Diacylglycerol's affair with protein kinase C turns 25

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This year marks the 25-year anniversary of the discovery by Nishizuka and co-workers that diacylglycerol activates the ubiquitous signal transducer protein kinase C. This discovery placed the lipid second messenger–protein kinase C signaling pathway center stage alongside the cAMP–protein kinase A pathway, which was already established as a fundamental mechanism for transducing extracellular signals.

'A small quantity of unsaturated diacylglycerol (DG) sharply decreased the Ca^{2+} and phospholipid concentrations needed for full activation of a Ca^{2+}-activated, phospholipid-dependent multifunctional protein kinase described earlier.' Accurate, to the point, and without any pomp and circumstance, so began an article by Takai, Kishimoto, Kikkawa, Mori and Nisizuka that was published in 1979 [1]. This basic finding, presented as a series of dose–response curves, has emerged as one of the most important breakthroughs in understanding cellular signaling (Figure 1). Twenty-five years of research, described in >40 000 research articles, emanated from the seminal discovery that diacylglycerol is the second messenger that triggers protein kinase C (PKC) signaling.

Lipids as second messengers

In 1952, Hokin and Hokin set sail for Canada from England, taking with them a rack of ethanol–ether extracts of lipids from ^{32}P-labeled pancreatic slices. These samples, which Hokin and Hokin had not had time to analyze before the ship set sail, allowed them to unearth the first clues that lipids transduce signals [2]. Specifically, they discovered that acetylcholine-mediated stimulation of pancreatic slices promoted the incorporation of ^{32}P from radiolabeled ATP into phospholipids.

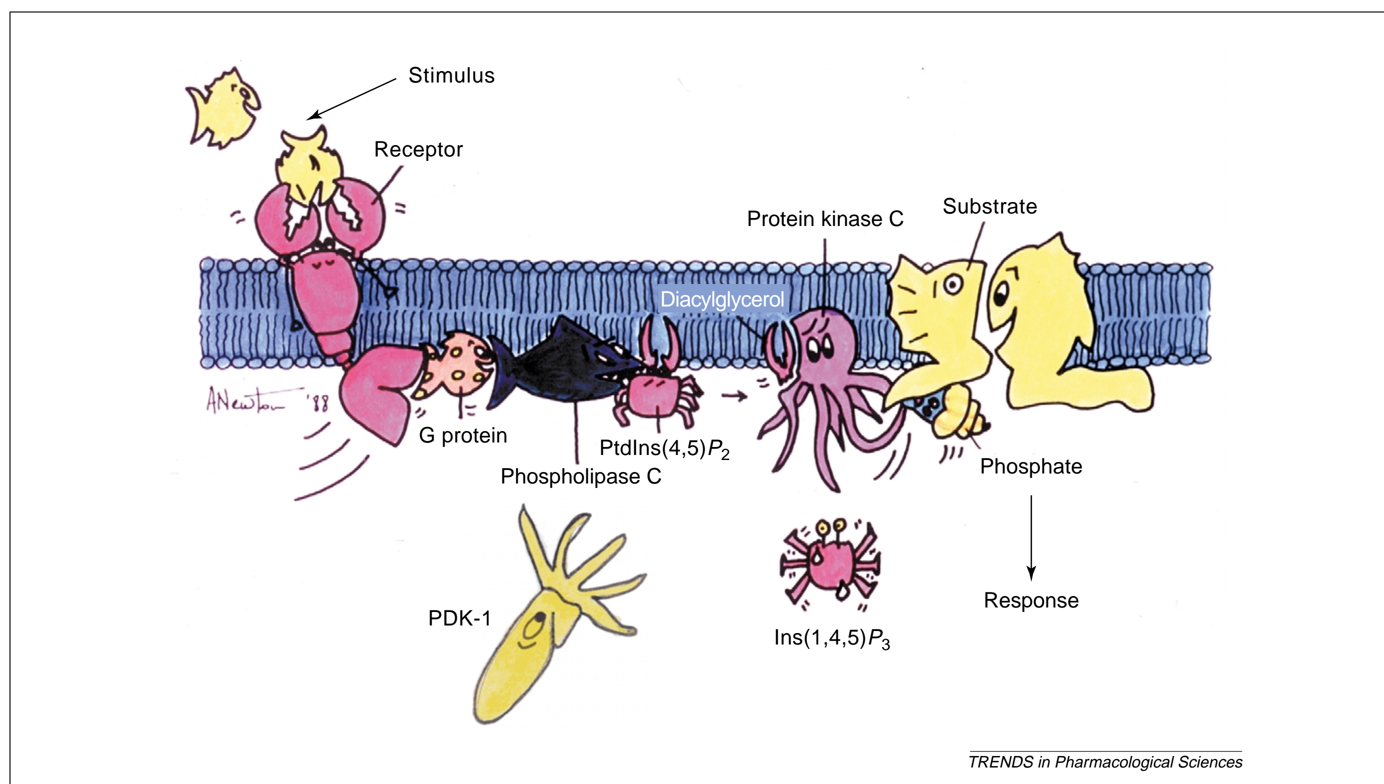

Figure 1. Cartoon showing that diacylglycerol, generated by receptor-mediated hydrolysis of phospholipids, tethers protein kinase C (PKC) to the membrane. Hydrolysis of phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2 (crab)] generates two second messengers: diacylglycerol, which engages the C1 domain of PKC to membranes, and inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3], which releases intracellular Ca^{2+} causing the C2 domain of conventional isozymes of PKC to become tethered to the membrane. Although there are other ways to regulate PKC activity [29], this scheme represents the classic pathway culminating from the discovery of Nishizuka and co-workers, which is celebrated in this article. Also shown is the upstream phosphoinositide-dependent kinase PDK-1 (see main text), which phosphorylates PKC as part of its maturation process. Note that there are other targets for diacylglycerol, although activation of PKC remains the primary target of this second messenger [24].

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(The observed increase in ^{32}P labeling represented the recycling of hydrolyzed phospholipids). It soon became apparent that phosphoinositides, a minor component of cell membranes, were hydrolyzed following stimulation of cells by a large variety of extracellular signals. How lipid hydrolysis transduced information within the cell remained a mystery for the next 25 years.

In the 1970s, Yasutomi Nishizuka and his team, working in the foothills above Kobe, Japan, were studying protein kinases. They chromatographically purified a protein kinase that appeared to be constitutively active, requiring nothing other than Mg^{2+} for activity. Without much else to base the name on, this kinase was called protein kinase M (PKM) [3]. Working long and hard in the cold room, the Nishizuka team found that much higher levels of this novel kinase could be purified from stocks of frozen brain rather than from the brains of freshly sacrificed rats. In addition, yields improved even more when, for economy, protease inhibitor levels were reduced. These insightful investigators then drew on their experience with protein kinase G, which they had shown several years earlier to become constitutively active following proteolysis. The Nishizuka team hypothesized that they were studying a constitutively active proteolytic fragment that had lost its auto-inhibition, and set out to identify the 'pro-enzyme' and the activators that conferred phosphotransferase activity.

In 1979, the Nishizuka group identified the pro-enzyme and reported that it was activated by membrane phospholipids, in particular phosphatidylserine. They also determined that it was activated by the Ca^{2+}-dependent protease calpain [4] and therefore named it PKC. The group soon noticed that crude extracts of phospholipids from brains, rather than pure lipids, were the most effective at activating PKC and set out to uncover the nature of the 'impurity' that activated PKC. The activating component was diacylglycerol, leading Nishizuka to make the conceptual breakthrough that PKC might be the target for one of the products of the lipid hydrolysis pathway discovered by Hokin and Hokin.

Following on the heels of the seminal finding that diacylglycerol activated PKC directly were the discoveries by Michell's laboratory that the substrate for the phospholipase that catalyzed the production of diacylglycerol was a minor phospholipid, phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] [5], and the findings by Schultz, Irvine and Berridge that the inositol (1,4,5)-trisphosphate headgroup produced by agonist-stimulated hydrolysis functioned as a second messenger to release internal Ca^{2+} [6]. Thus, PtdIns(4,5)P_2 hydrolysis produced two second messengers, and both regulated the activation of PKC.

Protein kinase C in the past 25 years

Nishizuka's finding that diacylglycerol was the second messenger that activated PKC has been followed by several other landmark findings that are summarized briefly here. In 1982, Nishizuka's team, working with Castagna, reported that phorbol esters directly bind and activate PKC [7]. This finding was made possible by the synthesis in Peter Blumberg's laboratory of relatively hydrophilic phorbol esters, notably phorbol dibutyrate (PDBu), that allowed the demonstration of specific, saturable and high-affinity binding of phorbol esters to cell membranes [8]. Soon after the report that PKC binds phorbol esters was the discovery by Anderson, Sando, and co-workers that phorbol esters cause the rapid redistribution of PKC activity from the cytosol to the membrane faction of cells [9,10]. This 'translocation' has served as the hallmark of PKC activity ever since.

In the mid-1980s, biochemical fractionation studies by Huang and co-workers led to the first suggestion that PKC activity results from a heterogeneous population of proteins [11]. This was confirmed in 1986, when the molecular cloning of PKC led to the realization that PKC comprises a family of isozymes, related by a conserved kinase core and differing in the domain composition of the regulatory moiety [12,13]. We now know there are 10 mammalian isozymes of PKC grouped into conventional, novel and atypical subfamilies. In the late 1980s, it was first appreciated in a report from the Fabbro laboratory that PKC is a phospho-protein [14]. This led to the realization that PKC isozymes are processed by a series of ordered phosphorylations that are required before the enzyme can respond to lipid second messengers [15,16]. In 1998, three groups reported that the newly discovered upstream kinase for Akt (protein kinase B), the phosphoinositide-dependent kinase PDK-1, was the upstream kinase for PKC family members [17–19]. Research during the 1990s also led to the appreciation that the location of PKC in the cell is under the control of anchoring or scaffold proteins, an idea pioneered by Mochly-Rosen [20,21]. Knockout studies during the past few years by Leitges, Messing and others have also established isozyme-specific functions of the PKC family [22,23]. In the past decade, it has also become apparent that non-PKC molecules are targets for diacylglycerol; although some of these molecules contain the structural domain that binds diacylglycerol (C1 domain), others do not [24]. Advances in cell imaging have allowed visualization of agonist-stimulated translocation [25,26]. Most recently, novel technologies to visualize PKC activity in real time in live cells have revealed a tightly controlled balance between cofactors and phosphatases that can result in oscillatory phosphorylation in the signaling by this key enzyme [27].

The molecular mechanisms that control the function of PKC in cells are now understood with much clarity. The major challenge now is to understand the cellular function of individual family members. A series of reviews in the *Biochemical Journal* succinctly summarize the current understanding of signaling by specific family members (see [28] and reviews therein).

Protein kinase C and pharmacology

In the late 1970s, studies of signal transduction mechanisms revolved around protein kinase A. This kinase was well recognized as being a crucial player in transducing signals from G-protein-coupled receptors following the activation of adenylyl cyclase. The increases in cAMP levels following receptor activation were well established as activating protein kinase A, whose activity was known to regulate glycogen phosphorylase and other enzymes

involved in intermediary metabolism. Nishizuika's finding that PKC is activated by another endogenous second messenger, this one being formed in response to activation of phospholipase C by what turned out to be a distinct family of G-protein-coupled receptors, led to the recognition that this kinase also plays a crucial role in signal transduction.

PKC is a therapeutic target for pathological signaling through hormones, growth factors, neurotransmitters and an abundance of other inputs. Although it has been difficult to ascribe specific functions to specific PKC isozymes and thus to target them for therapeutic intervention, the next 25 years are likely to provide key insight into the raison d'être and selectivity of this ubiquitous family of enzymes.

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p53: 25 years after its discovery

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Since its discovery 25 years ago, the p53 protein has emerged as a key tumor suppressor protein at the cross-roads of cellular stress response pathways. Through these pathways, which can lead to cell-cycle arrest, DNA repair, cellular senescence, differentiation and apoptosis, p53 facilitates the repair and survival of damaged cells or eliminates severely damaged cells from the replicative pool to protect the organism. Because of

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