COMMENTARY

New insights into the families of PLC enzymes: looking back and going forward

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A study in this issue of the *Biochemical Journal* by Harden and colleagues, in association with one published in the *Biochemical Journal* very recently [Hwang, Oh, Shin, Kim, Ryu and Suh (2005) *Biochem. J.* **389**, 181–186], have defined a new member of the superfamily of PLC (phosphoinositide-specific phospholipase C) enzymes, PLC η . Two isoforms, PLC η 1 and PLC η 2, and their splice variants add to the molecular diversity of PLC enzymes. The studies of PLC η regulation suggest that at least some splice variants of PLC η 2 could be regulated by the G-protein subunits G $\beta\gamma$. As two other families, PLC β and PLC ϵ , are also regulated

through heterotrimeric G-proteins, this finding reveals further complexity and possible interplay between different PLC families and their regulatory networks. At this point, when it is likely that the PLC η family completes the effort of identifying new members of this related group of PLC enzymes, I also discuss some more general concepts of PLC regulation and catalysis, and challenges awaiting their further studies.

Key words: G-protein, phosphoinositide, phospholipase C (PLC), second messenger, signalling.

PLC (phosphoinositide-specific phospholipase C) isoforms found in eukaryotes comprise a related group of proteins that cleave the polar head group from PtdIns(4,5) P_2 . The best-documented consequence of this reaction is generation of two second messengers: inositol 1,4,5-trisphosphate, a universal calcium-mobilizing second messenger, and diacylglycerol, an activator of several protein kinase C isoforms [1,2]. Historically, the PLC enzymes have been studied since the 1950s. However, an interest in their molecular characterization followed the observations that established PLC as a key enzyme in agonist-stimulated phosphoinositide metabolism and signalling. In the late 1980s and early 1990s, several mammalian PLC isoforms were isolated, and their corresponding cDNA sequences determined. According to sequence similarity, these isoforms were classified into three families: β , γ and δ (see Figure 1). The isoforms within each family (β 1– β 4, γ 1 and γ 2, and δ 1, δ 3 and δ 4) were found to be encoded by different genes, with further molecular diversity generated by splice variants derived from a single gene. Paralleling this work, a number of PLC regulators were identified, especially the GTP-binding (G α_q and G $\beta\gamma$) subunits of heterotrimeric G-proteins and protein tyrosine kinases. Based on molecular characterization of PLC isoforms and studies of their regulation, it was proposed that the isoforms within established families not only had greater sequence similarity and a common domain organization, but also shared a general regulatory mechanism specific to this family. Regulation of PLC γ isoforms through receptor and non-receptor tyrosine kinases distinguished this family from regulation of PLC β isoforms by G-protein subunits. This concept remained unchallenged over the following 10 years.

More recently, new PLC-related sequences have been identified using sequences conserved between β , γ and δ isoforms to analyse databases with a wealth of information incoming from genome sequencing projects. These searches have identified proteins that, despite close sequence similarity in selected regions, lack the critical catalytic residues and PLC activity {PLC-like proteins known as PLC-L1 and -L2, or PRIPs (phospholipase-

C-related, catalytically inactive proteins)-1 and -2} [3], a sperm-specific PLC designated as PLC ζ that is implicated in calcium mobilization required for fertilization [4], and a novel PLC ϵ family (reviewed in [5,6]; Figure 1). Studies of the regulation of PLC ϵ revealed not only a regulatory link that could be unique for this PLC, but also a complexity and possible interplay between different families and their regulatory networks. These studies suggest that, uniquely among PLC families, PLC ϵ is directly regulated by small GTPases from the Ras family; however, PLC ϵ enzyme activity can also be stimulated by subunits of G-proteins and small GTPases from the Rho family [6]. With these new findings, it can no longer be assumed that, in different cellular systems, the stimulation of PtdIns(4,5) P_2 hydrolysis through tyrosine kinase receptors (such as the epidermal growth factor receptor) would be solely mediated by PLC γ isoforms, or that the activation of GPCRs (G-protein-coupled receptors) would in turn activate only PLC β isoforms. Since Ras GTPases could be activated through both receptor types, PLC ϵ could also participate in the PtdIns(4,5) P_2 hydrolysis triggered by GPCR and tyrosine kinase receptors. Furthermore, some more direct upstream signalling components, such as G-protein subunits and Rho GTPases, could be common to PLC β and PLC ϵ isoforms.

Two new studies in this and a recently published issue of the *Biochemical Journal* [7,8] extend our knowledge of PLC isoforms. Together with observations by others [9,10], Zhou et al. [7] and Hwang et al. [8] define a new PLC family, PLC η . A similar approach applied by these groups again relied on database searches using well-conserved sequences that define the PLC superfamily. Since the available sequence databases are quite extensive and include several mammalian genomes, it is possible that the identification of PLC η 1 and PLC η 2 now completes the list of these related, phosphoinositide-specific PLC enzymes. Based on sequence comparisons, analysis of gene organization and functional studies, using methodology established in studies of other PLC isoforms, a number of important observations about PLC η were obtained relatively quickly. Together with previous

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Figure 1 Domain organization (A) and dendrogram (B) of mammalian PLC isoforms

The four domains of PLC δ , the PH domain, EF-hands (EF), catalytic domain (containing highly conserved X and Y regions) and the C2 domain (C2) are also present in most isoforms of other PLC families. The unique region of PLC γ , inserted through a flexible loop of the catalytic domain, includes the second PH domain, two SH2 (Src homology 2) regions and one SH3 (Src homology 3) region. PLC ϵ contains the domain with a guanyl-nucleotide exchange factor activity (CDC25) and two predicted RA (Ras association) domains that are implicated in the binding of small GTPases from the Ras family. In PLC β , the unique region (tail) is present at the C-terminus. The sequences unique to PLC η (tail) are, as in PLC β , located at the C-terminus. Although the domain organization of PLC η resembles that of PLC β (A), the analysis of PLC sequences (B) shows that PLC η is more closely related to PLC δ isoforms. The dendrogram was constructed according to [8].

extensive characterization of PLC families, this information brings the studies of PLC isoforms to a point where it is possible to consider more general and emerging themes of their relatedness, domain organization, catalysis and regulation.

Analysis of PLC η sequences revealed close similarity between the two new PLC isoforms (PLC η 1 and PLC η 2) that is higher than the similarity with any other PLC isoform from known PLC families. The predicted domains in the PLC η family include: (i) a PH (pleckstrin homology) domain; (ii) EF-hands; (iii) catalytic, highly conserved regions X and Y; and (iv) a C2 domain. These four domains represent the common core structure of most PLC isoforms. In other PLC families, features related to regulation are mostly contained within the unique regions. The region that is unique for PLC η is at the C-terminus beyond the C2 domain, positioned similarly to the unique region in PLC β . Interestingly, differential splicing seems to occur within the region at the

C-terminus, and sequences of splice variants of PLC η 1 and PLC η 2 differ in this region. Although none of these sequences reveals the existence of a known structural domain, the presence of the PDZ-binding sequence, Leu-Leu-Arg-Leu, is found in several splice variants of both PLC η 1 and PLC η 2 and seem to be conserved from fugu (the Japanese blow fish) to humans, suggesting a potential interaction site for other signalling proteins [7]. In some of the PLC β isoforms, the PDZ-binding domain is known to bind PDZ-containing scaffold proteins, bringing PLC β into a complex with components of GPCR signalling [11]. It is therefore possible that PLC η 1 and PLC η 2 could, through this interaction, be part of larger protein complexes with implications for intracellular localization and regulatory mechanisms. In addition to information obtained from sequence analysis, PLC η 1 and PLC η 2 proteins were expressed and characterized [7,8,10]. These functional studies demonstrate not only that these are active

PLC enzymes, but also that the common catalytic mechanism applies to this family. Mutation of an essential conserved histidine residue, implicated in the general acid–general base mechanism of substrate hydrolysis, abolished the enzyme activity of both PLC η 1 [8] and PLC η 2 [10]. Furthermore, a comprehensive functional analysis of possible regulatory components of PLC η 2 by Zhou et al. [7] have provided important insights into which of the known PLC activators are not likely to be involved and, more importantly, which could be involved in stimulation of PLC η 2 in cells. The panel included most (approx. 15) of the known G α and G $\beta\gamma$ subunits, and approx. 20 Ras and Rho family members. Among the tested components, only G $\beta\gamma$ has the ability to stimulate PLC η 2 when these proteins are co-expressed in COS cells. This observation reinforces the possibility, raised in studies of PLC ϵ , that some regulatory proteins can stimulate members of several PLC families. Thus it is possible that G-proteins, and in particular G $\beta\gamma$, can mediate activation of PLC β , PLC ϵ and PLC η isoforms. Further studies are, however, needed to compare different splice variants of PLC η 1 and PLC η 2 and to extend these observations to more physiological contexts.

With the end of the PLC isoform inventory in sight, challenges awaiting further studies of PLC η and other mammalian PLC families are also becoming apparent. There are several important areas of research where information is lacking. Despite the structural and mechanistic insights into the conserved core of domains involved in catalysis, little is known about the molecular mechanisms that underlie transition from the inactive into the active state following stimulation. To achieve this goal, further structural studies of PLC isoforms and their regulators are clearly required. It will also be important to obtain more information about the biological roles of PLC isoforms. So far, experiments using genetically modified mice have suggested that some closely related isoforms with the same general regulatory mechanism have a different function at the level of the organism. Similar experimental approaches should clarify possible biological differences between PLC η 1 and PLC η 2, and reveal whether their absence could be related to any of known disorders in humans. Since one of the splice variants of PLC η 2 may be selectively expressed in neurons [10], a genetic targeting of this PLC could clarify whether this PLC η 2 variant is critically involved in the regulation of brain function. Perhaps one of the biggest challenges in future studies of PLC will be to try to correlate our increasing knowledge of PLC enzymes with the ever-increasing functions of their common substrate, PtdIns(4,5) P_2 . Over the last 10 years,

functions of PtdIns(4,5) P_2 beyond its role as a precursor of second messengers have been recognized [12]. As for some other phosphoinositides, PtdIns(4,5) P_2 can bind to modular domains (e.g. PH domains) or other specific interaction sites, and therein play additional important roles in directly regulating membrane association and activity of a broad range of proteins. Thus, by changing local concentrations of PtdIns(4,5) P_2 and, consequently, of other phosphoinositides, PLC isoforms can potentially affect membrane association and the activity of many proteins at different subcellular locations. Controlling levels of PtdIns(4,5) P_2 could also affect the properties of those cellular membranes themselves. Thus it is important that we begin to unravel the functions of PLC η and other PLC isoforms with these diverse functions of PtdIns(4,5) P_2 in mind.

REFERENCES

- 1 Rebecchi, M. J. and Pentyala, S. N. (2000) Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiol. Rev.* **80**, 1291–1335
- 2 Rhee, S.-G. (2001) Regulation of phosphoinositide-specific phospholipase C. *Annu. Rev. Biochem.* **70**, 281–312
- 3 Uji, A., Matsuda, M., Maeda, K., Kanematsu, T. and Hirata, M. (2002) Molecules interacting with PRIP-2, a novel Ins(1,4,5) P_3 binding protein type 2: comparison with PRIP-1. *Life Sci.* **72**, 443–453
- 4 Saunders, C. M., Larman, M. G., Parrington, J., Cox, L. J., Royse, J., Blayney, L. M., Swann, K. and Lai, F. A. (2002) PLC zeta: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development* **129**, 3533–3544
- 5 Cullen, P. J. (2001) Ras effectors: buying shares in Ras plc. *Curr. Biol.* **11**, R342–R344
- 6 Wing, M. R., Bourdon, D. M. and Harden, T. K. (2003) PLC-epsilon: a shared effector protein in Ras-, Rho-, and G $\alpha\beta\gamma$ -mediated signaling. *Mol. Interv.* **3**, 237–280
- 7 Zhou, Y., Wing, M. R., Sondek, J. and Harden, T. K. (2005) Molecular cloning and characterization of phospholipase C-eta2. *Biochem. J.* **391**, 667–676
- 8 Hwang, J.-I., Oh, Y.-S., Shin, K.-J., Kim, H., Ryu, S. H. and Suh, P.-G. (2005) Molecular cloning and characterization of a novel phospholipase C, PLCeta. *Biochem. J.* **389**, 181–186
- 9 Stewart, A. J., Mukherjee, J., Roberts, S. L., Lester, D. and Farquharson, C. (2005) Identification of a novel class of mammalian phosphoinositol-specific phospholipase C enzymes. *Int. J. Mol. Med.* **15**, 117–121
- 10 Nakahara, M., Shimozawa, M., Nakamura, Y., Irino, Y., Morita, M., Kiudo, Y. and Fukami, K. (2005) A novel phospholipase C, PLC η 2, is a neuron-specific isozyme. *J. Biol. Chem.* **280**, 29128–29134
- 11 van Huizen, R., Miller, K., Chen, D.-M., Li, Y., Lai, Z.-C., Raab, R. W., Stark, W. S., Shortridge, R. D. and Li, M. (1998) Two distinctly positioned PDZ domains mediate multivalent INAD–phospholipase C interactions for G-protein-coupled signaling. *EMBO J.* **17**, 2285–2297
- 12 Toker, A. (2002) Phosphoinositides and signal transduction. *Cell Mol. Life Sci.* **59**, 761–779