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Review

Phospholipase C is a key enzyme regulating intracellular calcium and modulating the phosphoinositide balance

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ABSTRACT

Spatial and temporal activation of phosphoinositide turnover enables eukaryotic cells to perform various functions such as cell proliferation/differentiation, fertilization, neuronal functions, and cell motility. In this system, phospholipase C (PLC) is a key enzyme, which hydrolyzes phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) into two second messengers, inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) and diacylglycerol (DAG). $Ins(1,4,5)P_3$ triggers the release of calcium from intracellular stores, and DAG mediates the activation of protein kinase C (PKC). In parallel, $PI(4,5)P_2$ also directly regulates a variety of cellular functions, including cytoskeletal remodeling, cytokinesis, phagocytosis, membrane dynamics, and channel activity, in addition to its role as a substrate for PLC and phosphatidylinositol 3-kinase (PI3K), which generates $PI(3,4,5)P_3$. An imbalance of these phosphoinositides contributes to the pathogeneses of various human diseases. Therefore, strict regulation of the levels of $PI(4,5)P_2$ and $PI(3,4,5)P_3$ by PLC or other interconverting enzymes is necessary for cellular functions. In this review, we focus on the roles of PLC as a calcium-regulating enzyme and as a modulator of the phosphoinositide balance.

Contents

Abbreviations: PLC, phospholipase C; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; $PI(3,4,5)P_2$, phosphatidylinositol 3,4,5-bisphosphate; $Ins(1,4,5)P_3$, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; PI3K, phosphatidylinositol 3-kinase; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; TRP channel, transient receptor potential channel; PH domain, pleckstrin homology domain; SH domain, src homology domain; RA domain, Ras-associating domain; RasGEF, Ras-GTPase exchange factor; PDZ, PSD95/Dlg/ZO-1; KO mice, knock out mice; TPA, 12-*O*-tetradecanoylphorbol-13- acetate; ZP, zona pellucida; NFAT, nuclear factor of activated T cells.

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1. Introduction

An epoch-making discovery was made in about the middle of the 1980s, i.e., the hydrolysis of $PI(4,5)P_2$ by PLC generates two second messengers, $Ins(1,4,5)P_3$ and DAG, when cells are stimulated with hormones or neurotransmitters [1,2]. DAG mediates the activation of PKC, and $Ins(1,4,5)P_3$ triggers the release of calcium from intracellular stores. Following this discovery, it became clear that growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) also use this signaling pathway [3,4]. In 1989, another impressive discovery was reported by C. Cantley's group [5]. They found a novel phosphoinositide kinase, PI3K, that phosphorylates $PI(4,5)P_2$ at the 3-position of the inositol ring to generate $PI(3,4,5)P_3$. $PI(3,4,5)P_3$ transduces important signals that induce cell proliferation, motility, etc., and thus defects in the generation/degradation of $PI(3,4,5)P_3$ cause cancer, diabetes and inflammation [6,7].

We and another group have reported that $PI(4,5)P_2$ directly regulates a variety of cell functions. $PI(4,5)P_2$-regulated cytoskeletal reorganization has been extensively analyzed. There are many excellent reviews on $PI(4,5)P_2$ regulation of cytoskeletal molecules such as gelsolin, cofilin, profilin, α-actinin, vinculin, WASP, ERM, etc. [8–11]. $PI(4,5)P_2$ also regulates membrane trafficking, such as endocytosis of the EGF-receptor [12–14]. In addition, multiple functions of $PI(4,5)P_2$ have surprisingly been observed in regulation of transcription in nuclei [15,16], cytokinesis [17], and the gap junction in cell–cell adhesion [18]. Furthermore, a more remarkable function of $PI(4,5)P_2$ is regulation of ion channel activity [19]. $PI(4,5)P_2$ positively controls not only the activity of potassium channels [20], but also that of calcium channels including the transient receptor potential (TRP) channel [21,22]. Imbalances in this network often lead to the pathogeneses of human diseases [6,7,10,23–28]. Therefore, strict regulation of the levels of $PI(4,5)P_2$ and $PI(3,4,5)P_3$ by PLC or other converting enzymes is necessary for maintaining homeostasis of the body.

2. PLC isozymes

2.1. Structure and regulation of PLC isozymes

Thirteen PLC isozymes have been identified and categorized into six classes, the β (1–4), γ (1, 2), δ (1, 3, 4), ε, ζ, and η (1, 2) types, on the basis of structure and regulatory activation mechanisms (Fig. 1) [29–32]. Each isozyme is composed of subtype-specific domains and conserved domains. All PLC isozymes contain catalytic X and Y domains as well as various regulatory domains, including a pleckstrin homology (PH) domain, EF-hand motif, and C2 domain. Although the overall amino acid similarity between these isozymes is not high, the similarity between the domains of all isozymes is more than 40–50%. Subtype-specific domains contribute to specific regulatory mechanisms. These domains include the src homology (SH) domain in PLCγ [29,32], and the two Ras-associating (RA) domains and Ras-GTPase exchange factor-like domain in PLCε [30,32,33]. In addition, PLCβ type isozymes have C-terminal extensions of approximately 400 amino acids, including the PSD95/Dlg/ZO-1 (PDZ)-binding motif [32,35]. The PLCη-type also has an isozyme-specific long C-terminal region [36,37], which does not exhibit similarity with that of the PLCβ-type.

PLC is a soluble protein that is localized mainly in the cytosol and is translocated to the plasma membrane, where it hydrolyzes $PI(4,5)P_2$ in response to cell activation. Thus, targeting of PLC to the plasma membrane is a critical event for signal transduction. The regulatory mechanisms for β-type and γ-type PLCs have been analyzed extensively. The association with heterotrimeric G-proteins of the Gq family induces the activity of β-type PLCs [38]. Comparison of the ligand-binding affinities of different PLCβ isozymes revealed that PLCβ2 and PLCβ3 are sensitive to the βγ subunit, whereas PLCβ1 exhibits high affinity for Gαq. These findings suggest that each β-type isozyme is regulated differently by each subunit of Gq. Recently, Hicks et al. reported that a portion of the X–Y linker occludes the active site of PLCβ2 and that deletion of an X–Y linker constitutively activates PLCβ2, indicating that PLCβ2 may have an autoinhibitory regulation mechanism [39]. With recruitment of PLCβ2 to the plasma membrane, the active site may become free.

γ-Type isozymes are regulated primarily by receptor and cytosolic tyrosine kinases [3,4]. When growth factors such as PDGF, EGF, and nerve growth factor interact with their respective receptors, increases in cellular calcium and activation of multiple protein cascades are observed. The SH2 domains of PLCγ1 are required to target it to tyrosine-autophosphorylated receptors in this process. PLCγ1 is then tyrosine-phosphorylated and activated. Rhee's group indicated that PDGF-induced generation of $PI(3,4,5)P_3$

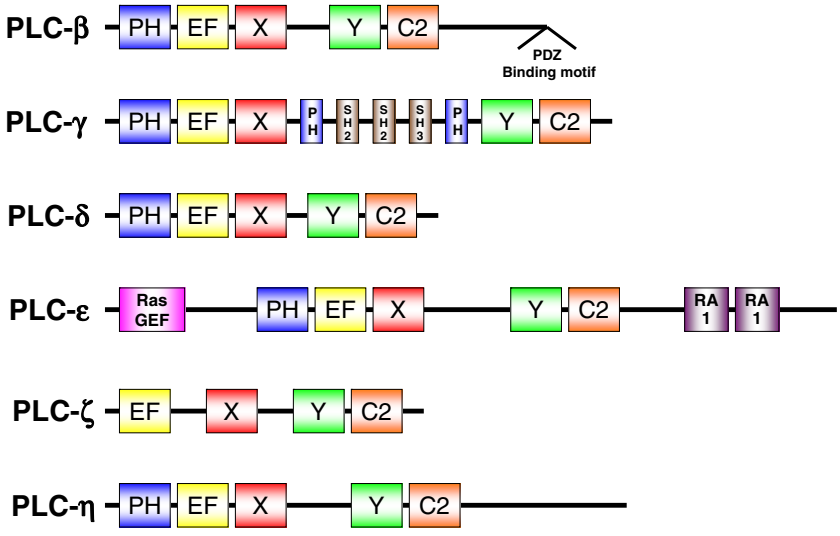

Fig. 1. Domain structure of each type PLC. Catalytic and regulatory domains, and their interacting molecules are shown. PH, pleckstrin homology domain; EF, EF-hand domain; X and Y domains, PLC catalytic domains; C2,C2 domain; PDZ, PDZ-binding motif; SH, src homology domain; RasGEF, Ras-GTPase exchange factor-like domain; RA, Ras-associating domain.