**To the heart of the matter:**

The many roles of protein kinase C-delta

**Biological processes are regulated by complex networks of interacting messages called signaling pathways. To occur at rates fast enough to sustain life, these biological processes need enzymes — biological catalysts that speed up chemical reactions. One important family of enzymes crucial for regulating cell function is the protein kinase C (PKC) family. PKCs have been shown to be involved in a diverse range of biological processes including the regulation of cell growth, differentiation, metabolism, and cell death (apoptosis). As well as their role in normal biological processes, PKCs have been implicated in maladaptive pathological responses that drive a wide range of clinical disorders including diabetes, Alzheimer’s disease and cardiovascular disorders. Consequently, the PKC family has been the focus of intensive research ever since its discovery in 1977.

**ONE ENZYME, MANY FORMS**

PKC was originally thought to be a single protein. We now understand that PKCs comprise of a family of structurally related subtypes (called isoforms); they are classified depending on their structure, function, and cofactor (helper molecule) requirements. PKCs are serine/threonine kinases, they exert intricate control over various aspects of protein function in health and disease by transferring a phosphate to the hydroxyl (OH) group of either a serine or threonine residue in an acceptor protein. This process is called phosphorylation.

One particular PKC isoform – protein kinase C-delta (PKCδ) – is the focus of research for Professor Susan Steinberg, Professor of Pharmacology at Columbia University Irving Medical Center. An important enzyme, PKCδ has the ability to phosphorylate multiple target proteins involved in a diverse range of biological processes in the heart, both in health and disease, including regulation of cardiac muscle contraction, the severity of ischemia/reperfusion injury, and the pathogenesis of cardiac hypertrophy and failure. Elegantly combining a wide range of reductionist molecular and cell biological approaches, Professor Steinberg has revolutionized the thinking behind the intricate roles of PKCδ in health and disease. Her ultimate aim is to develop potential therapies targeted to PKCδ-dependent pathological cardiac remodeling.

**CONTEXT-SPECIFIC ROLES OF PKCδ**

Professor Steinberg’s early interest in PKCδ was sparked by intriguing differences that set it apart from other enzymes in the PKC family. The hallmark of activation for a conventional PKC is its translocation to the cell’s plasma membrane. After docking to the membrane, PKC sits near target substrates where it carries out its catalytic effects. Professor Steinberg noted that this mechanism failed to explain PKCδ’s actions in heart cells, where PKCδ phosphorylates proteins away from the cell membrane. Professor Steinberg also observed that PKCδ has diverse and in some cases opposing actions – for example its role in both ischemic/reperfusion injury and cardioprotection. This did not fit with the idea that PKCδ’s catalytic ability is an inherent property of the enzyme, which remains unaltered when the enzyme is activated.

To address this quandary Prof Steinberg embarked on a series of careful experiments in heart cells (cardiomyocytes). She provided compelling evidence that PKCδ is activated in a stimulus-specific manner in cardiomyocytes. Specifically she showed that growth factor stimuli activate PKCδ at lipid membranes, but PKCδ becomes a lipid-independent enzyme during oxidative stress. Importantly, this change means it is poised to phosphorylate substrates throughout the cell. An additional observation suggested a molecular mechanism to explain this change in enzyme function: that oxidative stress results in the activation of Src family tyrosine kinases (a family of enzymes that phosphorylate target proteins on tyrosine residues), and that PKCδ is unique among PKC family enzymes in that it is a target for regulatory tyrosine phosphorylation by Src kinases. In a series of carefully designed experiments, Professor Steinberg used molecular and biochemical approaches to show that oxidative stress leads to an increase in PKCδ phosphorylation at tyrosine-311, a tyrosine residue in the hinge region of the enzyme that is the major site for PKCδ phosphorylation by Src. She then showed that phosphorylation at this tyrosine residue converts PKCδ from a lipid-dependent serine kinase (an enzyme that acts primarily to phosphorylate target substrates on serine phosphoacceptor sites) to a lipid-independent serine/threonine kinase (an enzyme that can now phosphorylate substrates on either serine or threonine).

**Professor Steinberg has revolutionised the thinking behind the intricate roles of PKCδ in health and disease.**

**REVISED MODEL: DISTINCT MODES FOR PKCδ ACTIVATION.**

**Caspease-3 Cleavage site**

<table>
<thead>
<tr>
<th>REGULATORY DOMAIN</th>
<th>KINASE DOMAIN</th>
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<tr>
<td>C1A</td>
<td>C1B</td>
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**G-Loop**

**C-tail**

**αC2**

**Y311**

**S643 S662**

**S357**

**C2**

**C1A**

**C1B**

**Kinase**

**Lipid Membrane**

**GPCR Agonists**

**Oxidative Stress**

**Src**

**ALLOSTERIC ACTIVATION - CARDIOPROTECTION**

**Lipid-dependent Ser kinase**

**ACTIVATION VIA Y311 PHOSPHORYLATION – I/R INJURY**

**SER/THR kinase**

**Physiologic stimuli lead to the generation of lipid cofactors that allosterically activate PKCδ at membranes. Oxidative stress leads to the activation of Src family kinases and the phosphorylation of PKCδ at Y311. This generates a docking site for the C2 domain of PKCδ, resulting in a C2 domain–αC2 interaction that induces long-range conformational changes that culminate in Ser357 dephosphorylation. PKCδ is converted into a lipid-independent serine/threonine kinase as a result of the decrease in Ser357 phosphorylation.**
PROTEOLYTIC ACTIVATION DURING APOPTOSIS

KD is a lipid-independent enzyme. PKCδ is converted into a constitutively active catalytic domain fragment as a result of caspase-3 cleavage during apoptosis.

Steinberg continues to unravel the agonist-specific PKCδ signalling ‘modes’ that link the enzyme to functionally distinct cellular responses.

References


Personal Response

Your research has revolutionised our understanding of the many actions of PKCδ. What has been the highlight of your career so far?

There have been several ‘Ah-Ha’ moments in my career, particularly as it relates to studies of PKCδ. The satisfaction of conjuring up a unifying simple model to explain previously confusing experimental data and then being able to guide the many talented members of my laboratory as they painstakingly pursue various experimental strategies to ultimately substantiate our initial hypothesis cannot be overstated. Similarly, the ability to use biochemical approaches to identify novel molecular determinants of PKCδ – in essence using a biochemical strategy to infer structural information typically defined using crystallographic methods that directly interrogate protein structure – also has been extremely rewarding.