Protein Kinase C Family

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Protein kinase C is a family of enzymes that has a central role in transducing information from external stimuli to cellular responses. Members of this family of serine/threonine kinases respond to signals that cause lipid hydrolysis. Protein kinase C isozymes phosphorylate an abundance of substrates, leading to both short-term cellular responses such as regulation of membrane transport and long-term responses such as memory and learning.

Historical Perspective

Protein kinase C was discovered in the late 1970s by Yasutomi Nishizuka and colleagues at Kobe University, Japan. Their initial discovery was of a constitutively active enzyme that required only Mg^{2+} for activity (and hence was named protein kinase M, PKM). Further studies revealed that PKM was a proteolytic product of a full-length enzyme; this enzyme was named protein kinase C because its enzymatic activity could be released by a Ca^{2+}-dependent protease. The subsequent discovery that protein kinase C is activated by the phospholipid hydrolysis product, diacylglycerol, was a major finding in biology: it provided the molecular mechanism for how lipid hydrolysis, discovered 25 years earlier to be triggered by stimuli such as acetylcholine, couples to cellular signaling pathways.

But the discovery that catapulted research on protein kinase C to the forefront of cellular signaling was the finding that it is the receptor for the potent tumor-promoting phorbol esters. Phorbol esters are present in the milky sap exuded from plants of the Euphorbiaceae family; the oil from the seeds of one member of this family, in particular croton tiglium, has particularly strong irritant properties and, as such, has been used over the millennia for purposes as varied as poison for hunting arrows to medicinal purposes. In the 1960s, the active ingredient in the oil was found to be a family of diesters of the tetracyclic diterpene phorbol. Phorbol esters were shown to be extremely potent tumor promoters, and classic studies revealed that painting phorbol esters on the skin of mice allowed otherwise subthreshold amounts of carcinogens to promote tumors. The finding that protein kinase C is the direct molecular target of phorbol esters placed this enzyme at the center of signaling pathways that control normal cell function and carcinogenesis.

Protein Kinase C Family Members

There are ten mammalian isozymes of protein kinase C that share in common a carboxyl-terminal kinase domain linked to an amino-terminal regulatory moiety (Figure 1). The regulatory moiety, in turn, contains a number of functional modules and it is the composition of these functional modules that further defines the three subfamilies of the protein kinase C isozymes. These modules are an autoinhibitory-pseudosubstrate sequence that maintains the enzyme in an inactive conformation, and one or two membrane-targeting modules that direct protein kinase C to the membrane following generation of the appropriate second messengers. Specifically, the C1 domain binds diacylglycerol and phorbol esters and the C2 domain binds Ca^{2+}; each event promotes the binding of the respective domain to membranes.

Conventional protein kinase C isozymes (α, βI, βII, and γ), have a C1 and a C2 domain and respond to both diacylglycerol and Ca^{2+}. Novel protein kinase C isozymes (δ, ε, η, and θ/L) have a C1 domain that binds diacylglycerol, but an impaired C2 domain that does not bind Ca^{2+}. These isozymes respond to cellular increases in diacylglycerol but not Ca^{2+}. Atypical protein kinase C isozymes (ζ and ι/λ) have an impaired C1 domain and no C2 domain and bind neither diacylglycerol nor Ca^{2+}. Thus, stimuli that elevate intracellular diacylglycerol activate conventional and novel protein kinase C family members, with conventional isozymes being additionally regulated by Ca^{2+}.

Protein Kinase C Phosphorylation

Before protein kinase C is competent to signal, it must first be processed by a series of ordered phosphorylations. The first is mediated by an upstream kinase, the phosphoinositide-dependent kinase, PDK-1.

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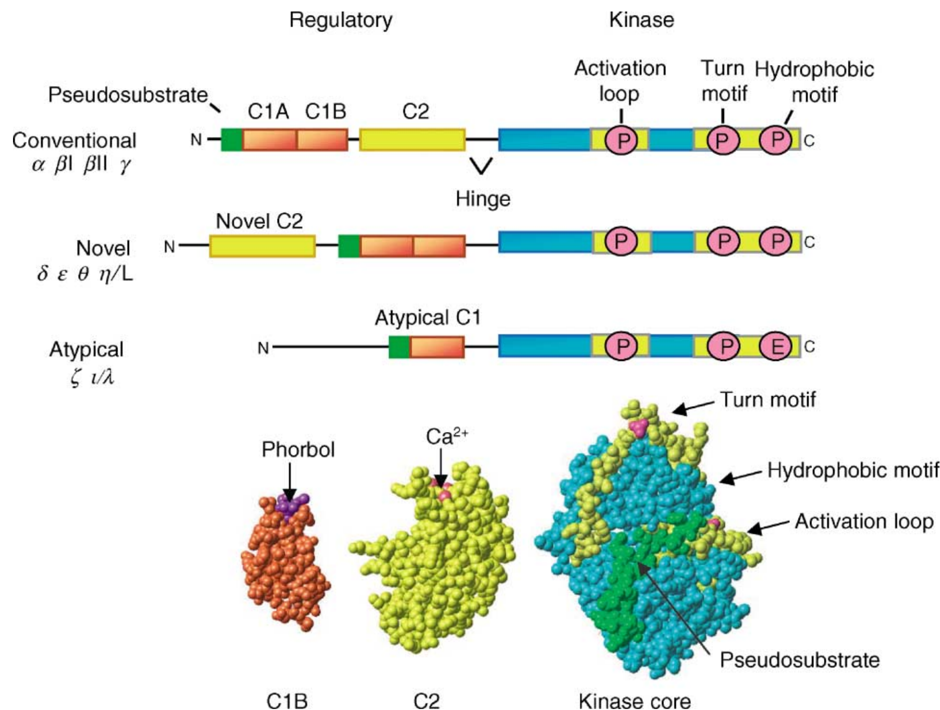

FIGURE 1 Primary structure and domain composition of protein kinase C family members. The amino terminal regulatory moiety contains the autoinhibitory pseudosubstrate sequence (green), the C1 domain, which binds diacylglycerol/phorbol esters (orange; present as a tandem repeat in conventional and novel protein kinase C isozymes), and the C2 domain, which binds Ca^{2+} (yellow). The C2 domain in novel protein kinase Cs and the C1 domain in atypical protein kinases are nonligand-binding variants. The carboxyl terminal catalytic moiety contains the kinase core which has two phosphorylation segments, the activation loop segment and the carboxyl-terminal segment (yellow), with a total of three phosphorylation sites (pink circles; the negatively charged amino acid glutamate (E) occupies the position of the phospho-acceptor position of the hydrophobic motif in atypical protein kinase Cs). The 3D structures of the domains are shown below the primary structure. Reproduced from Newton, A. C. (2003). Regulation of the ABC kinases by phosphorylation: Protein kinase C as a paradigm. *Biochem. J.* **370**, 361–371, by permission of the Biochemical Society.

This kinase has a pivotal position in cell signaling because it provides the activating phosphorylation to many other protein kinases, including the prosurvival kinase, Akt/protein kinase B. PDK-1 phosphorylates a conserved segment near the entrance to the active site referred to as the activation loop (Figure 1), an event that structures the active site for substrate binding and catalysis. The phosphorylation of the activation loop by PDK-1 triggers two intramolecular autophosphorylation reactions at two conserved positions in the carboxyl terminus, the turn motif, and hydrophobic motif (Figure 1). These phosphorylations lock protein kinase C in its mature and catalytically competent conformation. It is this species of protein kinase C that is activated by lipid hydrolysis and transduces signals.

Protein Kinase C Translocation

Mature (i.e., phosphorylated) protein kinase C is typically localized to the cytosol where it bounces on and off the membrane by diffusion-controlled mechanisms. It is maintained in an inactive conformation because the pseudosubstrate sequence occupies the substrate-binding cavity. For conventional protein kinase C isozymes, generation of Ca^{2+} and diacylglycerol target protein kinase C to the membrane by binding the C2 and C1 domains and thus tethering the enzyme to membranes. The membrane-bound species adopts an active conformation by removal of the pseudosubstrate from the substrate-binding cavity, allowing substrate binding, phosphorylation, and downstream signaling. Finding a membrane-embedded ligand (diacylglycerol) by diffusion from the cytosol is a low-probability event, and, in the case of conventional protein kinase Cs, nature has chosen a clever mechanism to increase the efficiency of this. Binding of Ca^{2+} to the C2 domain essentially pretargets protein kinase C to the membrane, where it can now initiate a much more effective search for its membrane-embedded ligand, diacylglycerol. As a consequence, conventional protein kinase Cs translocate to membranes ~1 order of magnitude faster than novel protein kinase C isozymes, which do not have the advantage of pretargeting by a Ca^{2+}-responsive C2 domain.

The advent of fluorescent methodologies has allowed imaging of protein kinase C translocation, and, most recently, activity in real time in living cells (Figure 2).

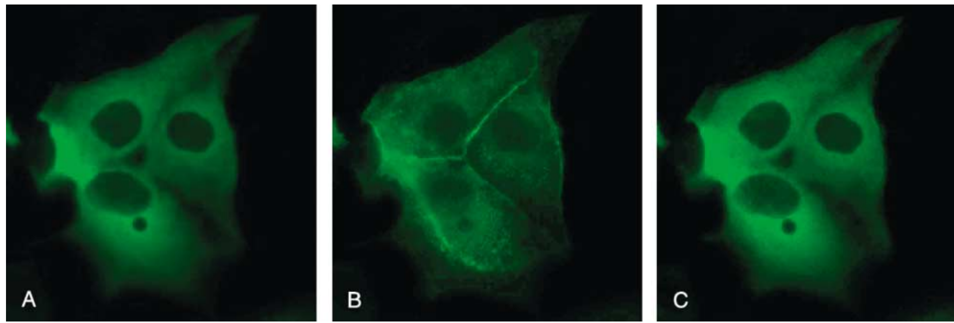

FIGURE 2 Protein kinase C was visualized in cells by expression of a construct of protein kinase C fused to a naturally fluorescent protein from the jellyfish *Aequorea victoria*, the green fluorescent protein (GFP). Panel A shows that protein kinase C is localized to the cytosol in unstimulated MDCK cells (diffuse fluorescence throughout the cell); panel B shows that protein kinase C translocates to the membrane (strong fluorescence intensity at cell peripheri) following 1 min of treatment with the agonist UTP, which induces phospholipid hydrolysis and generation of the two second messengers for protein kinase C:Ca^{2+} and diacyglycerol. Panel C shows that protein kinase C has redistributed back to the cytosol 5 min after UTP treatment; second messenger levels have returned to resting levels. Images courtesy of Jon Violin.

In general, protein kinase C translocation and activity mirrors the generation of its second messengers. For example, histamine stimulation of HeLa cells results in oscillations in protein kinase C substrate phosphorylation that are phase-locked with Ca^{2+} oscillations.

Protein Kinase C Scaffolds

Correct subcellular location is essential for normal signaling by protein kinase C. An abundance of scaffold proteins that tether protein kinase C near its substrates, activators, and regulatory proteins, such as phosphatases, has been described. The importance of correct subcellular location is perhaps best illustrated in the *Drosophila* visual cascade, where mutants lacking the protein kinase C-binding scaffold, InaD, are defective in visual transduction because components of the signaling cascade are mislocalized.

Protein Kinase C Downregulation

Prolonged activation of protein kinase C by treatment of cells with phorbol esters results in degradation of protein kinase C, a phenomenon referred to as down-regulation. In fact, prolonged treatment of cells with phorbol esters is a commonly used approach to deplete cells of all except atypical protein kinase Cs (these are resistant to phorbol ester-dependent down-regulation because they do not bind phorbol esters). The molecular mechanism of this down-regulation involves dephosphorylation of activated protein kinase C, followed most likely by ubiquitination and proteolysis. The molecular chaperone HSP70 has recently been shown to protect protein kinase C from down-regulation by allowing rephosphorylation of the enzyme and sustaining its signaling lifetime.

Protein Kinase C Signaling

Protein kinase C phosphorylates an abundance of substrates, including membrane proteins, cytoskeletal proteins, cytosolic proteins, and nuclear proteins. Yet identifying the precise cellular role and cellular targets of protein kinase C remains elusive. Genetic deletion of specific isozymes results in subtle phenotypic differences, suggesting functional redundancy of the isozymes. Nonetheless, sifting through the abundant studies on protein kinase C function reveals a few defined themes in addition to the general involvement in cell growth and proliferation. Notably, animals deficient in protein kinase C isozymes are deficient in adaptive responses. For example, mice lacking protein kinase C ε have reduced anxiety and have reduced tolerance to alcohol. Mice lacking protein kinase C γ have reduced pain perception, and mice lacking protein kinase C β II have reduced learning abilities and an impaired immune response. This theme carries over to the molecular level where many of the substrates of protein kinase C are receptors which become desensitized following phosphorylation by protein kinase C.

Isozyme-specific functions have been most clearly delineated for novel and atypical protein kinase C isozymes. For example, protein kinase C δ activation has been shown to play a role in apoptosis. Protein kinase C θ plays a key role in immune responses, and mice deficient in this isozyme have impaired T cell signaling and interleukin 2 production. Defined functions have also been established for protein kinase C ζ: this isozyme is required for maintenance of cell polarity and, in addition, regulates cell growth, DNA synthesis, and activation of the transcription factor, NFκB. Much less is known about defined physiological substrates and functions of conventional protein kinase C isozymes. Defining the precise raison d'etre for this

multi-membered class of kinases is one of the pressing issues in biological chemistry.

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Glossary

diacylglycerol The membrane-retained lipid backbone released from phospholipids following activation of appropriate phospholipases, enzymes that hydrolyze phospholipids. Diacylglycerol is considered a second messenger because it transfers information from stimuli such as hormones to protein kinase C, which transduces the signal by phosphorylating protein substrates.

kinase The class of enzymes that covalently transfer phosphate from ATP to hydroxyl groups of proteins.

phosphorylation The covalent attachment of phosphate from the cellular energy currency, ATP to hydroxyl residues of proteins, a modification that changes the properties of the protein.

Further Reading

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Biography

Alexandra Newton is a Professor in the Department of Pharmacology at the University of California, San Diego, where her research team investigates the biology and chemistry of signaling by protein kinases, with particular focus on the molecular mechanisms of protein kinase C. She holds a Ph.D. in chemistry from Stanford University and received her postdoctoral training with Daniel E. Koshland, Jr., at the University of California, Berkeley.