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Recruitment and activation of phospholipase C (PLC)-δ_1 in lipid rafts by muscarinic stimulation of PC12 cells: Contribution of p122RhoGAP/DLC1, a tumor-suppressing PLCδ_1 binding protein

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Introduction

Phospholipase C (PLC, EC3.1.4.11) is a key enzyme in phosphoinositide/Ca^{2+} signaling since it hydrolyzes PtdIns(4,5)P_2 to generate two second messengers, Ins(1,4,5)P_3 and diacylglycerol, that participate in intracellular Ca^{2+} mobilization and protein kinase C (PKC, EC2.7.1.37) activation, respectively (Berridge, 1993; Nishizuka, 1992). At present, six isozyme families, PLCβ, PLCγ, PLCδ, PLCε, PLCζ and PLCη, have been identified in the mammalian superfamily of PI-PLCs (Hwang et al., 2005; Lopez et al., 2001; Rebecchi and Pentyala, 2000;

Abbreviations: BHK, baby hamster kidney; BK, bradykinin; CCH, carbamylcholine; CIB, cell incubation buffer; *DLC1*, deleted in liver cancer 1; DSP, dithiobis-succinimidylpropionate; EGF, epidermal growth factor; GAP, GTPase activating protein; GPCR, G-protein-coupled receptor; IP_3, Ins(1,4,5)P_3; IP_3R, IP_3 receptor; MDCK, Madin–Darby canine kidney; NRK, normal rat kidney; PH, pleckstrin homology; PLC, phospholipase C; SOC, store-operated Ca^{2+} channel; START, steroidgenic acute regulatory protein-related lipid transfer; TG, thapsigargin; TRP, transient receptor potential.

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Rhee and Bae, 1997; Saunders et al., 2002; Song et al., 2001). PLCδ$_1$ is the most abundant and widely-expressed isozyme in mammalian tissues and is thought to be the most fundamental form in evolutionary terms, since most eukaryotic PLCs show homology with this isoform. Nevertheless, the regulation mechanisms and physiological function of PLCδ$_1$ are not well understood.

Cellular PtdIns(4,5)P_2 is localized mainly at the plasma membrane in various cell types (Watt et al., 2002). Since PLCδ$_1$ contains the pleckstrin homology (PH) domain that binds PtdIns(4,5)P_2 with high affinity, it is localized at the plasma membrane in many quiescent cells (Fujii et al., 1999). PLCδ$_1$, however, could show a diverse intracellular distribution. Previous studies have demonstrated that, although PLCδ$_1$ is predominantly localized at the plasma membrane, it translocates from one cellular site to another in response to extracellular stimuli or stress. For example, hypo-osmotic stress causes a rapid movement of PLCδ$_1$ from the plasma membrane to perinuclear regions in Madin–Darby canine kidney (MDCK) cells (Fujii et al., 1999). PLCδ$_1$ also shuttles between the cytoplasm and the nucleus in MDCK and normal rat kidney (NRK) cells, although its function in the nucleus is not clear (Okada et al., 2002; Yamaga et al., 1999).

The activation mechanism of PLCδ$_1$ *in vivo* is not well understood. Since the δ isoforms seem more sensitive to Ca^{2+} than any other PLC isoforms (Allen et al., 1997) except for PLCζ (Kouchi et al., 2004) *in vitro*, it is thought that PLCδ$_1$ ''senses'' a small rise in intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) and acts as a positive feedback regulator. For example, when rat pheochromocytoma PC12 cells were treated with bradykinin (BK) or carbamylcholine (CCH), which binds to a G-protein-coupled receptor (GPCR) that stimulates PLCβ (Rhee and Bae, 1997; Zhu and Birnbaumer, 1996), PLCδ$_1$ was activated by the capacitative Ca^{2+} entry following the activation of PLCβ (Kim et al., 1999). Thus, $[Ca^{2+}]_i$ is critical for the regulation of the PLCδ$_1$ activity *in vivo*.

Several proteins have been identified as specific PLCδ$_1$-binding proteins. Firstly, it has been reported that $G_h\alpha$, the α subunit of an unusual heterodimeric GTP-binding protein that shows transglutaminase activity, interacts with PLCδ$_1$ and transmits transmembrane signaling *via* receptors such as α_1-adrenoreceptors, thromboxane and oxytocin receptors (Chen et al., 1996; Feng et al., 1996; Murthy et al., 1999). Secondly, a 122-kDa GTPase activating protein (GAP), p122RhoGAP, has been cloned as a novel PLCδ$_1$-interacting protein that shows specific GAP activity on RhoA, a member of the Rho GTPase family, and enhances the PtdIns(4,5)P_2-hydrolyzing activity of PLCδ$_1$ *in vitro* (Homma and Emori, 1995). Furthermore, over-expression of the C-terminal region of p122RhoGAP alone can inhibit the lysophosphatidic acid-induced formation of actin stress fibers and focal adhesions and immediately induces elevation of intracellular Ca^{2+} levels (Sekimata et al., 1999). p122RhoGAP, therefore, has been recognized as a dual signal mediator protein acting as a GAP on Rho and a stimulator of PLCδ$_1$. Interestingly, p122RhoGAP is the prototypic member of anti-oncogenic RhoGAP family with approximately 1100 amino acids that has a unique structure from N-terminal to C-terminal; a sterile α motif (SAM: often implicated in protein–protein interaction), a focal adhesion-targeting domain (Kawai et al., 2004), a RhoGAP catalytic domain and a START (steroidgenic acute regulatory protein-related lipid transfer domain, implicated in lipid association and transfer (Chang et al., 2006; Hanada et al., 2007; Kanno et al., 2007)) domain. Three closely related human genes encoding these RhoGAP family proteins are designated as deleted in liver cancer (DLC)1–3 (Ching et al., 2003; Durkin et al., 2007; Yuan et al., 1998), and p122RhoGAP equivalent DLC1 is known to suppress tumor growth and its loss or silence is found in various human solid tumors (Yuan et al., 2004).