Studies and Perspectives of Protein Kinase C

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Protein kinase C, an enzyme that is activated by the receptor-mediated hydrolysis of inositol phospholipids, relays information in the form of a variety of extracellular signals across the membrane to regulate many Ca^{2+}-dependent processes. At an early phase of cellular responses, the enzyme appears to have a dual effect, providing positive forward as well as negative feedback controls over various steps of its own and other signaling pathways, such as the receptors that are coupled to inositol phospholipid hydrolysis and those of some growth factors. In biological systems, a positive signal is frequently followed by immediate negative feedback regulation. Such a novel role of this protein kinase system seems to give a logical basis for clarifying the biochemical mechanism of signal transduction, and to add a new dimension essential to our understanding of cell-to-cell communication.

RECEPTOR-MEDIATED HYDROLYSIS OF INOSITOL PHOSPHOlipids was recently realized to be a common mechanism for transducing various extracellular signals into the cell, such as those from certain hormones, neurotransmitters, antigens, some growth factors, and many other biologically active substances. In 1953, the response of inositol phospholipids to the stimulation of cell surface receptors was recognized by Hokin and Hokin (*1*), who first showed that acetylcholine induces a rapid incorporation of ^{32}P into phosphatidyl inositol (PI) and phosphatidic acid. It became evident later that this incorporation resulted from the enhanced breakdown and resynthesis of inositol phospholipids occurring in many stimulated cells (*2, 3*). Durell *et al.* (*4*) suggested a potential role of this PI response in receptor functions, and Michell (*2*) subsequently postulated that the phospholipid breakdown might open a Ca^{2+} gate. In 1983, Berridge and his co-workers (*5*) demonstrated that inositol-1,4,5-trisphosphate (I-1,4,5-P_3), one of the earliest products of phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis, serves as a mediator of Ca^{2+} mobilization from an internal store, probably in the compartment of the endoplasmic reticulum (*6*). Our studies provided evidence that 1,2-diacylglycerol, the other product of PIP_2 hydrolysis that remains in membranes, initiates the activation of a specialized protein kinase, protein kinase C, and that the signal pathway through this protein phosphorylation is separate from, and often synergistic to, the Ca^{2+} signaling pathway for the control of various cellular functions and proliferation (*7*). Several aspects of protein kinase C have been reviewed (*8, 9*).

Some Properties of Protein Kinase C

Protein kinase C was identified in 1977 as a proteolytically activated protein kinase (*10*), and is now known to be ubiquitous in tissues and organs (*11*). In most tissues other than brain (*12*), this enzyme is recovered mainly from the soluble fraction as an inactive form, and is apparently translocated to membranes in a Ca^{2+}-dependent fashion when cells are stimulated (*13*). However, the precise intracellular topography of this protein kinase is unknown, since the enzyme is usually extracted in the presence of high concentrations of a Ca^{2+} chelator to prevent proteolysis by the Ca^{2+}-dependent protease, calpain. Recent immunocytochemical analysis using monoclonal antibodies against protein kinase C indicates that intracellular localization of this enzyme varies with cell types, but that the enzyme appears to be absent or poorly represented in the nucleus (*14*).

Protein kinase C preparations from various tissues are similar to one another in their kinetic and catalytic properties. However, the enzyme isolated from brain tissues sometimes reveals a double band upon sodium dodecyl sulfate–polyacrylamide gel electrophoresis (*15*). The precise nature of this doublet is not known, but our recent analysis of its complementary DNA clones has raised the possibility that more than one gene exists for this protein kinase (*16*).

Protein kinase C requires Ca^{2+} and phospholipid, particularly phosphatidyl serine, for its activation (*8*). Diacylglycerol dramatically increases the affinity of this enzyme for Ca^{2+} and thereby renders it fully active without a net increase in the Ca^{2+} concentration (*17*). Thus, the activation of this protein kinase is thought to be biochemically dependent on Ca^{2+}, but under some conditions physiologically independent of Ca^{2+}. However, the enzyme can also be activated by the synergistic action of an increase in Ca^{2+} concentration and the formation of diacylglycerol.

Diacylglycerols containing a 1,2-*sn* configuration, with various fatty acids in different chain length, are capable of activating protein kinase C, with those having an unsaturated fatty acid being most active (*18*). However, two other stereoisomers, 2,3-*sn*-diacylglycerol and 1,3-diacylglycerol, neither activate nor inhibit the enzyme, suggesting that a highly specific lipid-protein interaction is needed for this enzyme activation (*19*). From experiments with a Triton X-100 mixed micellar assay system, it has been postulated that about one molecule of diacylglycerol can activate one molecule of protein kinase C in the presence of greater than four but less than ten molecules of phosphatidyl serine (*20*). Nevertheless, this 1:1 stoichiometry does not necessarily indicate that diacylglycerol binds directly to the enzyme through a ligand-ligand interaction, and the precise mechanism of this enzyme activation is not yet fully understood (*21*).

Protein kinase C can also be activated by limited proteolysis with calpain. The smaller component is enzymatically active totally independently of Ca^{2+}, phospholipid, or diacylglycerol. Membrane-bound protein kinase C is more susceptible to this proteolysis (*22*), which has been suggested to occur in intact platelets and neutrophils (*23*). However, the physiological significance of this proteolytic activation is not known.

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Transient Signal Passage

Phosphatidylinositol-4-phosphate (PIP) and PIP_2, minor components of inositol phospholipids, are produced from PI by sequential phosphorylation of the *myo*-inositol moiety (*24*). In various tissues, signal-induced breakdown of PIP_2 appears to precede the previously known PI response, and PIP_2 has recently been regarded as a prime target for ligand-induced breakdown (*25*). The primary products of PIP_2 hydrolysis are I-1,4,5-P_3 and diacylglycerol. Thus, the information of external signals flows from the cell surface into the cell interior through two pathways, Ca^{2+} mobilization mediated by I-1,4,5-P_3 and protein kinase C activation induced by diacylglycerol.

Evidence for I-1,4,5-P_3 as a mediator of Ca^{2+} mobilization has been provided by studying the effect of this compound on various permeabilized cells, and also by its microinjection into intact cells (*5*). On the basis of direct binding studies with liver microsomes, it has recently been suggested that I-1,4,5-P_3 acts on stored Ca^{2+}, probably through its own receptor (*26*). This inositol trisphosphate, once produced, disappears very rapidly, and a major mechanism for terminating this signal flow is thought to be removal of the 5-phosphate by the action of a specific phosphatase (*3, 5*). Although there might be more than one mechanism eventually leading to the required intracellular increase in the concentration of Ca^{2+}, the Ca^{2+} signal in most tissues is transient and returns quickly to basal or even below basal levels. This transient Ca^{2+} spike has been successfully observed in experiments with the photoprotein aequorin (*27*). Both Ca^{2+}-transport adenosinetriphosphatase (ATPase) and Na^+/Ca^{2+} exchange protein are known to be responsible for the extrusion of Ca^{2+} to maintain homeostasis (*28*). In addition, a considerable amount of the released Ca^{2+} is taken up again into its internal reservoir for storage.

Analogously, the appearance of diacylglycerol in membranes is also transient, and this neutral lipid disappears within a few seconds or at most several minutes of its formation (*3, 7, 14*). This rapid disappearance of diacylglycerol is due both to its conversion back to inositol phospholipids by way of phosphatidic acid (PI turnover) and to its further degradation to arachidonic acid, which in turn can generate other messengers such as prostaglandins (*29*). Thus, protein kinase C is active for only a short time after the stimulation of the receptor. However, the consequence of this enzyme activation may persist for a long period depending on the biological stability of the phosphate that is covalently attached to each substrate protein molecule. Although both Ca^{2+} and diacylglycerol signals are transient, the two pathways are essential and often synergistic for evoking subsequent cellular responses.

Diacylglycerol and Tumor Promoter

Some synthetic diacylglycerols, such as 1-oleoyl-2-acetylglycerol, are readily intercalated into intact cell membranes and activate protein kinase C directly (*30*). Recently, 1,2-dioctanoylglycerol and 1,2-didecanoylglycerol were shown to be permeable (*31*). Thus, these diacylglycerols are often used in studies to explore possible roles of the enzyme in stimulus-response coupling. When added to intact cells, the active permeable diacylglycerols appear to be metabolized rapidly and converted to the corresponding phosphatidic acids and probably further to inositol phospholipids (*30, 32*). Diacylglycerols having a 2,3-*sn* configuration are not active for intact cells (*33*). It is physiologically important that the diacylglycerols derived from triacylglycerol by the action of lipoprotein lipase and a heparin-releasable hepatic lipase have a 2,3-*sn* configuration (*34*).

Tumor-promoting phorbol esters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), have a structure very similar to diacylglycerol and activate protein kinase C directly both in vitro and in vivo (*35*). Like diacylglycerol, phorbol esters dramatically increase the affinity of this enzyme for Ca^{2+}, resulting in its full activation at physiological Ca^{2+} concentrations. Several lines of evidence provided by this and other laboratories support the notion that protein kinase C is the receptor of tumor promoters (*36*).

However, phorbol esters may sometimes be unsuitable for studies of the physiological activation of protein kinase C, since diacylglycerol is present only transiently in membranes, while phorbol ester is hardly degraded. Therefore, phorbol ester may extend a usually limited phase of cellular response, thereby distorting the normal sequence of events. As will become evident later, protein kinase C presumably has both positive and negative actions that depend upon the function of the target substrate protein. At an early phase of

Table 1. Possible roles of protein kinase C in cellular responses.

Tissues and cells	Responses	References
Endocrine systems		
Adrenal medulla	Catecholamine secretion	*38*
Adrenal cortex	Aldosterone secretion	*39*
	Steroidogenesis	*40*
Pancreatic islets	Insulin release	*41*
Insulinoma cells	Insulin release	*42*
Pituitary cells	Pituitary hormone release	*43*
	Growth hormone release	*44*
	Luteinizing hormone release	*45*
	Prolactin release	*46*
	Thyrotropin release	*47*
Parathyroid cells	Parathyroid hormone release	*48*
Thyroid C cells	Calcitonin release	*49*
Leydig cells	Steroidogenesis	*50*
Exocrine systems		
Pancreas	Amylase secretion	*51*
Parotid gland	Amylase and mucin secretion	*52*
Submandibular gland	Mucin secretion	*53*
Gastric gland	Pepsinogen secretion	*54*
	Gastric acid secretion	*55*
Alveolar cells	Surfactant secretion	*56*
Nervous systems		
Ileal nerve endings	Acetylcholine release	*57*
Neuromuscular junction	Transmitter release	*58*
Caudate nucleus	Acetylcholine release	*59*
PC 12 cells	Dopamine release	*60*
Neurons	Dopamine release	*61*
Muscular systems		
Vascular smooth muscle	Muscle contraction	*62*
	Muscle relaxation	*63*
Inflammation and immune systems		
Platelets	Serotonin release	*30, 64*
	Lysosomal enzyme release	*65*
	Arachidonate release	*66*
	Thromboxane synthesis	*67*
Neutrophils	Superoxide generation	*68*
	Lysosomal enzyme release	*65, 69*
	Hexose transport	*70*
Basophils	Histamine release	*71*
Mast cells	Histamine release	*72*
Lymphocytes	T-lymphocyte activation	*73*
	B-lymphocyte activation	*74*
Metabolic and other cell systems		
Adipocytes	Lipogenesis	*75*
	Glucose transport	*76*
Hepatocytes	Glycogenolysis	*77*
Epidermal cells	Inhibition of gap junction	*78*
Fibroblasts	Inhibition of gap junction	*79*
Hepatocytes	Inhibition of gap junction	*80*

cellular responses, this enzyme probably acts synergistically with Ca^{2+} as part of a positive response, but more frequently it acts as a negative feedback control, as in the down-regulation of some receptors, which occurs immediately upon stimulation. Thus, it is essential to follow up in turn a temporal sequence of events occurring in different phases of a cellular response. In addition, its concentration should be given special consideration when attempting to evaluate the exact contribution of phorbol ester to physiological responses. There is no proof at present for protein kinase C being the sole target of tumor promoters, particularly at their higher concentrations. Therefore, it should not be surprising if slightly different effects are obtained from stimulation of living cells with diacylglycerol as opposed to phorbol ester. For instance, HL-60 leukemia cells have been reported to be differentiated by phorbol ester but not by permeable diacylglycerol (*37*).

Short-Term and Long-Term Responses

The role of protein kinase C in stimulus-response coupling was first demonstrated for the release of serotonin from platelets (*30*), and subsequently shown for the release, secretion, and exocytosis of cellular constituents from a variety of endocrine and exocrine tissues and for the activation of many other cellular functions (Table 1) (see also *38–80*). Under appropriate conditions, the two signal pathways, protein kinase C activation and Ca^{2+} mobilization, can be induced selectively and independently by the application of a permeable diacylglycerol or phorbol ester for the former and a Ca^{2+} ionophore such as A23187 or ionomycin for the latter. It became possible to show with this procedure that the two signal pathways are both essential and often act synergistically to elicit full cellular responses. Such a potential role of protein kinase C in signal transduction at the cell surface has also been extrapolated to neural functions, particularly transmitter release in both peripheral and central nervous systems, as well as modulation of membrane ion conductance. In fact, experiments with monoclonal antibodies have confirmed the presence of a protein kinase C–positive immunoreactive material in many regions of the cytoplasm, including axons and dendrites of neuronal cells (*14*); a typical example is shown in Fig. 1.

The two signal pathways, protein kinase C activation and Ca^{2+} mobilization, may also play roles in long-term responses such as gene expression and cell proliferation. For example, the two pathways are both essential and act synergistically to cause DNA synthesis in macrophage-depleted human peripheral lymphocytes (*73*). However, in order to stimulate rapid cell proliferation, some growth factor is still needed, indicating that an additional signal pathway is involved in eliciting the full activation of cell proliferation (Fig. 2). For instance, interleukin-1 presumably utilizes both Ca^{2+}- and protein kinase C–dependent signal pathways, and induces T lymphocyte proliferation by initiating the gene expression of interleukin-2 receptor and by facilitating the release of interleukin-2 itself (*81*). The latter appears to function much like a progression factor that causes continuous cell division by stimulating an additional signal pathway. Synergism between a permeable diacylglycerol and Ca^{2+} ionophore leads to proliferation of human B lymphocytes as well, and a parallel mechanism possibly exists in the growth promotion for these cells (*74*).

Phorbol ester and growth factor have been known for many years to act in concert to stimulate cell proliferation (*82*). Insulin is needed in addition to phorbol ester or permeable diacylglycerol for the growth response of Swiss 3T3 cell lines (*83*). Epidermal growth factor (EGF) appears to provoke inositol phospholipid breakdown in only a few tissues, such as human A431 epidermoid carcinoma cells (*84*). However, several growth factors, including platelet-derived growth factor (PDGF) (*85*), induce rapid breakdown of inositol phospholipids (*86*). Inversely, some short-term agonists, such as bombesin and α_1-adrenergic agents, occasionally exert long-term effects and stimulate cells to divide (*87*).

Tumor promoter and permeable diacylglycerol are known to induce gene expression of ornithine decarboxylase (*88*), histidine decarboxylase (*89*), serotonin *N*-acetyltransferase (*90*), and probably γ-interferon (*91*), plasminogen activator (*92*), calcitonin (*93*), prolactin (*94*), and glycophorin (*95*). Some proto-oncogenes, such as c-*fos* (*96*) and c-*myc* (*97*), are potential targets of protein kinase C action. By analogy to lymphocyte activation, protein kinase C may induce expression of those genes that are related to the action of growth factors, thereby promoting long-term cell proliferation. In intact mouse lymphocytes it has been reported that histone H2B and H4 phosphorylation is enhanced by treatment with phorbol ester (*98*). However, as noted above, protein kinase C seems to be absent or to have only a weak presence in the nucleus. An additional step of signal transduction presumably is needed prior to the ultimate activation of nuclear events if protein kinase C really is involved.

Modulation of Membrane Functions

Several lines of evidence suggest that protein kinase C modulates ion conductance by phosphorylating membrane proteins such as channels, pumps, and ion exchange proteins. It has been proposed that protein kinase C plays a role in extrusion of Ca^{2+} immediately after its mobilization into the cytosol, and that Ca^{2+}-transport ATPase is a possible target of this protein kinase. Studies with many

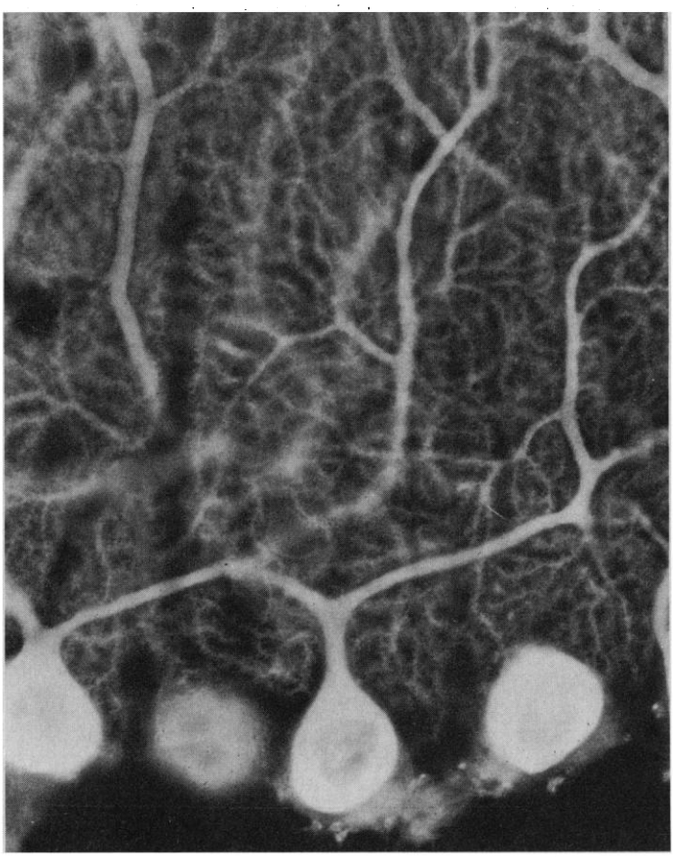

Fig. 1. Immunofluorescence of rat Purkinje cells stained with monoclonal antibody against protein kinase C. The properties of the antibody used and the detailed conditions for this staining will be described elsewhere.

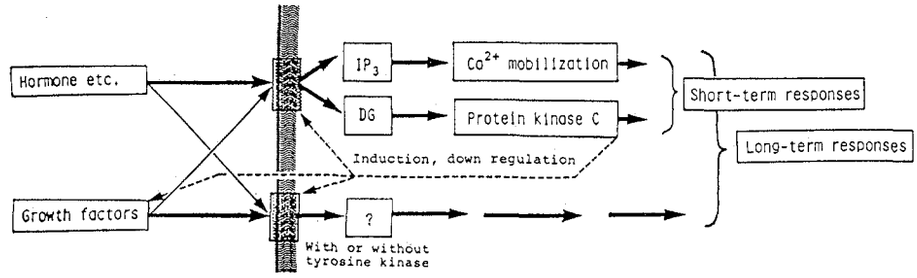

Fig. 2. Schematic representation of signal pathways for short-term and long-term cellular responses. Abbreviations: IP_3, inositol-1,4,5-trisphosphate; DG, 1,2-diacylglycerol.

cell types, including vascular smooth muscle and platelets, have shown that, when receptors are stimulated, the appearance of Ca^{2+} is transient (*14, 27*). In various cell types, the cytosolic Ca^{2+} concentration is frequently decreased by the addition of phorbol ester (*71, 99, 100*). From experiments with sarcoplasmic reticulum preparations from heart, it has been proposed that Ca^{2+}-transport ATPase is activated by the addition of protein kinase C (*101*). This protein kinase may also take part in the enhancement of Ca^{2+} entry, since microinjection of phorbol ester or protein kinase C itself into bag cell neurons from *Aplysia* enhances the voltage-sensitive Ca^{2+} current (*102*).

Similarly, a possible role of protein kinase C in activating Na^+-transport ATPase in peripheral nerve has been suggested (*103*). The Na^+/H^+ exchange protein appears to be another target that is activated by phorbol ester or by permeable diacylglycerol and, thus, protein kinase C may function to increase cytoplasmic *p*H (*104*). An additional example is obtained with photoreceptor cells of *Hermissenda* (*105*) and rat hippocampal pyramidal neurons (*106*). In these cells, treatment with phorbol ester or permeable diacylglycerol, as well as intracellular injection of protein kinase C, reduces Ca^{2+}-dependent K^+ conductance. This protein kinase has been proposed to play a role in the expression of plasticity, particularly in hippocampal neural activity (*107*) and learning of *Hermissenda* (*105*). It is worth noting that the phosphate attached to the substrate proteins of protein kinase C is sometimes resistant to the action of phosphatases and, therefore, the consequence of these phosphorylation reactions may persist for long periods (*14*). Ca^{2+} may initiate the physiological responses, and protein kinase C may help to ensure that the resulting biological events persist. It is also possible that other active membrane transport systems are modulated by protein kinase C.

Feedback Control and Down-Regulation

In biological systems positive signals are normally followed by immediate negative feedback control to prevent overshoot, and to allow responses to subsequent signals. A major function of protein kinase C appears to be intimately related to such feedback control of cell surface receptors, termed down-regulation. The stimulation of α_1-adrenergic receptor is well known to induce inositol phospholipid breakdown. Recent evidence obtained for hepatocytes (*108*) and smooth muscle (*109*) strongly suggests that protein kinase C exerts negative feedback control on the receptors of epinephrine (α_1 agonists) and angiotensin II. A similar feedback control by protein kinase C over the receptors that are coupled to inositol phospholipid breakdown has been suggested for many other cell types, including platelets (*14, 110*), neutrophils (*111*), PC12 cells (*112*), pituitary cells (*99*), astrocytoma cells (*113*), and hippocampal slices (*114*). The biochemical mechanism of this negative feedback control is a subject of great interest.

The feedback control or down-regulation by protein kinase C also extends to the receptors of other signaling systems. Protein kinase C phosphorylates the EGF receptor with a concomitant decrease in both its tyrosine-specific protein kinase and growth factor–binding activities (*115*). Phorbol ester markedly reduces the EGF binding in many mitogenically responsive cell types (*116*). In an analogous fashion, the receptors of insulin (*117*), somatomedin C (*117*),

Table 2. Proposed substrate proteins of protein kinase C.

Substrate proteins	References
Receptor proteins	
Epidermal growth factor receptor	*115*
Insulin receptor	*117*
Somatomedin C receptor	*117*
Transferrin receptor	*118*
Interleukin-2 receptor	*119*
Nicotinic acetylcholine receptor	*121*
β-adrenergic receptor	*122*
Immunoglobulin E receptor	*123*
Membrane proteins	
Ca^{2+}-transport ATPase	*101*
Na^+/K^+ ATPase	*103*
Na^+ channel protein	*124*
Na^+/H^+ exchange system	*104*
Glucose transporter	*125*
GTP-binding protein	*126*
HLA antigen	*127*
Chromaffin granule-binding protein	*128*
Synaptic B50 (F1) protein	*129*
Contractile and cytoskeletal proteins	
Myosin light chain	*63*
Troponin T and I	*130*
Vinculin	*131*
Filamin	*132*
Caldesmon	*133*
Cardiac C-protein	*134*
Microtubule-associated proteins	*135*
Enzymes	
Glycogen phosphorylase kinase	*136*
Glycogen synthase	*137*
Phosphofructokinase	*138*
β-Hydroxy-β-methylglutaryl–coenzyme A reductase	*139*
Tyrosine hydroxylase	*140*
NADPH oxidase	*141*
Cytochrome P450	*142*
Guanylate cyclase	*143*
DNA methylase	*144*
Myosin light chain kinase	*63*
Initiation factor 2	*145*
Other proteins	
Fibrinogen	*146*
Retinoid-binding proteins	*147*
Vitamin D–binding protein	*148*
Ribosomal S6 protein	*149*
GABA modulin	*150*
Stress proteins	*151*
Myelin basic protein	*152*
High-mobility group proteins	*153*
Middle T antigen	*154*
$pp60^{src}$ protein	*155*

transferrin (*118*), and interleukin-2 (*119*) may be targets of protein kinase C action. However, the function of phosphorylation of these receptors has not yet been elucidated. There is a suggestion that protein kinase C inhibits the internalization of EGF (*120*).

Negative feedback control by protein kinase C has been proposed also for intracellular processes, such as smooth muscle contraction. Myosin light chain and its kinase have been shown to serve as substrate proteins of protein kinase C in vitro and in vivo, and both reactions appear to counteract the Ca^{2+}-induced actin-myosin interaction (*63*).

Target Proteins

A potential role of protein kinase C has been postulated for the control or modulation of many metabolic and other processes. Table 2 lists some functionally defined proteins that have been proposed to serve as substrates for protein kinase C on the basis of in vitro experiments (*121–155*). This list of the phosphate acceptor proteins is expanding rapidly, although the physiological significance of many of these phosphorylation reactions remains to be explored.

Protein kinase C and cyclic adenosine monophosphate (AMP)–dependent protein kinase usually relay information along different signal pathways within the cell, but these two enzymes sometimes cause apparently similar cellular responses (*156*). These protein kinases often react with the same phosphate acceptor proteins, and even phosphorylate the same seryl and threonyl residues in a single protein molecule. For example, in myelin basic protein some seryl residues are phosphorylated by these two protein kinases equally (*157*). It is unclear why these two signaling systems normally exert different but sometimes similar effects on biochemical processes within a single activated cell. Extensive work by many investigators (*158*) has shown that the amino acid sequence in the vicinity of the phosphorylation site is a primary factor for substrate recognition. Although attempts have been made with some synthetic peptides (*159*), information about the substrate recognition of protein kinase C is still limited. The topographical arrangement or subcellular localization of the enzyme and its target proteins appears to be another crucial factor for determining substrate specificity.

Network of Signaling Systems

The signaling systems used by cells often display extensive heterogeneity, and many variations exist from tissue to tissue. Most tissues seem to have at least two major receptor classes for transducing information across the cell membrane. One class depends on the generation of cyclic AMP as a second messenger, while the other class of receptors induces rapid turnover of inositol phospholipids as well as mobilization of Ca^{2+}. Stimulation of the latter class normally leads to the release of arachidonate and often increases cyclic guanosine monophosphate (GMP). Thus, protein kinase C activation, Ca^{2+} mobilization, arachidonate release, and cyclic GMP formation appear to be integrated into a single receptor cascade (*8*).

Cellular responses may be divided into several modes (Fig. 3) (*160–174*). In bidirectional control systems the two classes of receptors appear to counteract each other, whereas in monodirectional control systems one receptor class may potentiate the other one (*175*). In cell types such as platelets, neutrophils, and lymphocytes, the signals that induce inositol phospholipid breakdown activate cellular functions such as release reactions and proliferation, but the signals that produce cyclic AMP usually antagonize such activation. In those cells the signal-induced breakdown of inositol phospholipids and the subsequent events eventually leading to cellular responses are all profoundly blocked by cyclic AMP. Conversely, in another group of cell types, such as avian erythrocytes, Leydig cells, and glioma C6, protein kinase C inhibits and desensitizes the adenylate cyclase system. In several other cell types, including pinealocytes, pituitary cells, and lymphoma S49, protein kinase C greatly potentiates cyclic AMP production. There is as yet no obvious example of a tissue in which cyclic AMP potentiates signal-induced turnover of inositol phospholipids. However, in many endocrine cells such as pancreatic islets, these two signal transduction pathways frequently act in concert to induce hormone secretion. The evidence presented thus far is still incomplete, but it is reasonable to assume that various combinations of the two receptor systems cooperate positively and thereby intensify responses in many physiological processes. Tumor promoters are well known often to enhance cell differentiation rather than cell proliferation. Additional major interactions of the signaling systems exist between calcium and cyclic AMP (*176*). Further exploration of various interactions and the network of the signaling systems in individual cell types will be of crucial importance for understanding the molecular basis of transmembrane control of cellular functions, growth, and differentiation.

Second Messenger Cascade and Coda

The available evidence on protein kinase C seems to indicate its crucial role in signal transduction for the activation of many cellular functions and for the control of cell proliferation. In fact, this

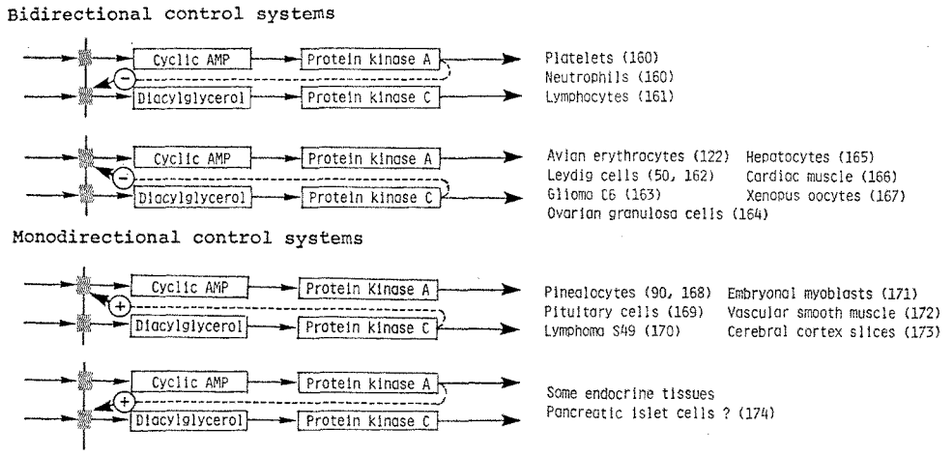

Fig. 3. Modes of interaction of two major signal-transducing systems. Protein kinase A, cyclic AMP–dependent protein kinase. Numbers in the parentheses indicate the references cited.

research field has seen explosive growth over the past few years, and recent results suggest that both of the two messengers, diacylglycerol and I-1,4,5-P_3, are each metabolized further to produce several compounds that may be additional new messengers. Diacylglycerol produces a series of biologically active arachidonic acid metabolites (*177*), among which lipoxin A and some other eicosanoids activate protein kinase C to some extent (*178*). I-1,4,5-P_3 is phosphorylated to form a series of inositol polyphosphates: inositol tetrakisphosphate, pentakisphosphate, and hexakisphosphate (*87, 179*). Inositol-1,3,4-trisphosphate, an isomer of I-1,4,5-P_3, was also shown to occur several minutes after stimulation of cell surface receptors; this isomer is derived from inositol tetrakisphosphate by the removal of its 5-phosphate (*180*). In addition, 1,2-cyclic phosphate analogs of some of these inositol polyphosphates have been identified (*181*). The biological functions of these compounds are not yet understood, but it is an attractive speculation that these second messenger cascade reactions constitute positive forward and negative feedback controls over a variety of sequential events occurring in physiological and pathological cellular responses.

Although the bifurcating pathway starting from PIP_2 hydrolysis appears to be firmly established in signal transduction, the mechanism of this receptor-mediated lipid hydrolysis is unclear. It is possible that GTP and its binding protein are involved in this process (*182*), but the physiological picture in various tissues may not be explained by a unified single mechanism, since there appear to be considerable tissue variations in the mode of interaction of cell surface receptors, as noted above. It also seems premature to discuss the detailed relation between the function of Ca^{2+} and that of protein kinase C. Each of these signal pathways may play diverse roles in controlling biochemical reactions. In particular, the protein phosphorylation catalyzed by protein kinase C may exert profound modulation of various Ca^{2+}-mediated processes, such as release reactions and exocytosis, cell proliferation and differentiation, membrane conductance and transport, potentiation and desensitization of other receptor systems, smooth muscle contraction, and some metabolic processes. Further exploration of the role of this protein kinase, especially its target proteins in each tissue, may provide clues to understanding the biochemical basis of signal transduction and cell-to-cell communication.

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Studies on Environmental Chemical Carcinogenesis in Japan

TAKASHI SUGIMURA

The historical background of studies in Japan on chemical carcinogenesis from environmental sources is described from personal experience.

IN 1915, YAMAGIWA FIRST SUCCEEDED IN PRODUCING CANcers in experimental animals at Tokyo Imperial University. This opened the door for studies of environmental chemical carcinogenesis throughout the world. He and his co-worker Ichikawa repeatedly applied coal tar that had been dissolved in benzene to the ears of rabbits. After persistent experimentation, squamous cell carcinoma–like tumors were produced (*1*). Yamagiwa was inspired (*1*) by Virchow's irritation theory of carcinogenesis and by Pott's observation of the high incidence of scrotum cancers in chimney sweeps. This was followed by the work of Tsutsui (*2*), of Chiba Medical College, who produced cancers in a shorter time by painting coal tar on the skin of mice.

In England, scientists under the direction of Kennaway fractionated coal tar into the active compounds that caused cancer with Tsutsui's method as a bioassay. The compound 1,2,5,6-dibenzanthracene was synthesized as the first pure chemical substance capable of producing mouse skin cancers in 1930 (*3*). Benzo[*a*]pyrene was also isolated by the same group from coal tar in 1932 (*4*). The scientific atmosphere in Japan at Yamagiwa's time did not encourage him to pursue collaborative work with scientists from different disciplines. Nevertheless, his contribution in demonstrating the first man-made cancer in

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