating that this lysophospholipid interacts with the PKC pathway. Other lysophospholipids are ineffective except for lysophosphatidylethanolamine, which is much less effective than lysophosphatidylcholine. Upon stimulation of platelets with thrombin (59) and T lymphocytes with an antigenic signal (11), lysophosphatidylcholine accumulates in a time-dependent manner.

The biochemical mechanism of the effects of lysophosphatidylcholine remains unexplored, but it may result partly from direct potentiation of the diacylglycerol-dependent activation of PKC (60). However, kinetic analysis in vitro and in intact cell systems has revealed that, unlike cis unsaturated fatty acids, lysophosphatidylcholine does not increase Ca^{2+} sensitivity of PKC activation (11). The results outlined above seem to indicate that agonist-induced activation of phospholipase A_2 may influence cell signaling through the PKC pathway. Addition of phospholipase A_2 to intact cells potentiates cellular responses that are elicited by diacylglycerol and ionomycin (57).

Several soluble phospholipases A_2, both intracellular and extracellular, have been identified in mammalian tissues (7, 49). The molecular size of intracellular phospholipase A_2 varies among different cells, ranging from 30 kD to 110 kD (61–63). The enzymes are active in the presence of Ca^{2+} concentrations less than 1 μM, and appear to translocate to membranes in response to agonists that mobilize Ca^{2+} such as bradykinin, histamine, ATP, and thrombin (61, 62). There seem to be arachidonic acid–selective and nonselective phospholipases A_2 within the cell (63). However, only the arachidonic acid–selective phospholipases A_2 have been well characterized (62), and one enzyme of 85 kD has been cloned (64).

Phospholipase A_2 is activated in cells treated with some growth factors, such as epidermal growth factor and platelet-derived growth factor (65). Studies with intact cell systems have suggested that PKC functions in the agonist-stimulated pathway leading to activation of phospholipase A_2. Phorbol esters and membrane-permeant diacylglycerols provoke arachidonic acid release, sometimes in synergy with physiological agonists (66). In neutrophils, phorbol esters activate both arachidonic acid–selective and nonselective enzymes, thereby increasing the intracellular amounts of various unsaturated fatty acids (67).

Further evidence for the role of PKC in the activation of intracellular phospholipase A_2 has been obtained with membrane preparations of mouse spleen macrophages (68) and Chinese hamster ovary (CHO) cells (69). Phospholipase A_2 isolated from cells pretreated with physiological agonists such as thrombin is phosphorylated on seryl residues, and both this phosphorylation and the activation of the enzyme in situ are blocked by staurosporin (69). The intracellular 85-kD phospholipase A_2 contains consensus phosphorylation sites for PKC and mitogen-activated protein kinase (MAP kinase) (64, 70). However, activation of PKC alone does not appear to be sufficient to cause release of fatty acids. Intermediary proteins such as phospholipase A_2–activating protein (PLAP) (71) may also take part in activation of this enzyme.

Although the mechanism by which agonists cause the activation of intracellular phospholipase A_2 remains unclear, it seems likely that activation of PKC by diacylglycerol leads to increased hydrolysis of phosphatidylcholine by phospholipase A_2. This pathway would enhance PKC activation by providing not only arachidonic acid but also other cis-unsaturated fatty acids and lysophosphatidylcholine (Fig. 1). The cessation of this phospholipid degradation may depend on the amount of diacylglycerol in membranes and on the duration of agonist action.

Calcium Signals and Phospholipid Degradation

In most stimulated cells, the concentration of Ca^{2+} increases only transiently, whereas physiological responses persist long after the Ca^{2+} concentration returns to basal amounts. The prolonged activation of PKC is thought to be responsible for maintaining such cellular responses (72). Ca^{2+} mobilization and PKC activation act synergistically to cause a variety of cellular responses, but the biochemical mechanism of this synergism is not fully understood (73). Ca^{2+} mobilization and phospholipid degradation are intimately interrelated, and sometimes complementary to each other. In the presence of high concentrations of Ca^{2+}, activation of PKC requires less phospholipid degradation, whereas in the presence of intense phospholipid degradation, less Ca^{2+} is required to activate the enzyme. Also, at an early phase of cellular response, the agonist-induced hydrolysis of inositol phospholipids is down-regulated as a result of activation of PKC (44, 73, 74) (Fig. 1).

Activation of cell surface receptors often results in the generation of regular oscillations in the intracellular Ca^{2+} concentration (75). Several mechanisms mediated by

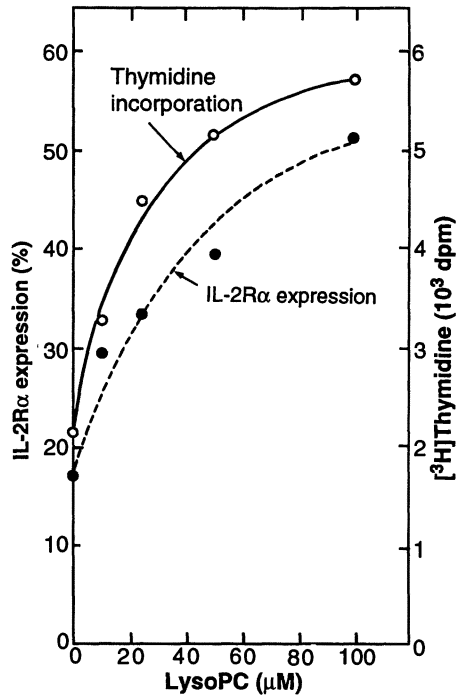

Fig. 3. Potentiation of T lymphocyte activation by membrane-permeant diacylglycerol and ionomycin. Purified human peripheral resting T lymphocytes were stimulated with 1,2-dioctanoylglycerol (50 μM) and ionomycin (0.5 μM) in the presence of various concentrations of lysophosphatidylcholine (LysoPC). The amount of the interleukin-2 receptor α-subunit (IL-2Rα) expressed and the amount of thymidine incorporated were measured after 16 hours and 30 hours, respectively (11). The incubation medium contained serum and thus, the effective concentration of LysoPC is much lower than indicated. LysoPC alone was inactive in the absence of diacylglycerol and ionomycin.

IP_3-induced Ca^{2+} release and Ca^{2+}-induced Ca^{2+} release have been proposed to explain this complex regulation of intracellular Ca^{2+} concentrations (76). On the other hand, PKC may participate in generation of Ca^{2+} oscillations by exerting negative feedback control over agonist-induced inositol phospholipid hydrolysis and setting up a new cycle of production of IP_3 (77). This hypothesis is primarily based on the observation that Ca^{2+} oscillations are inhibited by phorbol esters, and this inhibition is prevented by PKC inhibitors (77). A consequence of this proposed mechanism is that the activities of phospholipases and PKC and the amounts of diacylglycerol and IP_3 must also be oscillating with a rate or pattern similar to that of Ca^{2+}. In thrombin-stimulated human platelets, the amount of diacylglycerol does oscillate (78). It will be important to explore further the relationship between Ca^{2+} mobilization and phospholipid degradation, both of which may exert diverse actions eventually

Table 1. PKC subspecies in mammalian tissues.

Subspecies	Amino acid residues	Molecular size (calcd.) (daltons)	Activators*	Tissue expression	References
			Group A: classical PKCs (cPKC)		
α	672	76,799	PS, Ca^{2+}, DG, FFA, LysoPC†	Universal	(*79, 80*)
βI	671	76,790	PS, Ca^{2+}, DG, FFA, LysoPC	Some tissues	(*79, 80*)
βII	673	76,933	PS, Ca^{2+}, DG, FFA, LysoPC	Many tissues	(*79, 80*)
γ	697	78,366	PS, Ca^{2+}, DG, FFA, LysoPC	Brain only	(*79, 80*)
			Group B: new PKCs (nPKC)		
δ	673	77,517	PS, DG	Universal	(*79, 81–83*)
ϵ	737	83,474	PS, DG, FFA	Brain and others	(*79, 83–86*)
η(L)	683	77,972	?‡	Lung, skin, heart	(*83, 87, 88*)
θ	707	81,571	?	Skeletal muscle (mainly)	(*89*)
			Group C: atypical PKCs (aPKC)		
ζ	592	67,740	PS, FFA	Universal	(*79, 83, 90–92*)
λ	586	67,200	?	Ovary, testis, etc.	(*93*)

*The activators for each subspecies are determined with calf thymus H1 histone and bovine myelin basic protein as model phosphate acceptors. †DG, diacylglycerol; PS, phosphatidylserine; FFA, cis unsaturated fatty acid; LysoPC, lysophosphatidylcholine. ‡The detailed enzymological properties of the η(L), θ, and λ subspecies have not yet been clarified.

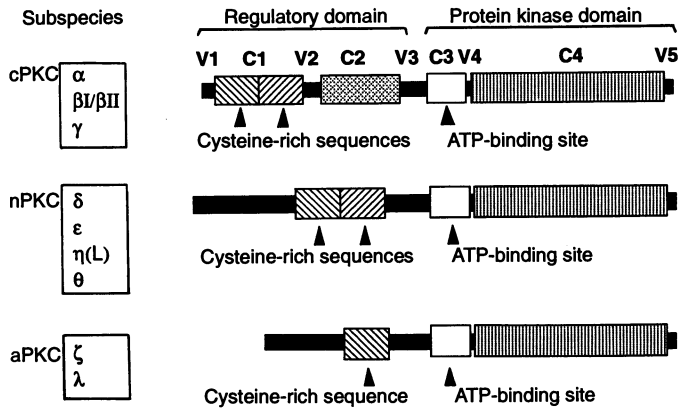

Fig. 4. Structure of PKC subspecies. Four conserved (C_1 to C_4) and five variable (V_1 to V_5) regions of the cPKC group are indicated. Details are outlined elsewhere (*79*), and in the text. The βI and βII subspecies are derived from a single gene by alternative splicing.

leading to the long-lasting control of physiological processes in a synergistic manner.

Calcium and Lipid Requirements of PKC Subspecies

Multiple discrete subspecies of PKC have been defined. These subspecies show subtly different enzymological properties, differential tissue expression, and specific intracellular localization (*79*). Ten subspecies of PKC have been identified in mammalian tissues (Table 1), but some of these subspecies do not show typical characteristics of the classical PKC enzymes in their mode of activation. An integrated nomenclature has been developed to categorize the PKC subspecies (*79–93*). Group A consists of four classical or conventional PKCs (cPKC): α, βI, βII, and γ (*79, 80*). Group B consists of four new PKCs (nPKC): δ, ϵ, η(L), and θ (*79–89*). Group C consists of two atypical PKCs (aPKC): ζ and λ (*79, 83, 90–93*).

The cPKC enzymes of group A have four conserved (C_1 to C_4) and five variable (V_1 to V_5) regions (Fig. 4). The C_1 region is a putative membrane-binding domain (*94*). The C_2 region appears to be related to the Ca^{2+} sensitivity of the enzyme. The C_3 region contains the catalytic site. The C_4 region seems to be necessary for recognition of the substrate to be phosphorylated. The cPKC enzymes are activated by Ca^{2+}, phosphatidylserine, and diacylglycerol or phorbol esters, and this activation is enhanced further by cis unsaturated fatty acids and lysophosphatidylcholine.

The nPKC enzymes of group B, which lack the C_2 region, do not require Ca^{2+}. The enzymes are activated by micelles composed of phosphatidylserine and diacylglycerol or phorbol esters (*79–86*). The ϵ subspecies is activated by cis unsaturated fatty acids but the δ subspecies is not. The δ and ϵ subspecies exist in phosphorylated forms in native tissues and appear as doublet bands after electrophoresis (*82, 86*). It is possible that the group B enzymes are integrated directly or indirectly in a protein kinase cascade that is initiated by the activation of some growth factor receptors, eventually leading to the regulation of nuclear events such as cell cycle control (*95*).

On the other hand, the aPKC enzymes of group C, which have only one cysteine-rich zinc finger–like motif (*79, 83, 90–93*), are dependent on phosphatidylserine but not affected by diacylglycerol, phorbol esters, or Ca^{2+} (*90–92*). The ζ subspecies is activated by cis unsaturated fatty acids (*92*). The signal to activate the group C enzymes remains unknown.

The known members of the PKC family all depend on phosphatidylserine, but show different requirements of phospholipid metabolites for their activation. Species of diacylglycerol that contain various fatty acids and are derived from phosphatidylinositol, phosphatidylcholine, or ether-linked diglycerides (*16*) can activate the PKC subspecies to various degrees (*17, 96, 97*). However, alkyl analogs of some membrane-permeant diacylglycerols appear to be inactive (*98*). The members of the PKC family probably respond differently to various combinations of Ca^{2+}, phosphatidylserine, diacylglycerol, and other phospholipid degradation products. Thus, the patterns of activation of the enzymes may vary in extent, duration, and intracellular localization. Detailed spatiotemporal aspects of phospholipid degradation and activation of PKC enzymes within the cell are poorly understood.

Implications and Perspectives

Despite extensive studies, our knowledge of specific functions of the individual PKC subspecies is still limited. However, in the nucleus, the signal pathway through PKC appears to be indispensable for the control of gene expression and the cell cycle. Treatment of CHO cells overexpressing the δ subspecies with phorbol esters prevents progression of the cell cycle through M phase, suggesting that some members of the PKC family may function in the regulation of specific points of the cell cycle (*99*). In the membrane, crucial roles have been assigned to PKC in down-regulation of receptors, modulation of ion channels, release of hormones and neurotransmitters, and exocytosis (Fig. 5) (*73*). Biochemical and electro-

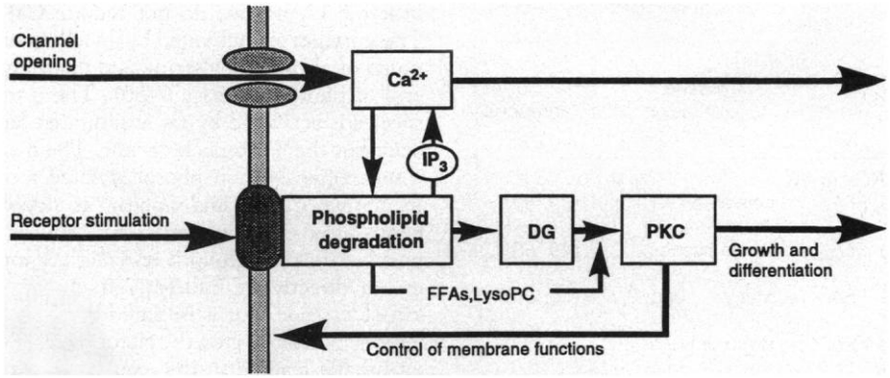

Fig. 5. Potential roles of PKC pathway in cellular regulation. IP_3, inositol 1,4,5-trisphosphate; FFAs, free cis unsaturated fatty acids; and LysoPC, lysophosphatidylcholine.

physiological evidence for a role of PKC in modulating Na^+ channels has been presented (*100*). The distinct modes of activation, together with the apparent patterns of tissue expression and intracellular localization of the various PKC subspecies, implies their specialized functions in cell signaling.

PKC participates in inflammatory processes and immune responses. The signaling pathway through PKC is essential for the activation of platelets, neutrophils, macrophages, lymphocytes, and fibroblasts, and for the function of vasculoendothelial systems (*73*). When added to intact cells, the extracellular, secreted phospholipase A_2 (group II) potentiates the agonist-induced activation of cellular functions such as antigen-induced T lymphocyte activation (*101*). Phospholipase A_2 alone is inactive. The secreted phospholipase A_2 may take part in the propagation of inflammatory responses by hydrolyzing cell membrane phospholipids directly to produce cis unsaturated fatty acids and lysophosphatidylcholine. The phospholipase A_2 (group II) is secreted by a variety of cells such as platelets, neutrophils, and mast cells at various sites of inflammation (*63, 102*). This enzyme requires more than 1 mM Ca^{2+} for activity, and releases various fatty acids from membrane phospholipids (*63*). Such an extracellular phospholipase A_2 has been identified in neuronal cells (*103*), where it may function in synaptic transmission. The cis unsaturated fatty acids such as arachidonic and oleic acids together with diacylglycerol may function in long-term potentiation of synaptic processes (*53, 104*).

Because of its diverse functions in signal transduction, research on the PKC family has spread over many fields of biology and medicine. The signaling pathway through PKC interacts with several other pathways that include cyclic adenosine monophosphate–dependent protein kinase and many other protein kinases. Further exploration of the networks of various signaling pathways such as those described in this article is important to our understanding of the dynamic aspects of cellular regulation.

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107. A part of this article was presented as the Third Hans Bloemendal Award Lecture on 21 May 1992 at the University of Nijmegen, The Netherlands The author is grateful to S. Nakamura, Y. Asaoka, K. Ogita, and M. Furuta for careful reading and preparation of this manuscript and to S. Nishiyama, Y. Kimura, and Y. Yamaguchi for their skillful secretarial assistance. Supported in part by research grants from the Special Research Fund of Ministry of Education, Science and Culture, Japan; Yamanouchi Foundation for Research on Metabolic Disorders; Sankyo Foundation of Life Science; Merc Sharp & Dohme Research Laboratories, U S.A., Central Laboratories of Takeda Chemical Industries; New Lead Research Laboratories of Sankyo Company; and Terumo Life Science Foundation.

The Economics of Health Care in Japan

Naoki Ikegami

Japan's health care system balances universal coverage at reasonable cost. The government has taken on the responsibility of acting as insurer and subsidizing health care spending for the employees of small enterprises and the self-employed. Despite the fee-for-service form of payment, costs have been contained by the use of a nationally uniform fee schedule that is mandatory for all providers. However, the increasingly affluent and aging population is making new demands on the system that can only be met by a major restructuring.

By broad measures of performance, Japan's health care system appears to have achieved a paragon of success. The gross health indicies are the best in the world: the infant mortality rate is 0.46% of live births and the life expectancy at birth is 75.9 for males and 81.8 for females (*1*). There is universal coverage with virtually unlimited access to all health care facilities by every citizen. Moreover, because Japan's per capita rates of computer-aided tomography (CAT) scans and patients undergoing renal dialysis are among the highest of all nations, there would seem to be no overt signs of rationing (*2*). What makes this record even more impressive is that the ratio of the gross domestic product (GDP) devoted to health care is 6.8%, little more than half the ratio of the United States (*3*).

In this article, I briefly describe how the Japanese health care system works to provide some general context for international comparison (*4*). Is Japan's system really a paragon? In particular, how is equity achieved? Why has it been possible to contain costs under a fee-for-service system? Are there any negative effects coming from cost containment? What are the relations between cost, access, and quality?

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The Delivery System: Functionally Undifferentiated

About 81% of Japan's hospitals and 94% of its physicians' offices (referred to as clinics) are privately operated (*5*). Although hospital beds have recently become regulated, there are still no restrictions on any other form of capital investment. However, this entrepreneurism has been permitted only by private practitioners. Investor-owned hospitals that are operated for profit are prohibited, and the hospital's chief executive must always be a physician. These legal limitations have effectively constrained the development of multihospital systems. Most of the hospitals are small, physician-owned family concerns that have developed from clinics. Few of them have ventured into high technology medicine because of the restraints posed by the financing system, so that this area tends to be dominated by the smaller but more prestigious public sector.

Health care in Japan differs from the United States in that the physicians in clinics do not have any access to hospital facilities and must refer all patients needing care that they cannot provide within their own premises. On the other hand, hospitals employ their physicians on fixed salaries and maintain large outpatient departments from which they admit all their inpatients. Because of this mutually exclusive arrangement, both clinics and hospitals compete for patients, who have freedom to choose the facility that they feel best fits their needs. This situation means that there is little functional differentiation between clinics and hospitals, and their boundary is further blurred because a third of the clinics have a small number of beds (the distinction is primarily legal in that facilities with more than 20 beds are designated as hospitals, whereas those having fewer than 20 are called clinics). Neither is there much differentiation between acute and long-term care. Hospitals have taken on the function of nursing homes in Japan, and 45% of the inpatients over the age of 65 have been hospitalized for more than 6 months (*6*).

The Financing System: Strictly Regulated

In contrast to the basically laissez-faire policy taken toward the delivery system, the financing system is highly regulated. First, although there are multiple payers, consumers have virtually no choice over the selection of their plan. They must join the one statutory plan offered by their employers, or if they are self-employed, that administered by their local governments or trade associations. However, the lack of choice does not really affect the consumer because there is little flexibility. All plans offer basically the same set of comprehensive medical benefits, including medications, long-term care, dental care, and some preventive care.

Second, neither insurers nor providers have the freedom to negotiate individually a different fee schedule. The fee-for-service system operates under a minutely defined price schedule set by the government. All providers are paid exactly the same amount, inclusive of physician's fees, for the same service regardless of the physician's expertise or the facility's characteristics or geographical location.

Third, consumers cannot opt out of the statutory system, and private health insurance, which is mainly limited to cash compensations to cover incidental expenses during hospitalizations, remains insignificant. Providers are strictly prohibited from balance billing (charging more than the fee schedule allows). Extra charges are permitted only for private hospital rooms [only 10% of the rooms are of this status in Japan (*7*)] and a very restrictive range of new technology, which is still being evaluated.

The insurance plans (Table 1) can be broadly divided into two categories. First is the insurance system for employees and their dependents, in which the premiums are generally paid on an equal basis between