REPRODUCTION
ANNIVERSARY REVIEW

SPERM FACTORS AND EGG ACTIVATION

The structure and function relationship of sperm PLCZ1

Angelos Thanassoulas[1], Karl Swann[2], F Anthony Lai[1] and Michail Nomikos[1]

[1]College of Medicine, QU Health, Qatar University, Doha, Qatar and [2]School of Biosciences, Cardiff University, Cardiff, UK

Correspondence should be addressed to M Nomikos; Email: mixosn@yahoo.com or mnomikos@qu.edu.qa

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Abstract

In 2002, sperm-specific phospholipase C zeta1 (PLCZ1) was discovered and through these 20 years, it has been established as the predominant sperm oocyte-activating factor. PLCZ1 cRNA expression or direct protein microinjection into mammalian oocytes triggers calcium (Ca^{2+}) oscillations indistinguishable from those observed at fertilization. The imperative role of PLCZ1 in oocyte activation is revealed by the vast number of human mutations throughout the *PLCZ1* gene that have been identified and directly linked with certain forms of male infertility due to oocyte activation deficiency. PLCZ1 is the smallest PLC in size, comprising four N-terminal EF-hand domains, followed by X and Y catalytic domains, which are separated by the XY-linker, and ending with a C-terminal C2 domain. The EF hands are responsible for the high Ca^{2+} sensitivity of PLCZ1. The X and Y catalytic domains are responsible for the catalysis of the phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$] substrate to produce the Ca^{2+}-mobilising messenger, inositol 1,4,5-trisphosphate (IP_3), while the XY-linker plays multiple roles in the unique mode of PLCZ1 action. Finally, the C2 domain has been proposed to facilitate the anchoring of PLCZ1 to intracellular vesicles through its direct interactions with specific phosphoinositides. This review discusses recent advances in the structure and function relationship of PLCZ1 and the potential binding partners of this important sperm-specific protein in the sperm and oocyte. The unravelling of all the remaining hidden secrets of sperm PLCZ1 should help us to understand the precise mechanism of fertilization, as well as enabling the diagnosis and treatment of currently unknown forms of PLCZ1 -linked human infertility.

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From the 'Sperm Factor hypothesis' to the discovery of PLCZ1

Twenty years ago, in 2002, there was a pivotal event in the field of reproductive biology regarding the elucidation of the mechanism of oocyte activation during mammalian fertilization. It was the year that a novel sperm-specific protein, phospholipase C zeta1 (PLCZ1), was discovered (Cox *et al.* 2002, Saunders *et al.* 2002). This discovery was the culmination of many years of intensive search for an endogenous, physiologically relevant moiety that was compatible with the fundamental, uncharted mechanism of sperm-mediated oocyte activation. PLCZ1 met all the requirements to surpass the other candidate molecules and take the lead as the predominant 'sperm factor' (Cox *et al.* 2002, Saunders *et al.* 2002, Nomikos *et al.* 2012, Saleh *et al.* 2020). The 'sperm factor' hypothesis suggested that upon the fusion of sperm and oocyte membranes, a soluble factor diffuses from the attached spermatozoon directly into the oocyte cytoplasm and this factor is capable of activating the IP_3 signaling pathway, thus triggering the Ca^{2+} oscillations in fertilized oocytes that lead to oocyte activation (Swann 1990).

Oocyte activation is a term that is used to describe the completion of a series of biochemical and morphological events that occur in the oocyte, after sperm–oocyte fusion is achieved. These events initiate first with cortical granule exocytosis, that ensures the prevention of polyspermy, then continues with the resumption of the cell cycle. The fertilized oocyte is alleviated from metaphase II arrest, resumes meiosis and extrudes the second polar body. Pronuclear formation and DNA synthesis are the next events that take place, resulting in the completion of the first cell cycle and initiation of the first mitotic divisions in the new zygote (Stricker 1999, Runft *et al.* 2002, Nomikos *et al.* 2013, Yeste *et al.* 2016).

This very orderly series of events are orchestrated by the Ca^{2+} oscillations that are triggered by PLCZ1 (Nomikos *et al.* 2013, Saleh *et al.* 2020). PLCZ1 is a 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase that hydrolyses the substrate phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$) to produce

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the second messengers IP_3 and diacylglycerol (DAG). IP_3 binds to the inositol 1,4,5-trisphosphate receptor (IP_3R) located at the endoplasmic reticulum (ER) which causes a conformational change to its intrinsic Ca^{2+} channel, leading to Ca^{2+} efflux from the ER to the oocyte cytoplasm (Nomikos *et al.* 2013, 2017*a*). This increase in intracellular Ca^{2+} concentration is displayed in the form of distinctive Ca^{2+} oscillations that are necessary and sufficient for oocyte activation (Nomikos *et al.* 2013, Saleh *et al.* 2020).

The predominant role of sperm PLCZ1 in oocyte activation and human fertilization

The 'sperm factor' hypothesis has gained wide acceptance in the field, due to the plethora of data that were consistent with this proposal. Sperm soluble extracts directly injected into the oocyte cytoplasm were seen to induce Ca^{2+} oscillations similar to those observed during *in vitro* fertilization (IVF) (Swann 1990). The question that arose from those experiments was the precise identity of the putative 'sperm factor'. Over the intervening years, there have been proposed several 'sperm factor' candidates. Along with PLCZ1, a truncated c-kit tyrosine kinase (Sette *et al.* 2002), citrate synthase (Harada *et al.* 2007) and post-acrosomal WWP-domain-binding protein (PAWP) (Wu *et al.* 2007) have been reported to be involved in the oocyte activation, but none of these molecules was able to mimic the Ca^{2+} oscillations observed upon IVF of mammalian oocytes. The fact that isolated sperm extracts could lead to the production of IP_3 in a cell-free oocyte homogenate, indicated that the sperm factor might be a phospholipase C (PLC) isoform (Jones *et al.* 1998). Subsequent studies identified PLCZ1 as the sperm PLC responsible for the IP_3-generating capacity in sperm extracts (Saunders *et al.* 2002). *PLCZ1* cRNA expression or direct protein microinjection into mammalian oocytes triggers Ca^{2+} oscillations indistinguishable from those observed at fertilization. Importantly, it has been estimated that the amount of mouse PLCZ1 that should be microinjected, in order to trigger Ca^{2+} oscillations in a mouse oocyte (4–8 fg) is within the same range as the amount of PLCZ1 contained in a single spermatozoon (~20–50 fg) (Saunders *et al.* 2002). In contrast, the amount of human PLCZ1 that is required to initiate Ca^{2+} signals in mouse oocytes is much less (~1 fg) (Yu *et al.* 2008).

Intriguingly, two recent studies presented the *Plcz1* 'knockout' (KO) mouse phenotype (Hachem *et al.* 2017, Nozawa *et al.* 2018). Both studies reported that intracytoplasmic sperm injection (ICSI) from the *Plcz1* KO mouse into the oocytes fails to trigger any Ca^{2+} oscillations. This shows that PLCZ1 is responsible for the Ca^{2+} oscillations and oocyte activation after ICSI. However, during IVF and mating with *Plcz1* KO males, some oocytes are activated at fertilization and embryo development is observed. Success rates of IVF are lower and litter sizes are smaller with *Plcz1* KO males, but the IVF result contrasts with the ICSI result. The reason why IVF leads some oocytes to be activated with PLCZ1 KO sperm is that there are a few large Ca^{2+} oscillations that occur much later than expected, when compared to using WT sperm (Nozawa *et al.* 2018). The belated Ca^{2+} oscillations with *Plcz1* KO sperm lead to delayed oocyte activation and eventually delayed cortical granule exocytosis, so that polyspermy is not halted and many zygotes fail to develop because they are polyspermic (Swann 2020). Overall, the data suggest that PLCZ1 initiates the Ca^{2+} oscillations at fertilization, being responsible for the majority of Ca^{2+} oscillations that are observed, but that during IVF the sperm potentially has another mechanism (another sperm factor) to promote belated Ca^{2+} oscillations in the mouse (Jones 2018). Even if this is the case for mouse oocytes, it may not apply to human oocytes (Lu *et al.* 2018). This disparity can be explained by the fact that mouse oocytes are much more likely to show Ca^{2+} oscillations and they are more sensitive to IP_3R-induced Ca^{2+} release than human oocytes because they have higher ATP levels (Storey *et al.* 2021).

The importance of PLCZ1 in human fertilization is strongly supported by the plethora of mutations continuously being identified in the PLCZ1 gene of patients with oocyte activation deficiencies, resulting in fertilization failure. In 2009, Heytens *et al.* identified the first *PLCZ1* mutation in a non-globozoospermic patient. In this PLCZ1 mutant, a histidine residue within the Y catalytic domain (position 398) was substituted by a proline residue (Heytens *et al.* 2009). There have since been identified at least 20 more mutations in the *PLCZ1* gene. I120M is the only point mutation that is located in the EF-hand domain of PLCZ1 (Torra-Massana *et al.* 2019). Six-point mutations, R197H, L224P, H233L, L246F, L277 and S350P are located in the X catalytic domain and four-point mutations, A384V, H398, P420L, K448N are located in the Y catalytic domain. Finally, four point mutations, I489F, S500L, R553P, M578T are located in the C2 domain (Heytens *et al.* 2009, Escoffier *et al.* 2016, Dai *et al.* 2020, Mu *et al.* 2020, Yan *et al.* 2020, Yuan *et al.* 2020*a*,*b*). In Figure 1, all the aforementioned mutations are mapped onto the ribbon structure model of human PLCZ1. In addition to the point mutations, there are also four frameshift mutations identified in the *PLCZ1* gene of patients with fertilization failure. T324fs and V326Kfs∗25 are located at the X-Y linker region and N377fs and R412fs are located at the Y catalytic domain (Torra-Massana *et al.* 2019, Mu *et al.* 2020, Yan *et al.* 2020).

The explicit correlation of all the currently identified *PLCZ1* mutations with fertilization failure is another strong indication that, at least in humans, PLCZ1 is the predominant sperm–oocyte-activating factor responsible for the characteristic Ca^{2+} oscillations observed at mammalian fertilization.

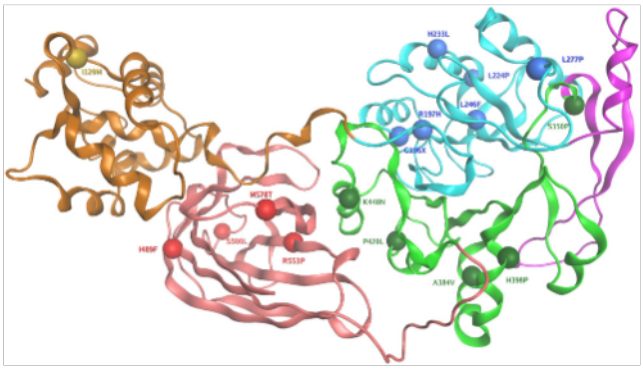

Figure 1 Known missense mutations mapped onto the ribbon structure model of human PLCZ1 (AlphaFold (Jumper *et al.* 2021) Entry: Q86YW0). Each sphere, colored by protein domain, represents a missense mutation associated with oocyte activation deficiency, reduced PLCZ1 levels in sperm and/or abnormal PLCZ1 localization. Yellow, blue, green and red spheres correspond to EF-hand, X, Y and C2 domains, respectively.

Structure and domain organization of PLCZ1

With a molecular weight of ~70 kDa, PLCZ1 is the smallest mammalian PLC isoform with the most compact domain organization. At the other extreme, phospholipase C epsilon 1 (PLCE1) is more than three times larger than PLCZ1 with a molecular weight of ~230 kDa and a much more complicated domain organization (Kelley *et al.* 2001). Among all PLC isoforms, PLCZ1 exhibits the highest homology (47% similarity and 33% identity) with phospholipase C delta 1 (PLCD1). A significant part of the research regarding PLCZ1 function and regulation in the oocyte has evolved from our knowledge of PLCD1 (Saunders *et al.* 2002), since the crystal structure of PLCD1 has been determined (Essen *et al.* 1996). The major difference between PLCZ1 and the other somatic PLC isoforms is the absence of a PH domain from its N-terminus. PLCZ1 is comprised of four EF-hand domains at the N-terminus, followed by the X and Y catalytic domains, which are separated by an unstructured XY-linker region and finally a C2 domain at its C-terminus (Nomikos *et al.* 2012).

EF hand domain of PLCZ1

EF hand domains in the tertiary structure of cognate proteins adopt a helix-loop-helix structure and primarily act as molecular switches regulated by Ca^{2+} concentration transients. Similar to PLCD1, PLCZ1 features four EF hands grouped into two pairwise lobes that seem to play an important role in its Ca^{2+} sensitivity, but in contrast to PLCD1, the Ca^{2+} sensitivity of PLCZ1 is 100-fold higher (Kouchi *et al.* 2005, Nomikos *et al.* 2005). In fact, PLCZ1 is one of the most Ca^{2+}-sensitive mammalian PLCs, since PLCZ1 is predicted to be half-maximally active at resting cytoplasmic Ca^{2+} levels (~100 nM) and maximally active at 1 μM [Ca^{2+}]. Deletion of one or both pairs of EF hands of PLCZ1 led to a complete loss of its Ca^{2+} oscillation-inducing activity in oocytes but had no effect on its *in vitro* enzymatic activity (Nomikos *et al.* 2005). Notably, the deletion of the first pair of EF hands raised the EC_{50} of PLCZ1 for Ca^{2+} from ~80 nM to ~700 nM and deletion of both pairs of EF hands further elevated the EC_{50} of PLCZ1 for Ca^{2+} from ~80 nM to ~30 μM. This implies that truncation of EF hands halts the activation of PLCZ1 in the oocyte since the basal [Ca^{2+}] in the oocyte is ~100 nM (Nomikos *et al.* 2005).

EF hand domains of PLC isoforms can also act as anchors through their interaction with membrane phospholipids, which facilitates proper access and binding of the substrate $PI(4,5)P_2$ in the active site. The first pair of EF hands of PLCD1 binds to negatively-charged membrane phospholipids through its positively charged residues. Arginine residues 182 and 186 and lysine residue 183 of PLCD1, which have been shown to bind to negatively charged membrane phospholipids, are all conserved in PLCZ1 (Cai *et al.* 2013). PLCZ1 has been shown to bind to $PI(4,5)P_2$ but not to phosphatidic acid or phosphatidylserine. Moreover, successive reduction of the net positive charge within the first EF hand domain of PLCZ1 reduces the interaction with $PI(4,5)P_2$ *in vitro*, and the Ca^{2+}-oscillation-inducing activity *in vivo*, without affecting its Ca^{2+} sensitivity (Nomikos *et al.* 2015).

Finally, it is worth noting that the EF hand domains of PLCZ1 have also been proposed to play a role in the nuclear translocation of PLCZ1. The biological significance of nuclear translocation of PLCZ1 is in potentially fine-tuning the Ca^{2+} oscillations in a cell cycle stage-dependent manner. Nuclear translocation is mediated by the nuclear localization sequence (NLS) that in the case of PLCZ1 is located in the XY-linker region. The NLS is a potential binding site for the nuclear transport receptor (NTR). Deletion of any EF hand from PLCZ1 abolishes the nuclear translocation ability of PLCZ1, probably by a conformational change that disturbs the NTR binding site (Kuroda *et al.* 2006).

X and Y catalytic domains and XY-linker of PLCZ1

The X and Y catalytic domains, which are centrally located between the EF hands and the C2 domain, form the active site that is common to all PLC isoforms. The PLCZ1 catalytic domain displays a 64% similarity with the catalytic domain of PLCD1 (Saunders *et al.* 2002). It has been already highlighted above that the majority of mutations in the *PLCZ1* gene are located in the X and Y catalytic domains; this is not surprising since these are the domains responsible for the primary function, that is, the enzymatic activity of PLCZ1 (Fig. 1). The effect of these mutations is to diminish the ability of PLCZ1 to catalyze the hydrolysis of its substrate $PI(4,5)P_2$, which is converted into the reduction of its Ca^{2+}-oscillation-inducing activity, and thus to oocyte activation deficiency.

The X and Y catalytic domains are separated by an unstructured region, the XY-linker. The XY-linker of PLCZ1 is extended in length and consists of more positively charged residues, compared to the corresponding XY-linker of PLCD1. The XY-linker region of PLCB, G, D and H mediates potent auto-inhibition of their enzymatic activity. These data are consistent with the electrostatic repulsion that may occur between the negatively charged XY-linker of these somatic PLC isoforms and the negatively charged substrate $PI(4,5)P_2$ (Hicks *et al.* 2008, Gresset *et al.* 2010). On the contrary, the positively charged XY-linker of PLCZ1 does not mediate auto-inhibition, since deletion of the XY-linker of PLCZ1 significantly diminishes $PI(4,5)P_2$ hydrolysis *in vitro*, and its Ca^{2+}-oscillation-inducing activity *in vivo* (Nomikos *et al.* 2011*b*).

The positively charged XY-linker of PLCZ1 may therefore be involved in the targeting of PLCZ1 to its membrane-bound $PI(4,5)P_2$ via electrostatic interactions, assisting in anchoring PLCZ1 to intracellular membranes (Nomikos *et al.* 2007, 2011*a*). Substituting three of the lysines for alanines (K374A, K375A and K377A) produced a sequential reduction of net positive charge within the XY-linker, which was found to reduce both the efficacy of $PI(4,5)P_2$ interaction *in vitro* as well as Ca^{2+}-oscillation-inducing activity *in vivo* (Nomikos *et al.* 2011*a*). Interestingly, porcine PLCZ1 remains functionally active even after proteolytic cleavage within its XY-linker, suggesting that an intact XY-linker is not essential for the enzymatic activity of PLCZ1 (Kurokawa *et al.* 2007).

C2 domain of PLCZ1

C2 domains are generally involved in targeting proteins to cell membranes. Deletion of the C2 domain from PLCZ1 leads to partial loss of enzymatic activity, but has no effect on the Ca^{2+} sensitivity of the enzyme *in vitro*. Nevertheless, microinjection of PLCZ1 lacking the C2 domain led to inability of PLCZ1 to elicit Ca^{2+}-oscillation-inducing activity in mouse oocytes. Moreover, microinjection of a PLCZ1 chimeric construct, where the C2 domain of PLCZ1 had been replaced by that of PLCδ1, did not result in any Ca^{2+} release in mouse oocytes (Theodoridou *et al.* 2013). All these data suggest that the C2 domain plays a major role in cellular PLCZ1 function (Nomikos *et al.* 2005, Nomikos 2015).

C2 domains bind to phospholipid-containing membranes, displaying different phospholipid selectivity (Rizo & Sudhof 1998). PLCZ1 interacts with phosphatidylinositol-3-phosphate (PI(3)P) and phosphatidylinositol-5-phosphate (PI(5)P) *in vitro*. It has been suggested that the association of the C2 domain with membrane-located PI(3)P may facilitate PLCZ1 targeting specifically to intracellular membranes and thus adopt the correct spatial arrangement on the endomembrane surface (Nomikos *et al.* 2011*a*,*b*).

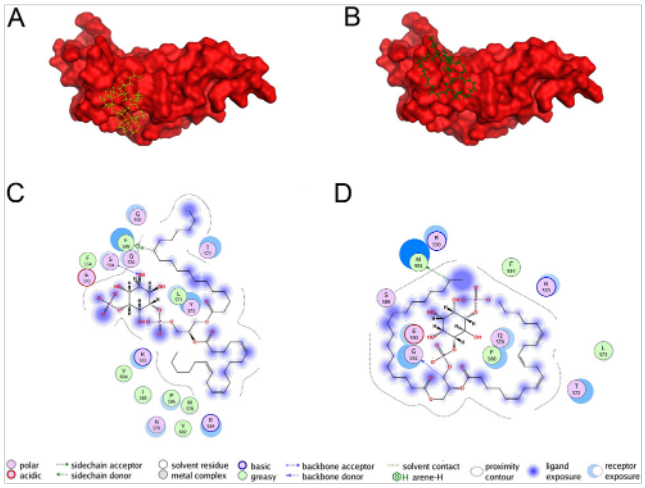

Figure 2 Panels (A) and (B): Superposition of the most probable structures for the PI(3)P and PI(5)P docking to the 465–608 aa region of human PLCZ1. This region contains the C2 domain of the protein and previous studies have demonstrated its capacity to interact independently with these phospholipids (Nomikos *et al.* 2011*a*). The ligands, PI(3)P (panel A) and PI(5)P (panel B), are shown as ball and stick models against the protein surface. Panels (C) and (D): 2D diagram of PI(3)P – C2 domain (panel C) and PI(5)P – C2 domain (panel D) interactions at the binding interface (cut-off <4.5Å).

For this review, a molecular docking approach was used to model the interactions between human PLCZ1 and PI(3)P, or PI(5)P, at the atomic level. Molecular docking simulations of PI(3)P and PI(5)P binding to the truncated region of human PLCZ1 (465–608 aa), corresponding to the C2 domain, were also performed. These data indicated that a protein patch comprising seven amino acid residues (F554, Q570, T572, L573, F588, G592, E593) within the C2 domain, is able to recognize both of these phospholipids with significant affinity (Fig. 2). Based on the PLCZ1 docking results, the binding free energy change is estimated to be −8.5 and −8.1 kcal/mol for the PI(3)P and PI(5)P phospholipids, respectively.

Another set of simulations was performed for the docking of PI(3)P and PI(5)P to the recently released full-length model of human PLCZ1 (AlphaFold Entry: Q86YW0). These docking results reveal that both phospholipids bind at the same region; however, they adopt a new configuration at the binding interface due to new interactions and steric restrictions from neighboring domains (Fig. 3). Residues F588 and G592, that are part of the above-described C2 patch, are still able to interact with both PI(3)P and PI(5)P in the full-length protein simulations. The binding free energy change for the full-length PLCZ1:PIP complexes is estimated to be −9.3 and −8.9 kcal/mol for PI(3)P and PI(5)P, respectively. The increased affinity of the phospholipids for full-length PLCZ1, as compared to the C2 region simulations, stems from better screening of the hydrophobic aliphatic chains from the solvent and new favorable interactions from the other domains.

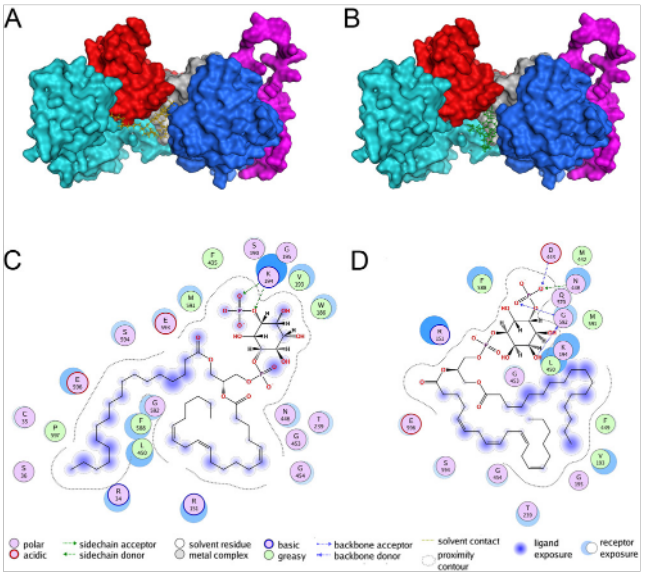

Figure 3 Panels (A) and (B): Superposition of the most probable structures for the PI(3)P and PI(5)P-PLCZ1 complexes. The ligands, PI(3)P (Panel A) and PI(5)P (Panel B), are shown as ball and stick models against the protein surface, which is colored according to the following sequence pattern: cyan (residues 1–154, contains EF hands), blue (residues 155–299, PI-PLC X-box), magenta (residues 300–349, XY-linker), gray (residues 350–465, PI-PLC Y-box), red (residues 466–608, contains C2 domain). Panels (C) and (D): 2D diagram of PI(3)P – PLCZ1 (panel C) and PI(5)P - PLCZ1 (panel D) interactions at the binding interface (cut-off <4.5Å).

PLCZ1 and its binding partners in the sperm

Despite all the significant advances in the elucidation of the structure and function of PLCZ1, the precise gamete cell-specific regulation of its activity remains unclear and its putative binding partners within the sperm are still unknown. However, there are tantalizing hints that some specific proteins could potentially interact with PLCZ1 within the sperm. The most prominent candidate is calmodulin (CaM) (Leclerc *et al.* 2020). CaM is a highly conserved eukaryotic protein that acts as the primary sensor of intracellular Ca^{2+} levels. It is able to bind a large variety of target sequences without a defined consensus sequence and this is the reason CaM interacts with a vast number of proteins (Tadross *et al.* 2008).

Previous studies reported that CaM interacts with phospholipase C beta1 (PLCB1), phospholipase C beta3 (PLCB3) and phospholipase C delta1 (PLCD1) and regulates their activity, but not in the same manner with all of these PLCs. Most specifically, CaM interacts with the NH_2-terminal region of PLCB1 and PLCB3 includes the PH and the EF-hand domains and enhances PLCB1 and PLCB3 activity, possibly by increasing access to the $PI(4,5)P_2$ substrate (McCullar *et al.* 2003). On the contrary, CaM interacts with the XY-linker region of PLCD1 and inhibits PLCD1 activity, most likely because CaM hinders the catalytic domain's access to its substrate, PIP_2 (Sidhu *et al.* 2005).

In a more recent study, a 'pull-down' assay was used to identify calmodulin-binding proteins within the mammalian spermatozoa and one of the proteins that was identified was PLCZ1 (Leclerc *et al.* 2020). Moreover, CaM and PLCZ1 localization within the sperm is very similar. CaM has been localized at the acrosomal and post-acrosomal areas of the head and along the tail of the sperm (Jones *et al.* 1980) and PLCZ1 has been localized mainly at the acrosomal, equatorial and post-acrosomal regions of the sperm head (Grasa *et al.* 2008, Yoon *et al.* 2008, Heytens *et al.* 2009).

Even though the amino acid sequences recognized by CaM do not contain a defined consensus sequence, they are typically characterized by a high propensity to form α-helix, have a net positive charge and contain two hydrophobic residues that serve as a hydrophobic anchor (Yap *et al.* 2000, Hoeflich & Ikura 2002). Furthermore, it has been proposed that many CaM binding sites are intrinsically disordered before interacting with CaM (Wall *et al.* 1997, Radivojac *et al.* 2006). The XY-linker region of PLCZ1 contains amino acid sequences that satisfy all these criteria. To investigate further the structural and energetic characteristics of a potential PLCZ1-CaM complex via the XY-linker, a series of docking simulations between CaM and region 289–353 aa of human PLCZ1 was performed (Fig. 4). The results of the simulations indicate the formation of a stable complex, with a free energy change of −8.9 kcal/mol. Further experiments are required to confirm and delineate the potential importance of the PLCZ1-CaM interaction in the regulation of physiological function.

Interestingly, some recent studies have suggested possible involvement of PLCZ1 with two other proteins, actin-like protein 7A (ACTL7A) and actin-like protein 9 (ACTL9). ACTL7A and ACTL9 belong to the highly conserved family of actin-related proteins (Arps), which have extensive sequence and structural similarity to actin. All Arps characterized so far are components of large multimeric complexes associated with chromatin or the cytoskeleton. ACTL7A and ACTL9 have not yet been fully characterized and they are called 'orphan'

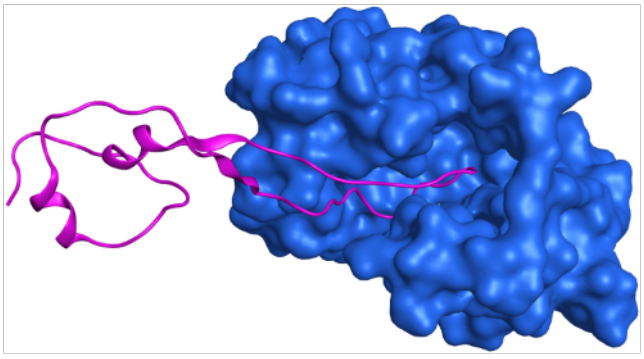

Figure 4 Docking pose predicted for the binding of the 289–353 aa region of human PLCZ1 (magenta ribbon model) to human Calmodulin (Blue space-filling model, PDB entry 2wel).

Arps (Boeda *et al.* 2011). They appear to be mostly testis-specific, located at the sub-acrosomal layer of the sperm that links the acrosomal membrane to the nuclear envelope, consistent with their potential role in maintaining the ultrastructure of the acrosome (Dai *et al.* 2020). It has been shown that mutations in the *ACTL7A* and *ACTL9* genes lead to defects in acrosome integrity, since the inner acrosomal membrane is detached from the nuclear envelope, and this results in oocyte activation deficiency and total fertilization failure. The PLCZ1 in *ACTL7A* and *ACTL9* mutant sperm was found to be expressed at much lower levels and significantly, it is no longer present in the head of the sperm, but observed only in the neck of the sperm. The significant reduction of PLCZ1 expression, and the absence of PLCZ1 from the head of the sperm, which is the crucial region that fuses with the oocyte, may be responsible for the oocyte activation deficiency and total fertilization failure that was observed in oocytes injected with both *ACTL7A* and *ACTL9* mutant sperm (Xin *et al.* 2020, Wang *et al.* 2021).

In search of the 'egg factor'

Almost all known mammalian PLCs are activated by interaction with other proteins, either in the cells where they are expressed or in the cells to which they are transported (Suh *et al.* 2008). PLCZ1 exhibits two characteristics that make the idea of sperm PLCZ1 possibly interacting with one or more egg (oocyte) factors, very realistic.

One distinct feature of PLCZ1 is that it triggers Ca^{2+} oscillations only in oocytes and not in somatic cells (Phillips *et al.* 2011). This fact suggests that there might be a specific egg (oocyte) factor, which is exclusively expressed in the oocyte cytoplasm that interacts with PLCZ1 in order for the latter to be activated and trigger the Ca^{2+} oscillations (Phillips *et al.* 2011, Nomikos *et al.* 2013).

Another distinct feature of PLCZ1 is that it targets intracellular, and not plasma membrane $PI(4,5)P_2$ sources (Yu *et al.* 2012, Sanders *et al.* 2018). The prototype, PLCδ1, binds to its substrate, $PI(4,5)P_2$, on the plasma membrane through direct interaction with its PH domain (Katan 1998, Rebecchi & Pentyala 2000). Since PLCZ1 lacks a PH domain, it should follow an alternative pathway to bind to its substrate, $PI(4,5)P_2$, that is primarily localized on intracellular vesicles within the oocyte cytoplasm. PLCZ1 may target these $PI(4,5)P_2$-containing vesicles by directly interacting with a specific egg (oocyte) factor, potentially a cytosolic binding protein or a membrane receptor (Swann & Lai 2013). Importantly, the unique targeting of PLCZ1 to intracellular vesicles plays an important signaling role, in allowing the Ca^{2+} waves to propagate through the oocyte cytoplasm (Sanders *et al.* 2018).

More research is required to identify a putative PLCZ1-binding partner (protein/receptor) within the fertilized egg. The identification of such an egg protein or 'receptor' would not only shed more light on the physiological mechanism of PLCZ1 action inside the oocyte, but it might also lead to the identification of other unidentified forms of female infertility that arise due to defects (mutations or reduced expression) of this PLCZ1 receptor.

Conclusion

The discovery of PLCZ1 was a significant milestone in the fields of reproductive biology and medicine. Up to date, PLCZ1 is the only moiety that has successfully fulfilled the strictest requirement to be the 'sperm factor', namely the triggering of Ca^{2+} oscillations indistinguishable to those observed during mammalian fertilization. Accumulating evidence shows that PLCZ1 is the predominant sperm oocyte-activation factor. Even though PLCZ1 is the smallest of all the PLCs, its crystal structure has not yet been resolved. Defining the 3D structure of PLCZ1 is of outmost importance, as it will facilitate our ability to decipher the exact mechanism of PLCZ1 action in the oocyte activation. In addition, it will enable us to move faster toward the exploitation of PLCZ1 as an important tool for the detection and the treatment of PLCZ1-linked cases of male factor infertility.

Declaration of interest

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Author contribution statement

M N prepared the first draft of the manuscript, which was revised by A T, K S and F A L. A T conducted the molecular docking simulations. All authors approved the manuscript.

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