Recombinant Phospholipase C-zeta Has High Ca^{2+}-sensitivity and Induces Ca^{2+} Oscillations in Mouse Eggs*

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Abbreviations used are: PLCζ, phospholipase C-zeta; PLCζΔEFX, PLCζ lacking three EF hand domains and catalytic X domain; [Ca^{2+}], calcium ion concentration; [Ca^{2+}]i, intracellular calcium ion concentration; ER, endoplasmic reticulum; PtdInsP₂, phosphatidylinositol 4,5-bisphosphate; InsP₃, inositol 1,4,5-trisphosphate; PH domain, pleckstrin homology domain
SUMMARY

Sperm-specific phospholipase C-zeta (PLCζ) is known to induce intracellular Ca^{2+} oscillations and subsequent early embryonic development, when expressed in mouse eggs by injection of RNA encoding PLCζ (Saunders et al., Development 129, 3533-3544, 2002). The present study addressed characteristics of purified mouse PLCζ protein that was synthesized using baculovirus/Sf9-cell expression system. Microinjection of recombinant PLCζ protein into mouse eggs induced serial Ca^{2+} spikes quite similar to those produced by injection of sperm extract, probably due to repetitive Ca^{2+} release from the endoplasmic reticulum caused by continuously produced InsP₃. Recombinant PLCδ1 also induced Ca^{2+} oscillations, but 20-fold higher concentration was required, compared with PLCζ. In the enzymatic assay of PtdInsP₂-hydrolyzing activity in vitro at various [Ca^{2+}], PLCζ exhibited a significant activity at [Ca^{2+}] as low as 10 nM and had 70% maximal activity at 100 nM [Ca^{2+}] that is usually the basal [Ca^{2+}]_{i} level of cells. On the other hand, the activity of PLCδ1 increased at [Ca^{2+}] between 1 and 30 µM. EC₅₀ was 52 nM for PLCζ and 5.7 µM for PLCδ1. Thus, PLCζ has approximately 100-fold higher Ca^{2+}-sensitivity than PLCδ1. The ability of purified PLCζ protein to induce Ca^{2+} oscillations qualifies PLCζ for a proper candidate of the mammalian egg-activating sperm factor. Furthermore, such high Ca^{2+}-sensitivity of PLC activity as PLCζ that can be active in cells at the resting state is thought to be an appropriate characteristic as the sperm factor which is introduced into the ooplasm upon sperm-egg fusion, first triggers Ca^{2+} release, and maintains Ca^{2+} oscillations.
INTRODUCTION

Fertilized mammalian eggs exhibit repetitive transient rises in [Ca\textsuperscript{2+}]_i called Ca\textsuperscript{2+} oscillations due to repeated Ca\textsuperscript{2+} release from the ER mainly through the InsP\textsubscript{3} receptor (1). The [Ca\textsuperscript{2+}]_i rises are a pivotal signal for egg activation and responsible for early embryogenesis (2, 3). Accumulated evidence suggests that Ca\textsuperscript{2+} oscillations are induced by cytosolic sperm factor introduced into the ooplasm upon sperm-egg fusion (2, 4). Therefore, the identification of the Ca\textsuperscript{2+} oscillation-inducing sperm factor, that is the egg-activating sperm factor, is a current central subject to elucidate the mechanisms of fertilization. Recently, Saunders et al. (5) have reported a novel type of PLC (the enzyme that produces InsP\textsubscript{3} and diacylglycerol from membrane PtdInsP\textsubscript{2}), PLC\(\zeta\) that is specifically expressed in the mammalian sperm. Injection of RNA encoding PLC\(\zeta\) into mouse eggs causes Ca\textsuperscript{2+} oscillations and subsequent early embryonic development, by expressed PLC\(\zeta\) at an estimated level comparable to the content in a single sperm (5). The Ca\textsuperscript{2+} oscillation-inducing activity of sperm extract (4, 6) is lost when pretreated with an antibody against PLC\(\zeta\) (5). Thus, PLC\(\zeta\) is considered as a strong candidate of the sperm factor. To assure this possibility, it is primarily necessary to examine whether purified PLC\(\zeta\) protein induces Ca\textsuperscript{2+} oscillations in the egg.

It has been shown that PLC\(\beta\)\textsubscript{1} (7), \(\gamma\)\textsubscript{1} (8, 9), \(\gamma\)\textsubscript{2} (8), \(\delta\)\textsubscript{1} (10) and \(\delta\)\textsubscript{4} (7) are expressed in the mammalian sperm and that recombinant PLC\(\beta\)\textsubscript{1}, \(\gamma\)\textsubscript{1}, \(\gamma\)\textsubscript{2} and \(\delta\)\textsubscript{1} failed to cause Ca\textsuperscript{2+} release in the ooplasm (11). PLC\(\zeta\) is the smallest PLC identified to date, lacking the N-terminal PH domain (Fig. 1A) (5) that is found in all isoforms of PLC\(\beta\), \(\gamma\) and \(\delta\), and is the site for interaction with membrane phospholipids (12). Since PLC\(\zeta\) as well as PLC\(\delta\) lacks a regulatory domain such as the G protein-binding site of PLC\(\beta\) or the SH domain of PLC\(\gamma\) for phosphorylation by tyrosine kinase, the activation mechanism of PLC\(\zeta\) and PLC\(\delta\) is unknown. Therefore, it is also necessary to access how PLC\(\zeta\) undergoes the active state for production of InsP\textsubscript{3}. Here we first show that recombinant PLC\(\zeta\) protein induces Ca\textsuperscript{2+} oscillations in mouse eggs and that PLC\(\zeta\) possesses an extremely high Ca\textsuperscript{2+} sensitivity in the PtdInsP\textsubscript{2}-hydrolyzing activity to be active even at the resting state of
cells.
EXPERIMENTAL PROCEDURES

Cloning of PLCζ and PLCδ1 — cDNA encoding full-length PLCζ (Accession No. AF435950) was cloned from cDNA library originated from mouse testis mRNAs. PLCζ cDNA was amplified by PCR using Pfu polymerase and the following primers; 5’-GAC AAGCGGCCCAGATCATG-3’ (forward primer), 5’-GGAAATTCATATGGAAAGCC AACTTCATGAG-3’ (internal forward primer involving an EcoRI site), 5’-CTAACGCG GTCAGTTACATGCG-3’ (reverse primer), 5’-GTCTAGATTACTCTCTGAAAGGTACC AAACATATAAATAC-3’ (internal reverse primer involving an XbaI site). cDNA of PLCδ1 (No. AF133125) derived from the mouse testis was amplified using 5-GGAA TTCTATGGACTCCGGTCGGGAC-3 involving an EcoRI site and 5-GCTCTAGATTAGTCCTGGATGGAGATCTTC-3 involving an XbaI site. PLCζ and PLCδ1 were subcloned into pBluescript II SK(+) (Stratagene) using EcoRI and XbaI sites. A PLCζ variant lacking three EF-hand domains (Accession No. AK006672), designated as s-PLCζ, was cloned using the same primers as those for PLCζ except internal forward primer 5-GGAATTCATATGGAGATCGATCACTCTGATTC-3. A mutant PLCζ lacking three EF hand domains and catalytic X domain, PLCζΔEFX, was constructed using 5-GGAATTCATATGGAGATCGATCAGTCTCTGATT-3 involving an EcoRI site, 5-GTCTAGATTACTCTTCTGGAATGACCAACATCAAATAAAAC-3 involving an XbaI site, 5-GTGGGTACCTTATCTGAAACCCACGAG-3 involving a KpnI site, and the reverse primer. The correct nucleotide sequences of PLCζ (1), s-PLCζ, PLCδ1 (10) and PLCζΔEFX were confirmed using BigDye Terminator Sequencing kit (Applied Biosystems).

Synthesis and Purification of PLCζ and PLCδ1 — Recombinant PLCζ or PLCδ1 was prepared by a baculovirus expression system (13). Using EcoRI and XbaI sites, open reading frame of PLCζ, s-PLCζ, PLCδ1 and PLCζΔEFX were subcloned into a baculovirus expression vector, pFastBac HT (Life Technologies Inc.) that contains amino-terminal His-tag sequence. The recombinant baculoviruses were generated by transformation into DH10Bac, and the transformants containing a PLC-inserted bacmid.
were selected by blue/white screening (see Instruction Manual by Life Technologies Inc.). Sf9 cells were grown at 28°C in Sf900-II SFM medium (Life Technologies) containing 5% fetal bovine serum. The cells (1x10^6 cells/ml) were infected with baculoviruses and incubated for 3 days. The infected cells (3 l for PLCζ and 300 ml for PLCδ1) were lysed with French pressure cell in a lysis buffer (120 mM KCl, 10 mM imidazole, 0.2 mM phenylmethanesulfonyl fluoride, 2 µg/ml pepstatin, 5 µg/ml leupeptin, and 20 mM HEPES/KOH, pH 7.5). The lysate was centrifuged at 15,000 g for 20 min, and the supernatant filtrated through 0.22 µm filter was applied to Nickel-chelating sepharose (Amersham Pharmacia Biotech.). The column was washed with a wash buffer (400 mM KCl, 50 mM imidazole, and 20 mM HEPES/KOH, pH 7.5), and protein was eluted with an ‘elution buffer’ (400 mM KCl, 300 mM imidazole, and 20 mM HEPES/KOH, pH 7.5). Purified PLC was dialyzed against an ‘intracellular buffer’ (120 mM KCl and 20 mM HEPES/KOH, pH 7.5). The final solution was designated as ‘PLC solution’.

**Measurement of PLC Activity** — The PLC activity was assayed by hydrolysis of PtdInsP2 (13), by mixing 10 µl PLC solution with 30 µl Ca²⁺ buffer and 10 µl phospholipid micelle solution. The final concentration was 50 µM phosphatidylethanolamine (Sigma), 40 µM PtdInsP₂ (Sigma), 1 µCi/ml (ca. 100 nM) [³H]PtdInsP₂ (Perkin Elmer), 50 mM HEPES (pH 7.0), 100 mM KCl, 2 mM EGTA, 1 nM to 100 µM Ca²⁺, and 0.5 mg/ml BSA. Ca²⁺ buffers of various [Ca²⁺] were prepared by EGTA/CaCl₂ mixture (14). The reaction mixture containing 25,000 dpm of [³H]PtdInsP₂ was incubated at 37°C for 5 min, and the reaction was stopped by adding 2 ml chloroform: methanol (2: 1 v/v). Radioactive InsP₃ was extracted by adding 0.5 ml of 1N HCl (13), and radioactivity in the upper aqueous phase was measured for 1 min in a liquid scintillation counter. PLC for assay was used at the concentration that produced a maximal [³H]InsP₃ level of approximately 4,000 dpm at a certain range of [Ca²⁺]. Under these conditions, [³H]InsP₃ formation was linear as the function of time and enzyme concentration.

**Preparation of Eggs** — Mature eggs were obtained from the oviducts of B6D2F1 female
mice superovulated by i.p. injection of gonadotropins (see 6 for details). The eggs were collected into M2 medium supplement with BSA (4 mg/ml) (6). Eggs were loaded with the Ca$^{2+}$-sensitive fluorescent dye fura-2 acetoxy methyl ester (5 µM; Molecular Probes Inc.) for 8 min at 37°C. The eggs were transferred to a plastic dish mounted on an inverted fluorescence microscope and heated at 30-32°C.

Injection of PLC and [Ca$^{2+}$]$_i$ Measurement —— PLC solution was injected into eggs through a glass micropipette (15). The injected amount was 5 pl (egg volume, ~200 pl). [Ca$^{2+}$]$_i$ was measured by a conventional Ca$^{2+}$ imaging method using an image processor (Argus 200; Hamamatsu Photonics) (see 15 for details). Images were acquired every 20 s for 30-40 min by applying 340 and 380 nm lights and measuring fluorescence (F) of fura-2 at 510 nm. Data were processed to calculate the fluorescence ratio F$_{340}$/F$_{380}$. 
RESULTS AND DISCUSSION

PLCζ and PLCδ1 with N-terminal His tag were purified up to a major single band shown by silver staining (Fig. 1B). The molecular weight including His tag was ~78 and ~90 kDa, consistent with the full length PLCζ (647 amino acids) (5) and PLCδ1 (756 amino acids) (16), respectively.

Exogenously applied PLC is predicted to produce InsP3 continuously in the egg and, thereby, cause repetitive Ca^{2+} release from the ER. Microinjection of recombinant PLCζ into mouse eggs did induce Ca^{2+} oscillations (Fig. 2A) (n=10 eggs). The first Ca^{2+} transient lasting for 4-5 min was followed by sharp Ca^{2+} spikes at intervals of 2-3 min. The interspike interval was progressively prolonged. The pattern is very similar to that of Ca^{2+} oscillations induced by injection of sperm extract (2, 6), although Ca^{2+} oscillations of lower frequency occur in eggs fertilized with a single spermatozoon (17). The minimal PLCζ concentration in the injection solution for induction of Ca^{2+} oscillations was about 60 \mu g/ml, and that in the egg was calculated to be 1.5 \mu g/ml. The total amount injected was calculated as 300 fg per egg. It has been shown that low frequency Ca^{2+} oscillations similar to those at fertilization are produced after injection of PLCζ RNA, by expressed PLCζ of 45 to 75 fg per egg estimated from densitometric calibration using an antibody against PLCζ (5). The difference in the effective amount of PLCζ for induction of Ca^{2+} oscillations might be derived from that in some modification of the PLCζ molecule in the cell to a more active form. Injection with 2-fold lower PLCζ (0.75 \mu g/ml in the egg) produced only a single Ca^{2+} transient after a time lag of ~2 min (Fig. 2B; n=3). This Ca^{2+} response pattern is usually observed upon injection of diluted sperm extract (data not shown). The critical PLCζ concentration for induction of a single Ca^{2+} release was between 15 and 30 \mu g/ml in the injection solution (Fig. 2, B and C).

A protein similar to but shorter than PLCζ (s-PLCζ) is expressed in the mouse testis (AK006672 in EMBL). s-PLCζ lacks 110 amino acid residues from the N-terminus corresponding to EF1, EF2, and EF3 domains, but is identical to PLCζ in EF4 and the succeeding region (Fig. 1A). s-PLCζ gene has not been found in the mouse genome and
s-PLCζ is probably a splicing variant of PLCζ. Recombinant s-PLCζ elicited no Ca$^{2+}$ spike at the concentration 37-fold higher than that of PLCζ (Fig. 2D), indicating that EF domain(s) is significant in the induction of Ca$^{2+}$ oscillations.

Recombinant PLCδ1, which possesses basically similar domain features to those of PLCζ except the PH domain (Fig. 1A), induced Ca$^{2+}$ oscillations at the concentration of 1.2 mg/ml in the injection solution (30 µg/ml in the egg; Fig. 3A; n=6) and only a few Ca$^{2+}$ transients at 0.8 mg/ml (Fig. 3B; n=3). Thus, about 20-fold higher concentration was required for induction of Ca$^{2+}$ oscillations, compared with PLCζ. PLCδ1 induced no Ca$^{2+}$ response at 0.6 mg/ml (Fig. 3C) or at the lower concentration range at which PLCζ induced Ca$^{2+}$ oscillations (data not shown).

The PLC activity (PtdInsP2-hydrolysing activity) of PLCζ in vitro was measured at [Ca$^{2+}$] between 10$^{-9}$ and 10$^{-4}$ M, in comparison with that of PLCδ1 (Fig. 4). No PLC activity was detected for PLCζΔEFX at any [Ca$^{2+}$] (Fig. 4B), indicating that the samples from Sf9 cells had no intrinsic PLC activity. The assay was performed using the amount of PLC that was capable of generating maximal $[^{3}$H]InsP$_3$ of approximately 4,000 dpm, which corresponded to 0.32 n mol InsP$_3$, under the condition that retained the linearity of InsP$_3$ formation to the reaction time and the enzyme concentration during incubation for 5 min. The amount used was 50 ng for PLCζ and 1 ng for PLCδ1 in 50 µl reaction mixture, corresponding to 1 µg/ml and 0.02 µg/ml, respectively. This concentration of PLCζ was in the same range as the intracellular concentration of injected PLCζ that induced Ca$^{2+}$ release (Fig. 2, A and B). The specific activity of PLCζ was 1.3 µ mol/mg/min at 1 µM Ca$^{2+}$ and that of PLCδ1 was 65 µ mol/mg/min at 30 µM Ca$^{2+}$ (Fig. 4, A and B). The specific activity was 50-fold higher for PLCδ1. Nevertheless, PLCζ induced Ca$^{2+}$ oscillations at 20-fold lower concentrations than PLCδ1 (Figs. 2A and 3A). This implies that PLCζ has 1000-fold higher efficiency than PLCδ1 in induction of Ca$^{2+}$ oscillations in the egg, when calculated on the basis of the specific PLC activity in vitro under the assumption of comparable dependence on [Ca$^{2+}$].

To address advantageous characteristics of PLCζ for induction of Ca$^{2+}$ oscillations, the
dependence of PLC activity on \([\text{Ca}^{2+}]\) was examined \textit{in vitro}. The PLC activity of recombinant PLC\(\delta 1\) was substantially recognized at 1 \(\mu\text{M}\) Ca\(2^+\) (Fig. 4A). The activity steeply increased between 1 and 30 \(\mu\text{M}\) Ca\(2^+\) and attained a saturation level at 30 \(\mu\text{M}\) Ca\(2^+\). The \([\text{Ca}^{2+}]\) for giving a half maximal PLC activity, \(\text{EC}_{50}\), was obtained by fitting a curve to the data using Hill equation. It was calculated as 5.7 \(\mu\text{M}\) (Hill constant, 1.7). In contrast, the PLC activity of recombinant PLC\(\zeta\) was significantly recognized at \([\text{Ca}^{2+}]\) as low as 10 nM, and reached a maximum at 1 \(\mu\text{M}\) Ca\(2^+\) (Fig. 4B). \(\text{EC}_{50}\) was 52 nM (Hill constant, 0.9); that is, ~100-fold lower than that for PLC\(\delta 1\). It should be noted that PLC\(\zeta\) had 70\% activity of the maximal level at 100 nM Ca\(2^+\) which is the resting \([\text{Ca}^{2+}]_i\) level in mouse eggs (6,18) as well as somatic cells (19). This implies that PLC\(\zeta\) can be active even at the resting state of cells. PLC\(\zeta\) has the highest Ca\(2^+\)-sensitivity among PLC isoforms identified and characterized to date (20-24).

\([\text{Ca}^{2+}]_i\) increases from 100 nM up to 500-1000 nM in each Ca\(2^+\) spike during Ca\(2^+\) oscillations in fertilized mouse eggs (25). In Figure 4B, this \([\text{Ca}^{2+}]\) range does not involve a steep Ca\(2^+\)-dependence which may be favorable for a positive feedback and pulsatile rise of the PLC activity. The Ca\(2^+\)-dependence of PLC\(\zeta\) as a whole formed a bell-shape curve (Fig. 4B), which may cause oscillatory changes in the PLC activity associated with Ca\(2^+\) oscillations. However, this seems to be insignificant, since the PLC\(\zeta\) activity substantially decreased at \([\text{Ca}^{2+}]\) as high as 10 \(\mu\text{M}\). Ca\(2^+\) oscillations could occur on the basis of Ca\(2^+\)-dependence of the InsP\(_3\) receptor (1), even if any oscillatory change of PLC activity is absent. They are produced by artificial supply of InsP\(_3\) at a sustained low level in the ooplasm (26). A single injection of a nonhydrolysable agonist of InsP\(_3\), adenophostin B, into the mouse egg produces long-lasting Ca\(2^+\) oscillations (18).

The present study demonstrated two critical properties of PLC\(\zeta\) using purified recombinant PLC\(\zeta\) protein; the high Ca\(2^+\) oscillation-inducing activity in the egg and the high Ca\(2^+\)-sensitivity in the PtdInsP\(_2\)-hydrolysing activity. These properties may rely on the N-terminal EF hand domains of PLC\(\zeta\), since s-PLC\(\zeta\) lacking three EF hand domains
was incapable of inducing Ca^{2+} oscillations (Fig. 2D) and exhibited significant PtdInsP_{2}-
hydrolysing activity only when [Ca^{2+}] was over 1 \mu M (data not shown). The EF domains
of PLC\(\zeta\) might have a high affinity to Ca^{2+}. The higher Ca^{2+}-sensitivity of PLC\(\zeta\) is likely
to give the ability to trigger Ca^{2+} release in cells at the resting state. The two properties of
PLC\(\zeta\) described above are appropriate for a Ca^{2+} oscillation-inducing sperm factor, since
the sperm factor is thought to be introduced into the ooplasm upon sperm-egg fusion, first
triggers Ca^{2+} release, and maintain Ca^{2+} oscillations (2, 4). It has been shown that boar
sperm extract possesses one-third of the maximal PtdInsP_{2} hydrolyzing activity at 100 nM
Ca^{2+} (27). Thus, sperm-specific PLC\(\zeta\) is a strong candidate of the mammalian egg-
activating sperm factor. There might be an inactivation mechanism for PLC\(\zeta\) activity in
the sperm before it is introduced into the ooplasm.

PLC\(\delta\)I possessed the much higher enzymatic activity \textit{in vitro}, but had the much lower
Ca^{2+} oscillation-inducing activity \textit{in vivo}, compared with PLC\(\zeta\). PLC\(\delta\)I has been shown to be
expressed in mouse immature germ cells, spermatogonia, but not detected in differentiated
spermatides and spermatozoa (10). Thus, PLC\(\delta\)I is unlikely to be the sperm factor. The
superiority of PLC\(\zeta\) to PLC\(\delta\)I in the Ca^{2+} oscillation-inducing activity is thought to be
derived from the much higher Ca^{2+}-sensitivity (~100-fold difference in EC_{50}). However,
the large difference in the efficiency of inducing Ca^{2+} oscillations is not interpreted only in
terms of Ca^{2+}-dependent PLC activity \textit{in vitro}, and suggests additional advantage of
PLC\(\zeta\). There might be an egg factor that promotes the activation of PLC\(\zeta\), or special target
membranes for PLC\(\zeta\) in the ooplasm. Further studies are necessary to determine whether
PLC\(\zeta\) is the physiological sperm factor at fertilization as well as to elucidate the activation
and modification mechanism of PLC\(\zeta\) on the basis of the molecular structure.

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LEGENDS

Fig. 1. A, Schematic illustration of the domain features of PLCζ, s-PLCζ, and PLCδ1. B, SDS-polyacrylamide gel electrophoresis of purified mouse PLCζ and PLCδ1 expressed in Sf-9 cells. PLCζ (100 ng) and PLCδ1 (100 ng) were separated on 10% SDS-polyacrylamide gel and subjected to silver staining.

Fig. 2. Changes of [Ca\(^{2+}\)]\(_{i}\) in mouse eggs in response to injection of PLCζ or s-PLCζ.
The ordinate is fluorescence ratio of fura-2 in the egg excited by 340 and 380 nm lights, and reflects [Ca\(^{2+}\)]\(_{i}\). The vertical arrow indicates the time of injection. A, Ca\(^{2+}\) oscillations induced by injection of 60 µg/ml PLCζ (1.5 µg/ml in the egg). B, A single Ca\(^{2+}\) spike induced by 30 µg/ml PLCζ. C and D, No [Ca\(^{2+}\)]\(_{i}\) change upon injection of 15 µg/ml PLCζ and 1.1 mg/ml s-PLCζ (28 µg/ml in the egg), respectively. The concentration in the egg was calculated from injection volume (~5 pl) and egg volume (200 pl).

Fig. 3. Changes of [Ca\(^{2+}\)]\(_{i}\) in response to injection of PLCδ1. A, Ca\(^{2+}\) oscillations induced by injection of 1.2 mg/ml PLCδ1 (30 µg/ml in the egg). B, Three Ca\(^{2+}\) spikes induced by 0.8 mg/ml PLCδ1. C, No [Ca\(^{2+}\)]\(_{i}\) change upon injection of 0.6 mg/ml PLCδ1.

Fig. 4. The dependence of PtdInsP\(_2\)-hydrolyzing activity of PLCζ and PLCδ1 on [Ca\(^{2+}\)]. Values of the specific activity (µ mol InsP\(_3\) produced from [\(^{3}\)H]PtdInsP\(_2\)/min/mg PLC) are presented as mean ± SD in three experiments. A, PLCδ1 (0.02 µg/ml in 50 µl reaction mixture). B, PLCζ (1 µg/ml) and PLCζΔEFX (50 µg/ml).
Fig. 1

A

PLCζ  647 aa

s-PLCζ  537 aa

PH  EF  X  Y  C2

PLCδ1  756 aa

B

M  ζ  δ1

97
66
45
Fig. 3

A

PLCδ1 1.2 mg/ml

B

PLCδ1 0.8 mg/ml

C

PLCδ1 0.6 mg/ml

Time (min)
Fig. 4

A

Activity (μ mol/min/mg)

PLCδ1

B

Activity (μ mol/min/mg)

PLCζ

PLCζΔEFX

[Ca²⁺] (M)
Recombinant phospholipase C-zeta has high Ca\(^{2+}\)-sensitivity and induces Ca\(^{2+}\) oscillations in mouse eggs
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