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PLCε mediated sustained signaling pathways

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A B S T R A C T

Phospholipase C-ε (PLCε) integrates signaling from G-protein coupled receptors (GPCRs) to downstream kinases to regulate a broad range of biological and pathophysiological responses. Relative to other PLCs, PLCε is unique in that it not only serves a catalytic function in phosphoinositide hydrolysis but also functions as an exchange factor small the low molecular weight G-protein Rap1. PLCε is selectively stimulated by agonists for GPCRs that couple to RhoA, which bind directly to the enzyme to regulate its activity. Rap1 also regulates PLCε activity by binding to its RA2 domain and this generates a feedback mechanism allowing sustained signaling. As a result of its regulation by inflammatory ligands for GPCRs and its ability to promote chronic signals, PLCε has been implicated in diseases ranging from cancer to ischemia/reperfusion injury. This review will discuss the regulation of PLCε, molecular mechanisms that contribute to sustained signaling, and the role of the enzyme in various disease contexts.

Introduction

Phospholipase C-ε (PLCε) is the most recently discovered and arguably the most unique member of the PLC family of enzymes. PLCs are traditionally thought to catalyze hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to generate two important second messengers inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (Bunney and Katan, 2006). IP_3 regulates release of intracellular

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calcium stores while DAG activates protein kinase C (PKC) and protein kinase D (PKD), kinases that regulate a myriad of biological functions (Newton, 2009; Rozengurt, 2011). This review focuses on observations demonstrating that PLCε is directly regulated by low molecular weight or small G-proteins and that its compartmentalization, coupled with its secondary function as a GTPase for Rap1, positions it as a signaling node that effects a variety of biological and pathophysiological responses through sustained DAG generation.

Discovery

PLCε was discovered in 1998 and is the 13th isozyme of the PLC family of enzymes using a yeast two-hybrid screen in *Caenorhabditis elegans*. Kataoka's group first identified PLC210 or PLCε as a Let-60 Ras-binding protein (Shibatohge et al., 1998). When the protein sequence was determined, it became apparent that PLC210 contained the conserved X and Y catalytic motif shared by the PLC family members (Shibatohge et al., 1998). However, PLC210 contains an extended N-terminal region, which makes it considerably larger (210 kDA) than other PLCs. Importantly, the N-terminus contains a CDC25-like domain homologous to the mouse and drosophila Son of Sevenless (SOS), suggesting that this domain functions as a guanine nucleotide exchange factor for Ras-like family members (Shibatohge et al., 1998). In addition, the C-terminus of PLCε was determined to have two Ras associating (RA) domains. Thus, the identification of PLCε and its novel domains suggested unique regulation and function of PLCε compared to the other family members.

Regulation of PLCε

Following its discovery, several groups demonstrated how the novel structure of PLCε is able to integrate signals from large and small G-proteins to downstream pathways. In 2000, Lomasney's group cloned the human form of PLCε and demonstrated its activation using heterologous expression of PLCε and constitutively active heterotrimeric G proteins, including the $G\alpha_{12}$ and $G\alpha_{13}$ proteins, which leads to activation of RhoA (Lopez et al., 2001; Suzuki et al., 2009). Smrcka's group demonstrated that the small G-protein Ras also regulates PLCε activity. When activated Ras was co-expressed with PLCε in COS-7 cells, inositol phosphate production was increased 5.5-fold over basal (Kelley et al., 2001). Smrcka's group further determined that point mutation of a critical lysine residue in the RA2 domain of the enzyme abolished Ras binding to PLCε in a GTP-dependent manner (Kelley et al., 2001).

In addition to Ras, other small G-proteins of the Ras family have been shown to directly interact with PLCε to regulate its activity. These include Rap1, Rap2, and TC21 all of which when co-transfected with PLCε in COS-7 cells increased inositol phosphate production (Kelley et al., 2004). Rap1, Rap2, and TC21 induced inositol phosphate production was shown to require the RA2 domain since mutation of the RA2 domain abolished responses to these proteins (Kelley et al., 2004). In HEK-293 cells and N1E-115 neuroblastoma cell, β2-adrenergic stimulation of PLCε mediated inositol phosphate production was observed and shown to occur through the ability of cAMP to activate Epac (exchange protein directly activated by cAMP), and hence Rap (Schmidt et al., 2001).

Harden's group unraveled another level of regulation of PLCε. They identified Pleckstrin Homology (PH) and EF-hand domains within PLCε, and hypothesized that since PH domains function as recognition motifs for Gβγ, Gβγ might also regulate PLCε (Wing et al., 2001). Indeed, using COS-7 cells, they showed that co-transfection of PLCε with Gβγ resulted in inositol phosphate production to levels similar as that observed with $G\alpha_{12}$ and $G\alpha_{13}$ (Wing et al., 2001). The regulation of PLCε by Gβγ was also demonstrated to be a distinct event from the activation by Ras since a PLCε mutant that is unable to bind Ras still mediated Gβγ activation of inositol phosphate production by PLCε (Wing et al., 2001).

Harden's group also observed PLCε activation in COS7 cells heterologously expressing $G\alpha_{12/13}$ (Wing et al., 2001) in concordance with what was observed by the Lomasney group (Lopez et al., 2001). The $G\alpha_{12/13}$ proteins bind guanine nucleotide exchange factors (GEFs) for RhoA and hence signal through activation of RhoA (Siehler, 2009; Sternweis et al., 2007; Suzuki et al., 2009). The interaction of RhoA with PLCε was determined to be responsible for the stimulatory effects seen with expression of the $G\alpha_{12/13}$ proteins. Interestingly the response to RhoA did not involve binding to the RA domains of the enzyme (Wing et al., 2003). Instead RhoA was shown to bind to a 65 amino acid residue insert within

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