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Phospholipase Cβ interacts with cytosolic partners to regulate cell proliferation

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ABSTRACT

Phospholipase Cβ (PLCβ) is the main effector of the Gαq signaling pathway relaying different extracellular sensory information to generate intracellular calcium signals. Besides this classic function, we have found that PLCβ plays an important but unknown role in regulating PC12 cell differentiation by interacting with components in the RNA-induced silencing machinery. In trying to understand the role of PLCβ in PC12 cell differentiation, we find that over-expressing PLCβ reduces PC12 cell proliferation while down-regulating PLCβ increases the rate of cell proliferation. However, this behavior is not seen in other cancerous cell lines. To determine the underlying mechanism, we carried out mass spectrometry analysis of PLCβ complexes in PC12 cells. We find that in unsynchronized cells, PLCβ primarily binds cyclin-dependent kinase (CDK)16 whose activity plays a key role in cell proliferation. I*n vitro* studies show a direct association between the two proteins that result in loss in CDK16 activity. When cells are arrested in the G2/M phase, a large population of PLCβ is bound to Ago2 in a complex that contains C3PO and proteins commonly found in stress granules. Additionally, another population of PLCβ complexes with CDK18 and cyclin B1. Fluorescence lifetime imaging microscopy (FLIM) confirms cell cycle dependent associations between PLCβ and these other protein binding partners. Taken together, our studies suggest that PLCβ may play an active role in mediating interactions required to move through the cell cycle.

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1. Introduction

1.1. PLCβ mediates calcium signals through Gαq

Mammalian inositide-specific phospholipase Cβ (PLCβ) enzymes are the main effectors of the Gαq pathway and respond to hormones and neurotransmitters and other sensory signals (for review see (Suh et al., 2008)). PLCβ's major role in the cell is to hydrolyze the signaling lipid phosphatidylinositol 4,5 bisphosphate (PIP_2) leading to activation of protein kinase C and release of calcium from intracellular stores impacting the activity of a host of calcium-sensitive enzymes. There are 4 known isoforms of PLCβ and all are strongly activated by Gαq subunits while two (PLCβ2,3) are additionally activated by Gβγ subunits. Activation of PLCβ requires binding to G protein/GPCR complexes on the surface of plasma membranes to undergo conformational changes that allow for catalysis of the hydrolysis of PIP_2. Live cell imaging of PLCβ in cultured cells show most of the enzyme resides on the plasma membrane (Dowal et al., 2006). However, PLCβ's will localize in the cytoplasm (e.g (Dowal et al., 2006; Guo et al., 2010).) and two isoforms (PLCβ1,3) display nuclear localization. The role of this latter population has been extensively reviewed (Ratti et al., 2017; Poli et al., 2016; Cocco et al., 2016).

Several years ago, we set-out to determine the cellular role of the cytoplasmic population of PLCβ1. Since the activity of PLCβ1 is very low when it is not stimulated, and we could not detect Gαq in the cytoplasm, we initially searched for novel cytosolic PLCβ activators. We used the C-terminal region as bait in a yeast two hybrid study since this region is responsible for activation by Gαq (Park et al., 1993). These studies identified the nuclease *TRAX* (translin-associated protein X) (Aisiku et al., 2010). TRAX is a small helical protein that shuttles between the cytoplasm and nucleus to hydrolyze ssDNA or RNA (for review see (Jaendling and McFarlane, 2010)). TRAX is routinely found associated with the oligonucleotide binding protein *translin* to form the complex *C3PO* (component 3 of the RNA-induced silencing complex). C3PO has shown to be involved in many functions including RNA-induced gene silencing. Specifically, C3PO has been shown to bind to *RISC* (RNA-induced silencing complex) and promote RNA-induced silencing (Li et al., 2012; Liu et al., 2009). In cells, PLCβ binds to and inhibits C3PO thereby reversing RNA-induced silencing (Philip et al., 2012, 2013). Reversal of siRNA by PLCβ was found to be independent of its PIP_2-hydrolyzing activity. Additionally, since PLCβ binds to C3PO in the same region as Gαq, we find that over-expressing C3PO inhibits calcium signaling though PLCβ while increasing the level of activated Gαq reduces the ability of PLCβ to reverse RNA-induced silencing (Philip et al., 2012, 2016). These results suggest that PLCβ is in dynamic equilibrium with Gαq on the plasma membrane and C3PO-RISC in the cytoplasm (for review see (Scarlata et al., 2016). Thus, these multiple interactions connect external signals and G proteins to post-transcriptional gene regulation (Fig. 1).

2. Topics

2.1. PLCβ plays a role in PC12 cell differentiation

Recently, we used PC12 cells as a model system to study neuronal differentiation (Garwain and Scarlata, 2016). In their undifferentiated state, PC12 cells have a round morphology and are highly proliferative. Upon treatment with nerve growth

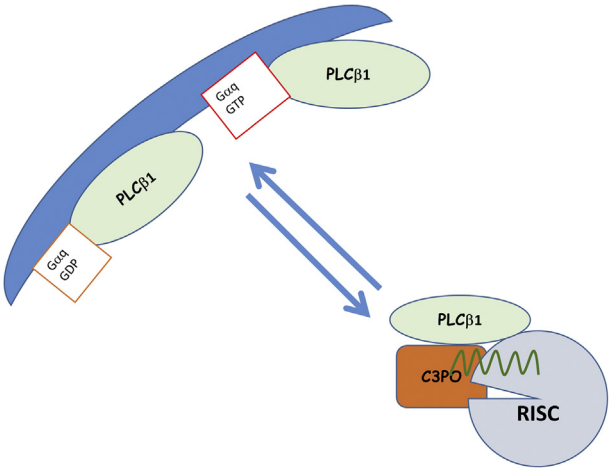

Fig. 1. Cartoon depicting the shuttling of PLCβ1 from Gαq on the membrane to C3PO and the RNA-induced silencing complex (RISC). Factors that affect the dynamics of this equilibria, such as Gαq activation or assembly of the RISC complex, will influence the amount of PLCβ1 involved in calcium signaling versus RNA-induced silencing.

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