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Measuring the Interaction of Protein Kinase C with Membranes

An Introduction

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1. Translocation: The Hallmark for Protein Kinase C (PKC) Activation

The translocation of PKC to membranes has served as the hallmark for PKC activation since the historic discovery that phorbol esters cause PKC to redistribute from the cytosol to the membrane ***(1,2)***. In the 20 years since this discovery, the literature has been flooded with reports on the intracellular redistribution of PKC after agonist or phorbol ester stimulation ***(3)***. In recent years, this translocation has been beautifully captured in real time in live cells with the advent of fluorescent technologies ***(4,5)***.

Extensive biophysical and biochemical studies have elucidated much of the molecular detail regarding the mechanism of translocation (reviewed in **ref. *6***). **Figure 1** shows a model based on these studies for the translocation of conventional PKC isozymes. In resting cells, PKC adopts a conformation in which the autoinhibitory pseudosubstrate (light grey rectangle; *see* **Fig. 1** in Chapter 1) is bound to the substrate-binding cavity; this inactive species bounces on and off the membrane by a diffusion-controlled mechanism. In the absence of ligands for its membrane-targeting modules, the C1 and C2 domains (*see* Chapter 1), the kinase is not retained at the membrane, and it diffuses back to the cytosol. (Note that scaffold or anchoring can retain PKC at specific intracellular locations, including the membrane, via protein:protein interactions; *see* Chapter 26.) The generation of signals that cause hydrolysis of phosphatidylinositol bis phosphate create two second messengers: inositol

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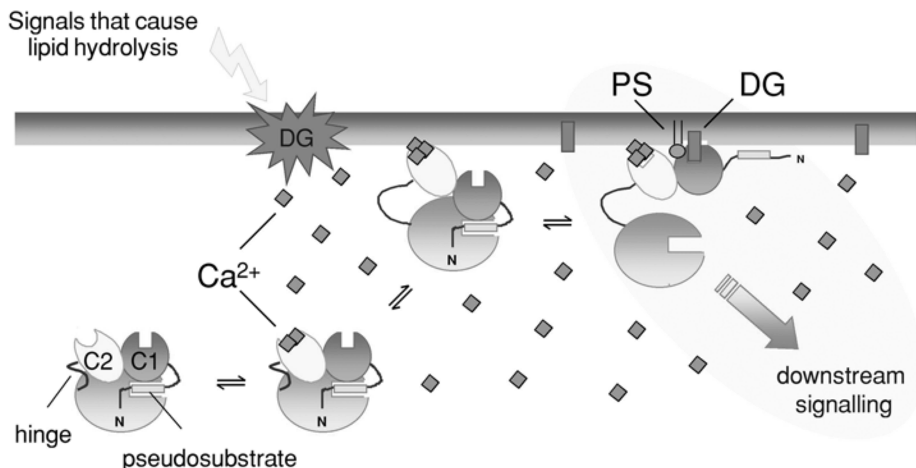

Fig. 1. Model describing the mechanism of translocation of conventional PKCs to membranes in response to signals that cause elevation of intracellular Ca^{2+} and diacylglycerol. In the absence of these stimuli, PKC localizes to the cytosol in a conformation in which the pseudosubstrate (light grey rectangle) blocks the substrate-binding cavity and a proteolytically labile hinge connecting the kinase domain (large circle with cleft) with the membrane targeting regulatory moieties is masked (species on far left). Elevation of intracellular Ca^{2+} (diamonds) recruits cytosolic PKC to the membrane by engaging the Ca^{2+}-bound C2 domain with anionic phospholipids, an event that unmasks the hinge but, because the interaction is of low affinity, does not result in release of the pseudosubstrate (middle). The membrane-bound enzyme then diffuses in the two-dimensional plane of the membrane searching for diacylglycerol; an encounter with this second messenger engages the C1 domain on membranes, with additional binding energy supplied by specific interaction of the C1 domain with phosphatidylserine (right). Engaging both domains on the membrane results in a high-affinity interaction that provides the energy to release the pseudosubstrate from the substrate-binding cavity, allowing phosphorylation and downstream signaling.

tris phosphate, which mobilizes intracellular Ca^{2+} (diamonds in **Fig. 1**), and diacylglycerol (dark grey rectangles in **Fig. 1**). Ca^{2+} binds the C2 domain of conventional PKCs (**Fig. 1**, second species from left), causing the affinity of this module for membranes to increase dramatically. Thus, on the next collision with the membrane, the kinase is retained at the membrane (**Fig. 1**, third species from left). Studies with the isolated C2 domain of a conventional PKC suggest that two Ca^{2+} ions bind cytosolic PKC and that a third Ca^{2+} ion bridges the C2 domain with anionic phospholipids in the membrane-bound species *(7)*. The enzyme then diffuses in the two-dimensional plane of the membrane until it encounters its membrane-localized ligand, diacylglycerol.

Binding of diacylglycerol to the C1 domain results in a high-affinity membrane interaction with a specific interaction between the diacylglycerol-bound C1 domain and phosphatidylserine, increasing the binding affinity of this module an additional 10-fold ***(8)***. The engagement of the C1 and C2 domains on the membrane provides the energy to release the autoinhibitory pseudosubstrate sequence from the substrate-binding cavity, allowing substrate binding and phosphorylation, propagating downstream signaling.

Novel PKCs do not have the advantage of Ca^{2+}-triggered pretargeting to membranes because their C2 domains do not bind Ca^{2+}. Thus, the encounter of these isozymes with diacylglycerol is considerably less efficient because it occurs from the cytosol rather than the plane of the membrane. Kinetic measurements of green fluorescent protein-tagged PKCs suggest that the rate of translocation of novel PKCs is an order of magnitude slower than that of conventional PKCs in vivo ***(9)***. Atypical PKCs have neither a C2 domain nor a ligand-binding C1 domain and thus respond to neither Ca^{2+} nor diacylglycerol.

Part IV describes the major techniques for studying the membrane interaction of PKC in vivo using fluorescence imaging (Chapter 9), and in vitro using model membranes for equilibrium (Chapter 10) and kinetic (Chapter 11) measurements. This part also describes the classic assay for measuring the binding of phorbol esters to PKC developed by Blumberg and co-workers (Chapter 12), whose synthesis of the relatively hydrophophilic phorbol dibutyrate was instrumental in studying PKC ***(10)***.

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