Emerging roles of phosphoinositide-specific phospholipases C in the ciliates Tetrahymena and Paramecium

George Leondaritis1 and Dia Galanopoulou2,*
1Department of Pharmacology; Medical School; University of Thessaly; Larissa Greece; 2Department of Chemistry; University of Athens; Athens, Greece

Phospholipases C (PLCs) that hydrolyze inositol phospholipids regulate vital cellular functions in both eukaryotic and prokaryotic organisms. The PLC superfamily consists of eukaryotic phosphoinositide-specific PLCs (PI-PLCs), bacterial PLCs and trypanosomal PLCs.1 PI-PLCs hydrolyze phosphatidylinositol-4,5-bisphosphate (PtdIns4,5P2) to produce inositol-1,4,5-trisphosphate (Ins1,4,5P3) and constitute a hallmark feature of eukaryotic cells. In metazoa, this reaction is coupled to receptor signaling via specific PI-PLC isoforms and results in acute increase of cytosolic Ca2+ levels by Ins1,4,5P3-sensitive Ca2+ channels (IP3-receptors, IP3Rs).2 A striking result of many studies so far has been the presence of a single PI-PLC gene in all unicellular eukaryotes investigated, as opposed to expansion of PI-PLC isoforms in metazoa;3 this has suggested that a single housekeeping PI-PLC represents an archetypal and simplified form of PI-PLC signaling.3 Several studies however have noted a unique expansion of PI-PLC/IP3R pathway components in ciliates.4,5 In a recent paper we showed the presence of multiple functional PI-PLC genes in Tetrahymena thermophila and biochemical characterization, pharmacological studies and study of their expression patterns suggested that they are likely to serve distinct non-redundant roles.4 In this report we discuss these studies and how they advance our understanding of PI-PLC functions in ciliates.

Unique Combinations of PLC Genes in Ciliates

Biochemical probing of PLC activities in homogenate and subcellular fractions from two Tetrahymena species revealed the presence of two distinct PLC activities utilizing PtdIns and PtdIns4,5P2. PtdIns was utilized by a Ca2+-insensitive/inhibited PLC, while PtdIns4,5P2 was hydrolyzed by a membrane-associated, low micromolar Ca2+-activated PLC. The latter activity was inhibited in vitro by the PI-PLC inhibitor U73122, indicating a bona-fide eukaryotic PI-PLC. These two activities were attributed to two sets of genes identified in the Tetrahymena genome that correspond to bacterial PtdIns-PLCs (TtBPLC1-2) and eukaryotic PI-PLCs (TtPLC1-3).4 In another ciliate, Paramecium tetraurelia, six eukaryotic PI-PLC genes are present revealing a unique expansion in ciliates (for the sake of simplicity we have utilized the assignment in ref. 4 instead of that in ref. 6 for Paramecium PLCs). Phylogenetic analysis indicates further diversity within the ciliate clade. The core of ciliate PI-PLCs appears to be composed of TtPLC1, TtPLC2, PtPLC1 and PtPLC2 (Fig. 1A and B). Two genes, TtPLC3 and PtPLC3, are evolutionarily divergent and, based on analysis of their PLCx domains, they cannot be consistently assigned to a specific PI-PLC group (see Fig. 5C and Sup. Fig. S2D in ref. 4 for details). TtPLC3 is a novel inactive PI-PLC isoform resembling PLC-I/PRIP proteins identified only in metazoa,4 but PtPLC3 apparently codes for an active PLCx domain.
enzyme. Adding to the diversity within ciliate PI-PLCs, three Paramecium genes (PtPLC4-6) form a distinct group (Fig. 1B) and they have been shown to be specifically involved in GPI anchor cleavage (a typical characteristic of bacterial and trypanosomal PLCs) rather than PtdIns4,5P2 hydrolysis. In Tetrahymena, a bacterial PLC gene, TtBPLC1, codes for a catalytically competent PtdIns-PLC, and may correspond to a secreted activity that participates in extracellular digestion of PtdIns; given the well-established activity of other bacterial PLCs in hydrolyzing GPI-anchored proteins, it may be a possible candidate for cleavage of GPI-anchors in Tetrahymena. In conclusion, although there is significant diversity of PLC genes within these two ciliates (Fig. 1), three PI-PLC genes in Tetrahymena (TtPLC3/PRIP apparently in an indirect manner), and at least two in Paramecium (since PtPLC2 is postulated to be a non-functional gene), are likely to participate exclusively in PtdIns4,5P2 hydrolysis and hence generation of Ins1,4,5P3 in vivo (Fig. 1).

Occurrence of PI-PLC-Generated Ins1,4,5P3 in Ciliates

Tetrahymena PI-PLC activity is actively involved in hydrolysis of PtdIns4,5P2 in vivo, since short-term treatment with U73122 results in significant upregulation of PtdIns4,5P2 levels. Furthermore, GTPγS exerted a modest activation of PI-PLC activity in vitro indicating that GTPases are likely to play a regulatory (activating) role. These data advance significantly our knowledge and validate our pharmacological tools for dissecting the roles of ciliate PI-PLCs in vivo. Nevertheless, we still lack definite evidence for stimulus-induced formation of Ins1,4,5P3 per se in either Tetrahymena or Paramecium. Use of Dowex chromatography for detection of Ins1,4,5P3 in Dictyostelium resulted in erroneous assumptions due to the presence of other co-eluted inositol trisphosphates, and it has yielded inconsistent results in detection of inositol-labeled Tetrahymena InsP3 compounds in vivo (our data). Furthermore, an early study utilizing HPLC in Paramecium has failed to present evidence for the presence of Ins1,4,5P3. Nevertheless, the use of a standard Ins1,4,5P3 mass assay has yielded positive results in Tetrahymena, where the abundance of Ins1,4,5P3 was estimated to be approximately 10 pmol/10⁶ cells (Leondaritis G and Galanopoulou D, unpublished data). This value is comparable to the abundance of PtdIns4,5P2 which is deduced to be in the range of 100 pmol/10⁶ cells (estimation based on the mass quantification of PtdIns and [3H]myo-inositol labeling, assuming isotopic equilibrium between all phosphoinositide pools). These compelling results however are faced with an early Paramecium study suggesting that the Ins1,4,5P3 mass assay may also detect other inositol trisphosphate isomers produced by a InsP₆-phosphatase activity (in vitro), with equal sensitivity.
Roles of PI-PLCs in Ciliates

In Tetrahymena, comparative studies of expression patterns and activity in three strains suggested a high level of co-ordination between expression levels of TtPLC2 (and probably TtPLC1) and total PI-PLC activity. What would be then the role of PI-PLC-generated Ins1,4,5P3 in Tetrahymena? Recent studies on the presence and functionality of IP3R genes in Paramecium, also present in Tetrahymena as well, suggest that Ins1,4,5P3 may indeed regulate Ca2+ homeostasis in ciliates. Study of a specific Paramecium IP3R (CRC-II-1a,b/PRIP/PRIP1,2) has shown that it regulates Ca2+ mobilization in a restricted subcellular location, the contractile vacuole complex, and it may have a latent role in regulation of basal Ca2+ homeostasis. The fact that uncaging of Ins1,4,5P3 induces only short and strictly localized Ca2+ mobilization in Paramecium suggests that it is unlikely to promote global and large mobilization of intracellular Ca2+. Instead, a compartmentalized function of PI-PLC-generated Ins1,4,5P3, as a Ca2+-mobilizing agent in a localized fashion in the contractile vacuole complex or other—yet unidentified—subcellular locations, rich in one or more of the approximately 16 IP3R-related CRC channels in Paramecium (and the like in Tetrahymena), seems more plausible with the current knowledge. In addition, the well-correlated expression patterns of TtPLC3/PRIP and TtPLC2 (but not TtPLC1) during Tetrahymena conjugation, point to a specific role for PI-PLC-generated Ins1,4,5P3 in this process during which Ca2+ dynamics and requirements may be quite different. Furthermore, circumstantial evidence from T. thermophila mutant strains and the phenotype of CRC-II-1a,b silencing in Paramecium point to the possibility that PI-PLC activity may impinge on secretory pathways, an aspect that would be interesting to study in detail in the future.

A last point deserves further attention. In S. cerevisiae, PI-PLC-generated Ins1,4,5P3 is the substrate for the synthesis of higher inositol phosphates that are recognized as important players in transcription and chromatin remodeling. The first step is catalyzed by a ubiquitous eukaryotic multifunctional enzyme known as IPK2/IPMK, to yield Ins(1,4,5,6)P4 and subsequently Ins(1,3,4,5,6)P5. In Tetrahymena, an intact IPK2/IPMK-like catalytic domain is encoded by the Tbd2 gene, a class II histone deacetylase. Cells defective in Tbd2 were shown to exhibit chromatin and cytological phenotypes indicative of a role for Tbd2 in chromatin maturation including the proteolytic processing of histone H3 in micronuclei, but the involvement of the IPK2/IPMK domain is unknown. Since TtPLC1-3 have nuclear export signals and TtPLC3 possesses additional nuclear localization signals, it would be interesting to examine the possibility of co-localization with Tbd2 in Tetrahymena nuclei.

In conclusion, several recent studies have highlighted the presence of PI-PLCs in the ciliates Tetrahymena and Paramecium and future experiments along these lines may well unravel novel functions and novel modes of regulation of PI-PLC signaling in eukaryotes. The connection with Ca2+ homeostasis seems quite solid, although several parts of the puzzle are still uncertain (some are also discussed in ref. 10 and 11). The characterization of the phenotypes of PLC-deficient cells will add significantly to our understanding of evolution of PI-PLC signaling in eukaryotic organisms and will probably require expertise in diverse fields of ciliate biology.

Acknowledgments

We thank our students, T. Sarri, A. Efstathiou and Dr. I. Dafnis for their help during distinct phases of this project. We also thank Professor W.F. Boss and Dr. I.Y. Perera, Department of Plant Biology, NCSU, USA, for their help with Ins1,4,5P3 assay. This work was partially supported by a University of Athens grant (KA 70/4/8779).

References

1. Heinz DW, Essen LO, Williams RL. Structural and mechanistic comparison of prokaryotic and eukaryotic phosphoinositide-specific phospholipases C. J Mol Biol 1998; 275:635-50.
2. Irvine RF, Schell MJ. Back in the water: the return of the inositol phosphates. Nat Rev Mol Cell Biol 2001; 2:327-38.
3. Michell RH. Inositol derivatives: evolution and functions. Nat Rev Mol Cell Biol 2008; 9:151-61.
4. Leondaritis G, Sarri T, Dafnis I, Efstathiou A, Galanopoulou D. Biochemical and genetic evidence for the presence of multiple phosphatidylinositol- and phosphatidylinositol-4,5-bisphosphate-specific phospholipases C in Tetrahymena. Eukaryot Cell 2011; 10:412-22.
5. Ladenburger EM, Sehring IM, Korn I, Plattert H. Novel types of Ca2+-release channels participate in the secretory cycle of Paramecium cells. Mol Cell Biol 2009; 29:3605-22.
6. Kloppel C, Müller A, Markter S, Simon M. Two isoforms of eukaryotic phospholipase C in Paramecium affecting transport and release of GPI-anchored proteins in vivo. Eur J Cell Biol 2009; 88:577-92.
7. van Haastert PJM, de Vries MJ, Penning LC, Roovers E, van der Kaay J, Erneux C, van Lookeren Campagne MM. Chemoattractant and guanosine 5′-y-thio)triphasphate induce the accumulation of inositol-1,4,5-trisphosphate in Dicytostelium cells that are labelled with [3H]inositol by electroporation. Biochem J 1989; 258:577-86.
8. Freund WD, Maier GW, Tietz C, Schultz JE. Metabolism of inositol phosphates in the protozoan Paramecium: Characterization of a novel inositol-hexakisphosphate-dephosphorylating enzyme. Eur J Biochem 1992; 207:359-67.
9. Leondaritis G, Galanopoulou D. Characterization of inositol phospholipids and identification of a mastoparan-induced polyphosphoinositide response in Tetrahymena pyriformis. Lipids 2000; 35:525-32.
10. Ladenburger EM, Korn I, Kaselieč N, Wassmer T, Plattert H. An Insl1,4,5P3 receptor in Paramecium is associated with the osmoregulatory system. J Cell Sci 2006; 119:3705-17.
11. Plattert H, Sehring IM, Schilde C, Ladenburger EM. Pharmacology of ciliated protozoan drug (in)sensitivity and experimental drug (ab)use. Int Rev Cell Mol Biol 2009; 273:163-218.
12. Shears SB. How versatile are inositol phosphates kinases? Biochem J 2004; 377:265-80.
13. Smith JJ, Torigo SE, Masson J, Fish LC, Wiley EA. A class II histone deacetylase acts on newly synthesized histones in Tetrahymena. Eukaryot Cell 2008; 7:471-82.