Biochemical and Biophysical Research Communications 450 (2014) 1204–1211

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Review

Sperm-induced Ca^{2+} release during egg activation in mammals

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ARTICLE INFO

Article history:
Received 7 April 2014
Available online 21 April 2014

Keywords:
Phospholipase C (PLC)
PLCzeta
Egg activation
Calcium oscillations
Sperm
Fertilization

ABSTRACT

This review discusses the role that the sperm-specific phospholipase C zeta (PLCζ) is proposed to play during the fertilization of mammalian eggs. At fertilization, the sperm initiates development by causing a series of oscillations in cytosolic concentrations of calcium [Ca^{2}] within the egg. PLCζ mimics the sperm at fertilization, causing the same pattern of Ca^{2+} release as seen at fertilization. Introducing PLCζ into mouse eggs also mimics a number of other features of the way in which the fertilizing sperm triggers Ca^{2+} oscillations. We discuss the localization of PLCζ within the egg and present a hypothesis about the localization of PLCζ within the sperm before the initiation of fertilization.

Contents

1. Introduction: the problem of egg activation

Following maturation, mammalian eggs remain arrested at metaphase of the second meiotic division (MII). Liberation from this MII arrest is an essential pre-requisite for cell division and subsequent embryogenesis, and is a key event of so called ‘*egg activation*’. Egg activation includes meiotic resumption, cortical granule exocytosis, prevention of polyspermy and pronuclear development. Such events are initiated by the fertilizing sperm, which accomplishes the activation process via a series of rises in the intracellular free Ca^{2+} concentration within the egg (see Fig. 1) [1,2]. These so called repetitive Ca^{2+} transients, or Ca^{2+} oscillations, at fertilization are essential for egg activation. Indeed, prevention of fertilization Ca^{2+} transients by Ca^{2+} chelators prevents activation and subsequent embryogenesis [3], while mimicking the Ca^{2+} oscillations using multiple electroporation pulses triggers all the main events of egg activation and induces embryonic development [4].

Currently, Ca^{2+} release in mammalian eggs at fertilisation is widely accepted to occur via hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2), resulting in inositol trisphosphate (IP_3)-mediated Ca^{2+} release from the endoplasmic reticulum (ER) through the IP_3 receptors (IP_3Rs). The injection of IP_3 or adenophostin A (an IP_3 analogue) results in Ca^{2+} release in all mammalian eggs examined [5]. Indeed, IP_3Rs in mouse and cow eggs undergo a clear down-regulation (loss in number) at fertilization, a phenomenon that only occurs following a substantial increase in IP_3 levels, indicating that the fertilizing sperm increases the levels of intracellular IP_3 within the egg [5–7]. Finally, injecting functionally inhibitory antibodies to IP_3Rs, or the premature down-regulation of IP_3Rs in mouse eggs, both block Ca^{2+} oscillations at fertilization [8]. There is currently no consistent evidence to suggest that other Ca^{2+} releasing messengers such as cyclic ADP ribose, or NAADP

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http://dx.doi.org/10.1016/j.bbrc.2014.04.078

cause physiological Ca^{2+} release at fertilization in mammalian eggs. Hence, the key problem of understanding how the sperm causes Ca^{2+} release and egg activation in mammals at fertilization comes down to understanding how the sperm initiates IP_3 production within the egg. In this review, we focus primarily on role of a soluble sperm factor in initiating the Ca^{2+} release that causes mammalian egg activation.

2. The 'soluble' sperm factor as egg activator

Gamete fusion precedes the initiation of Ca^{2+} oscillations in mouse eggs by about 1–3 min, and experiments that used low pH to inhibit fusion show that gamete fusion is essential for the initial Ca^{2+} release [9,10]. A simple explanation for these observations is that the sperm contains a soluble factor that diffuses into the egg following gamete fusion, which then initiates Ca^{2+} release. The term 'soluble' infers that the factor used by the sperm is able to diffuse throughout the egg cytosol to initiate Ca^{2+} release. Hence, it is soluble when it is active. Such a proposal is in contrast to the idea that the sperm factor exerts its effects upon a plasma membrane receptor, or otherwise diffuses within the 2-dimensional membrane compartment. The term 'soluble' here does not imply that the factor is held in an entirely soluble state within the sperm, or that it can be readily extracted from sperm into aqueous solution.

In support of the soluble sperm factor hypothesis, experiments show that the injection of sperm cytosolic extracts into eggs of mouse, human, pig, and cow triggers a prolonged series of Ca^{2+} oscillations similar to those seen at fertilization; injection of sperm cytosolic extracts also produces all the other events of egg activation [4,5,11]. Such Ca^{2+} oscillations are not stimulated by injecting Ca^{2+}, while injection of IP_3 or stimulating G-proteins leads to a dampened series of Ca^{2+} oscillations that are significantly dissimilar to those at fertilization [12]. The soluble factor was shown to be of high molecular mass, sensitive to proteases, and present specifically in cytosolic extracts from sperm [11]. Thus, it seems clear that the mammalian sperm delivers a specific soluble protein to the egg that in turn results in Ca^{2+} release. This idea is supported by the successful fertilization of eggs in both mouse and human eggs following intracytoplasmic sperm injection (ICSI). In these species, ICSI is also accompanied by a prolonged series of Ca^{2+} oscillations [13,14]. ICSI in mouse eggs also leads to IP_3R down-regulation and hence substantial IP_3 production [15].

Efforts to identify the soluble sperm factor have led to the identification of a number of candidates in the past. The first candidate was a 33 kDa protein (or oscillogen) that comigrated with the ability of sperm extracts to induce Ca^{2+} oscillations in eggs [16]. However, further investigations indicated that the recombinant 33 kDa protein is unable cause Ca^{2+} oscillations in eggs [17,18]. A subsequent candidate sperm factor included the truncated form of the c-kit receptor, tr-kit, which induced parthenogenetic mouse egg activation [19]. Further studies suggested that tr-kit activated phospholipase C (PLC) isoform gamma-1 (PLCγ1) through phosphorylation by a Src-like kinase, Fyn [20]. Another more recent proposed sperm factor is the post-acrosomal sheath WW domain-binding protein (PAWP), which upon microinjection into porcine, bovine, macaque, and *Xenopus* eggs resulted in pronuclear formation, an indicative event of successful egg activation [21]. It has also been implied that PAWP mediates its effects by interaction with Yes associated protein (YAP) that ultimately works through a Src-like kinase and hence PLCγ [21]. PAWP injection caused a Ca^{2+} increase when injected into *Xenopus* eggs, but did not appear to mimic the single large Ca^{2+} wave normally seen at fertilization in such eggs [22]. So far, no other research groups have independently verified the effects of either tr-kit, or PAWP in eggs. Most significantly, it is still not known whether either tr-kit or PAWP can cause Ca^{2+} oscillations similar to those observed at fertilisation in mammalian eggs. Without this information, it is difficult to make any clear assessment of their potential role, if any, during egg activation at fertilization.

Given the evidence for sperm-mediated Ca^{2+} release being caused by IP_3 signaling in mammalian eggs, we can expect at some point for a phosphoinositide (PI)-specific phospholipase C (PLC) to be stimulated in the signaling pathway. In principle, this might involve a sperm factor stimulating an egg-derived PLC activity. There are currently 13 known mammalian PI-specific PLC isozymes, that have been categorized based on their structure and regulatory mechanisms. These include 3 types of PLCdelta (PLCδ), 4 types of PLCbeta (PLCβ), 2 types of PLCgamma (PLCγ), PLCepsilon (PLCε), PLCzeta (PLCζ), and 2 types of PLCeta (PLCη) [23]. PLCγ has been implicated in fertilization in echinoderm eggs, since injecting SH2 domains to act as a dominant-negative suppressor blocks Ca^{2+} release at fertilization [24]. However, PLCγ is unlikely to be involved in Ca^{2+} release in mammalian fertilization as the same SH2 domains do not block Ca^{2+} oscillations at fertilization in mouse eggs [25]. The lack of effect of SH2 domains within mouse eggs raises further doubts over the role of tr-kit or PAWP in signaling at fertilization.

Other PLCs of the β and δ classes are either not detected in eggs, or do not seem to significantly affect female fertility because the knockout mice of such PLCs are fertile. The best candidate for an egg-derived PLC is PLCβ1 [26]. Knockdown of PLCβ1 levels in mouse eggs decreased the amplitude of Ca^{2+} oscillations at fertilization. However, over-expression prior to fertilisation inhibited the duration and frequency of Ca^{2+} oscillations at fertilization [26]. It is not yet clear how to interpret such data, as both under-expression (knockdown) and overexpression of PLCβ1 result in some form of inhibition of Ca^{2+} oscillations. It should also be noted that there is little if no indication that egg-derived PLCs can be directly stimulated by physiological Ca^{2+} increases in mammalian eggs. For example, strontium (Sr^{2+}) media can stimulate prolonged Ca^{2+} oscillations in unfertilized mouse eggs that in many ways resemble those occurring at fertilization [3]. However, unlike fertilization, Sr^{2+}-induced Ca^{2+} oscillations do not lead to IP_3R down-regulation and result in Ca^{2+} release independent of IP_3 production [6,7]. Studies using fluorescent probes for PIP_2 or diacylglycerol (DAG; the other product of PIP_2 hydrolysis by PLCs) suggest that PIP_2 hydrolysis is only stimulated by high, supra-physiological Ca^{2+} levels in unfertilized mouse eggs [27].

3. PLCζ and Ca^{2+} release in eggs

The sperm cytosolic extracts that trigger Ca^{2+} oscillations in mouse eggs have also been shown cause Ca^{2+} release and IP_3 generation in sea urchin egg homogenates [18,28]. Subsequent work suggested that the Ca^{2+} releasing factor in sperm was itself a highly active PLC. The idea that the sperm itself delivers a PLC circumnavigates some of the above issues in understanding the role of egg-derived PLCs at fertilization. The specific PLC isozyme responsible for Ca^{2+} releasing activity in eggs was first identified using mouse expressed sequence tag (EST) databases to describe a novel, testis-specific PLC, termed PLCzeta (PLCζ), a ~74 kDa protein [29]. Immunodepleting PLCζ from cytosolic sperm extracts suppressed their ability to release Ca^{2+} in mouse eggs or sea urchin egg homogenates [29]. Most significantly, injection of recombinant PLCζ protein or cRNA into mouse eggs causes Ca^{2+} oscillations similar in character to those observed at fertilization, supporting embryonic development to the blastocyst stage [29–31]. Quantifying the amount of PLCζ protein expressed in mouse eggs following cRNA injection indicated that the fertilization pattern of Ca^{2+} oscillations were elicited in the same range as the amount of PLCζ found in a single sperm [29]. In fact microinjection of tagged versions of PLCζ suggested that PLCζ is effective at ~40 fg per egg [29]. Such estimates correlate with the range of PLCζ currently thought to be within a single mouse sperm. Thus, the PLCζ in sperm singularly provides a sufficient explanation of the stimulus for at fertilization.

PLCζ is much more effective than its other PLC counterparts in producing Ca^{2+} oscillations in mouse eggs. PLCδ1 (the closest homologue to PLCζ) only induces low frequency Ca^{2+} oscillations at >1 pg per egg [29,32]. Other PLCs are even less effective, if at all, in causing Ca^{2+} oscillations in mouse eggs]. Finally, transgenic mice with disrupted PLCζ expression in the testis through RNA interference (RNAi) exhibited sperm which induced prematurely ending Ca^{2+} oscillations. While these mice were not infertile, mating experiments yielded significantly reduced litter sizes [33]. In addition to these data in mouse, there are now numerous reports of cases of failed fertilization after ICSI in humans, and in many cases this has been shown be due to a lack of an egg activating factor [34–36]. Indeed, sperm involved in such cases of ICSI failure exhibit a reduced or absent capacity to cause Ca^{2+} oscillations, and are deficient with regards to PLCζ [34–36]. Specific cases have also shown a deficit in PLCζ from the sperm or else deleterious PLCζ mutations present within the catalytic X or Y domains. These data all imply that PLCζ plays a role in activation during normal fertilization.

Mammalian orthologues of PLCζ have now been characterized in human, pig, cow monkey and horse sperm [31,37–39]. PLCζ exhibits characteristic X and Y catalytic domains that form the active site common to all PLCs, a single C2 domain, and four tandem EF hand domains. It is the smallest known mammalian PLC with a molecular mass of ~70 kDa in humans and ~74 kDa in mice [29,31].The activity of PLCζ is not species specific, since injection of cRNA encoding PLCζ from range of different mammalian species, as well non-mammals such as birds can trigger Ca^{2+} oscillations in mouse eggs [31,40]. Mutations have been made in the catalytic domain of PLCζ that completely abrogate Ca^{2+} release in mouse eggs [29]. This shows that the effects of PLCζ are mediated via the production of IP_3. Furthermore, the injection of PLCζ leads to a significant down-regulation of IP_3Rs in mouse eggs [41]. This down-regulation occurs even in the presence of the Ca^{2+} chelator, BAPTA, which implies that PLCζ causes the direct generation of IP_3.

One reason why PLCζ is so effective in eggs is due to its exquisite sensitivity to Ca^{2+} [30,42,43]. At resting cytosolic Ca^{2+} levels of around 100 nM in eggs, PLCζ is thought to be at least half maximally active. There is also a steep dependence of PLCζ activity on Ca^{2+} above 100 nM. Hence, unlike the unfertilized egg, once an egg is injected with PLCζ, small increases in Ca^{2+} are able to generate substantial quantities of additional IP_3. This implies a positive feedback loop of IP_3 production and Ca^{2+} release in an egg containing active PLCζ, an important feature of generating Ca^{2+} oscillations. The EF hands of PLCζ appear to be responsible for the Ca^{2+} sensitivity of PLCζ, and deletion or mutation of conserved Ca^{2+} binding residues within the EF hands resulted in a loss of Ca^{2+}-induced PLCζ activity. PLCζ also contains a C-terminal C2 domain, but there is no C2 Ca^{2+} binding site predicted for PLCζ, and deletion of the PLCζ C2 domain did not alter the Ca^{2+} sensitivity of PLCζ activity [42,43]. However, this domain seems to be important for PLCζ activity *in vivo*, as removal of the C2 domain leads to a loss of its ability to cause Ca^{2+} oscillations in eggs [42,43].

An additional structural feature that imparts significant impact upon PLCζ function is an unstructured region between the X and Y domains, referred to as the X–Y linker [44]. A distinctive feature of PLCζ is that unlike other PLC isozymes, it does not have a PH domain, which is thought to be an essential domain for PLCδ1 binding to PIP_2 at the plasma membrane [44,45]. However, the X–Y linker region of PLCζ has been shown to bind PIP_2 *in vitro*, in preference to other phospholipids [44].The X–Y linker region also contains a predicted nuclear localization signal (NLS) sequence that may play a role in the regulation of PLCζ [46].

4. PLCζ localisation in gametes

The localization of PLCζ within mouse eggs further supports the assertion that PLCζ is indeed the mammalian sperm factor. PLCs produce IP_3 through the hydrolysis of PIP_2, phospholipids that are present exclusively in membranes. One would then assume that PLCζ would target the egg plasma membrane, where cells normally contain the bulk of their PIP_2. However, at fertilization in mouse eggs there is an increase in PIP_2 levels at the plasma membrane rather than a decrease [47]. Also, there is no detectable DAG increase at the plasma membrane at fertilization or after PLCζ injection, despite the ability of DAG probes to detect changes following Ca^{2+} ionophore treatment [27]. Finally, tagging PLCζ with fluorescent protein shows no localization within the cortex [48]. These findings raise the question of how PLCζ can be so active in hydrolyzing PIP_2 when it is not apparently targeted to the major source of substrate in the plasma membrane.

The solution to these conundrums appears to be that PLCζ hydrolyzes PIP_2 in other parts of the cell. Recent immunocytochemistry experiments indicated that PLCζ is localized on small vesicles (no bigger than 1 μm) in the egg cytoplasm. PIP_2 in mouse eggs also appears to be localized in vesicles, suggesting that IP_3 can be generated from an intracellular source of PIP_2 [48]. Data in support of this was also found using a plasma membrane targeted inositol phosphate phosphatase [48]. Expressing such a phosphatase in the plasma membrane of mouse eggs reduces plasma membrane PIP_2, completely abolishing Ca^{2+} oscillations induced by injection of PLCδ1 [48]. However, the expression on the plasma membrane of inositol phosphate phosphatase did not exert significant effect on Ca^{2+} oscillations at fertilization, or following PLCζ injection. This data therefore suggest that both the sperm and PLCζ do not need to hydrolyze plasma membrane PIP_2 to cause Ca^{2+} oscillations in the egg. These data are consistent with previous studies that found that mammalian sperm extracts (containing PLCζ) hydrolyze PIP_2 in sea urchin egg homogenates. Furthermore, membrane subfractions of sea urchin egg homogenates that were able to generate maximal IP_3 were the ones richest in yolk, vesicles of which have been demonstrated to contain PIP_2 [49]. However, as mouse eggs do not contain yolk, the nature of the observed vesicles within mammalian eggs remains to be determined.

Following fertilization, Ca^{2+} oscillations persist in mouse eggs for ~3–4 h, after which time the oscillations cease, correlating with the formation of the pronuclei [50]. Following pronuclear formation, oscillations are absent during the interphase of the first cell cycle. However, further oscillations can be observed following the start of the first mitosis, coinciding with nuclear envelope breakdown (NEB), suggesting that the sperm factor that was delivered to the egg is sequestered into the newly-formed pronuclei, and re-released into the egg cytosol following NEB [50]. Indeed, Ca^{2+} oscillations and egg activation were induced in unfertilized eggs following transfer of female or male pronuclei from zygotes previously fertilized by sperm [51]. Furthermore, this activation transferability seems specific to the pronuclei of zygotes fertilized by sperm, as pronuclei from parthenogenetically-activated eggs, or cytoplasts from any source are ineffective. Thus, pronuclear localization is a key feature of an egg activating sperm factor (at least within mice) [51]. This is significant as in mouse eggs, pronuclear formation is accompanied by PLCζ translocation [46,52]. Mutation of positively-charged amino acid residues to negatively-charged ones within the NLS sequence of PLCζ resulted in the persistence of Ca^{2+} oscillations after the formation of the pronuclei showing that nuclear localization of PLCζ is essential for cessation of Ca^{2+} oscillations [46]. PLCζ release from pronuclei after nuclear envelope breakdown upon entry into mitosis provides a simple explanation for the existence of Ca^{2+} transients during mitosis in zygotes, and not during mitosis in parthenogenetically-activated embryos. PLCζ is accumulated in the nuclei of the two cell embryos [46]. However, such 'nuclear shuttling' only appears applicable to Ca^{2+} oscillations within mouse zygotes. It currently remains unclear how Ca^{2+}oscillations terminate in eggs of other species, particularly since bovine, rat and human PLCζ do not undergo nuclear localization, even following microinjection of the corresponding cRNA into mouse eggs [53], indicating that other mechanisms may be involved in other species. Nevertheless, the nuclear localization of PLCζ in early embryos is another example of how it mimics the known properties of the sperm factor used to cause Ca^{2+} release at fertilization.

If a factor is to trigger Ca^{2+} release in the egg at fertilization, any such factor is expected to reside in a compartment readily accessible to the egg shortly after gamete fusion. This is particularly likely in hamster sperm where the delay between gamete fusion and Ca^{2+} release appears to be in the order of 10 s [54]. The most appropriate region is the post-acrosomal region of the perinuclear theca (PT), a condensed layer of cytosolic protein surrounding the sperm nucleus [55]. The significance of the perinuclear theca is underlined by the finding that in mouse sperm this region is retained following detergent extraction of sperm heads, which also retain the ability to activate eggs in ICSI [56,57]. Notably, immunofluorescence studies in mouse and bull sperm have indicated that PLCζ is localized within the post-acrosomal region of the sperm. However, depending on the species, PLCζ has also been detected in the acrosomal and post-acrosomal regions of the sperm head [58–61], while in equine sperm PLCζ it is detectable in the sperm head as well as the principal piece of the flagellum [38]. This localization in horse sperm may be significant as microinjection of equine sperm tails into mouse eggs resulted in Ca^{2+} oscillations [38].

Interestingly, a recent study on PLCζ disagrees with the majority of the published localization data and suggests that PLCζ is localized to the acrosome of mouse and human sperm, with further populations on the surface of the sperm head, following secretion during sperm maturation within the epididymis [62]. However, the study by Aarabi et al. [62] used PLCζ polyclonal antibodies whose specificity remains a concern. More significantly these observations are clearly inconsistent with previous findings that PLCζ is biochemically detectable in extracts from the perinuclear theca of mouse sperm that cause egg activation [58]. Hence, the consensus of data still suggests that PLCζ is localized inside the sperm and predominantly within the PT of mouse sperm. This is certainly a part of the sperm which is exposed to the egg cytoplasm soon after gamete fusion.

The solubility of the sperm factor may perhaps be an issue related to localization. Early studies of the 'sperm factor' used cytosolic extracts from hamster or boar sperm [11]. Later studies of the 'sperm-borne oocyte activating factor' showed that mouse sperm heads, from which the soluble cytosolic proteins had been removed, could also activate eggs [56,57]. While such findings may have suggested the presence of multiple sperm factors localized in different regions of the sperm, we now know that the protein that causes Ca^{2+} oscillations in both cases is PLCζ. Thus, it is prudent to suggest that PLCζ is in both soluble and insoluble fractions of the sperm head. Potential reasons that may underlie previous discrepancies may be attributable to the use of different types of sperm, particularly the use of hamster versus mouse sperm. Ca^{2+} oscillations are initiated within seconds following sperm–egg fusion in hamsters [54], thus suggesting that hamster sperm contains a significant fraction of their PLCζ in a soluble form, consistent with the ease of extraction of the factor from hamster sperm [11]. In contrast, there appears to be a delay in the order of minutes (2–4 min) between mouse gamete fusion and the first Ca^{2+} increase [9,10]. Interestingly, cross-fertilization experiments (mouse sperm on hamster eggs) indicated that this long delay is a feature of the sperm, not the eggs [63]. This delay may be attributable to the poor solubility of mouse PLCζ, requiring more time to be released from the sperm head following fusion. Indeed, early studies failed to detect any egg activating activity from mouse sperm cytosol [64], and specific extraction protocols are required to extract the mouse sperm factor from the detergent-resistant perinuclear theca [57]. Porcine PLCζ may be somewhat intermediate between the above two species, as PLCζ has been detected in both soluble and insoluble fractions of porcine sperm [59]. Thus, it is judicious to suggest that insoluble PLCζ is expected to be in the sperm PT, while the soluble form of PLCζ may be less specifically localized. This idea is expressed in Fig. 2 as a schematic hypothesis.

It remains unclear whether a particular pattern of localization is required, or is indeed more physiologically relevant. However, considering that PLCζ has been shown to be present within the PT of mouse sperm [58], perhaps the equatorial and post-acrosomal populations of PLCζ are both physiologically valid. Considering that the equatorial region of sperm is one of the first areas of the sperm

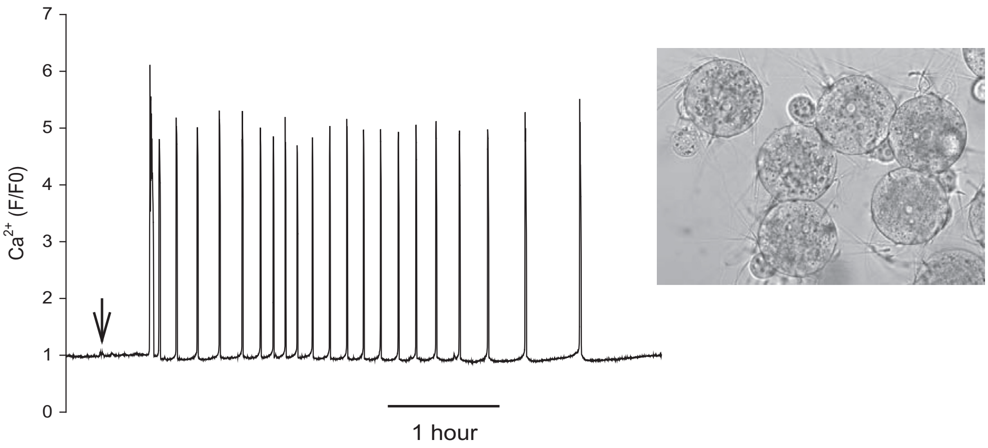

Fig. 1. Ca^{2+} oscillations at fertilization in mouse eggs. A recording of intracellular Ca^{2+} in a mouse egg undergoing *in vitro* fertilization. Intracellular Ca^{2+} is measured with Rhod dextran, which was microinjected into the egg. The fluorescence is expressed as a ratio of the intensity divided by the starting fluorescence value. Sperm were added to eggs at the arrow. This egg was one of several in the image frame taken at the end of the experiment on the right hand side. All the eggs were monospermic and formed two pronuclei.

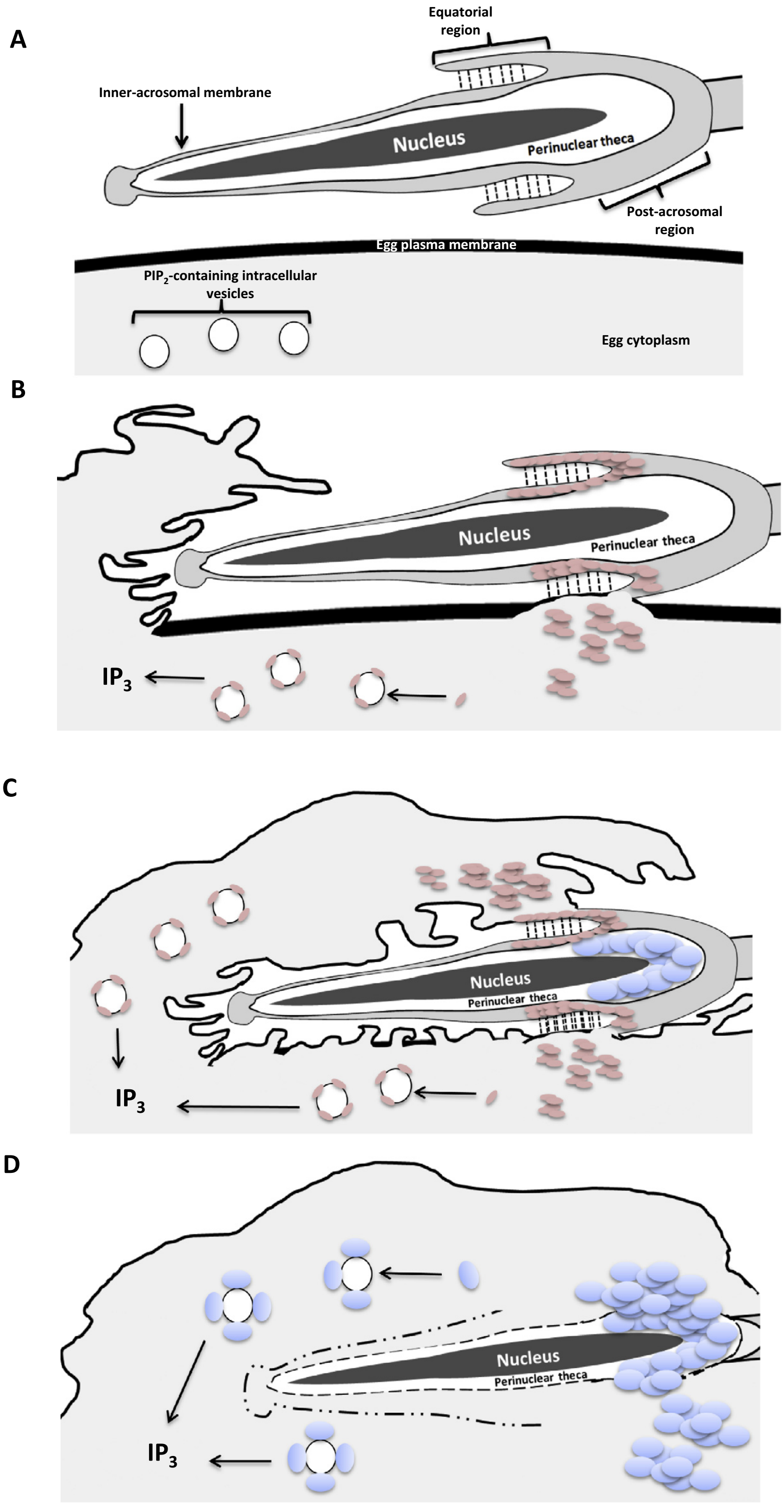

Fig. 2. Schematic representation of the proposed hypothesis by which soluble and insoluble fractions of PLCζ enter the egg during/after gamete fusion, following the series of events first described by Yanagimachi [55]. (A) Following the acrosome reaction, and prior to the initiation of gamete fusion, the inner acrosomal membrane is exposed, with the equatorial region of the sperm head being one of the first regions to be presented to the egg plasma membrane. (B) Immediately during gamete fusion, the equatorial region merges with the egg plasma membrane, and the egg begins to 'engulf' the sperm head, incorporating both cytoplasmic elements. It is at this stage that soluble PLCζ (pink) is released into the egg, which then hydrolyses PIP_2 present on intracellular vesicles to produce IP_3. Such may be the case for species such as hamster, whose sperm initiates the first Ca^{2+} increase at this stage, which would be explained by the assertion that soluble PLCζ rapidly diffuses into the egg immediately following gamete fusion. (C) As the sperm is gradually incorporated into the egg cytosol, soluble PLCζ is able to diffuse rapidly throughout the point of gamete fusion. However, the perinuclear theca is not solubilized at this stage, thus insoluble PLCζ (blue) remains bound within the sperm. This scenario may occur in sperm from species such as the pig, where PLCζ is reported in both soluble and insoluble states. (D) Once the sperm head has been completely incorporated into the egg, the postacrosomal region of the perinuclear theca is next to be solubilized, releasing insoluble PLCζ into the egg which then targets vesicular PIP_2. This scenario would explain what happens during mouse fertilization, where the first Ca^{2+} spike is not observed until several minutes post-fusion. Release of PLCζ from mouse sperm at this stage would explain this initial delay.

head to fuse with the egg, previous opinions have suggested that the equatorial pattern of PLCζ localization may be the most physiologically relevant as PLCζ localized to this region would be one of the first soluble sperm proteins to be introduced into the egg [16,55]. However, further populations within the post-acrosomal region may also be physiologically relevant depending upon the species being studied. Thus, it is possible that species exhibiting only an equatorial pattern of sperm PLCζ elicit Ca^{2+} rapidly upon sperm–egg fusion, whereas those species with the post-acrosomal population would initiate Ca^{2+} release in a relatively delayed manner. It is also possible that in species exhibiting more than one population of PLCζ within a sperm, these multiple populations may help ensure that the pattern of Ca^{2+} release can be sustained beyond gamete fusion. Indeed, following initial fusion of the equatorial region of the sperm head with the egg, the next region thought to merge with the egg is the post-acrosomal region [55]. It is also worth noting that the sperm tail also eventually becomes completely incorporated with the egg following gamete fusion so this may be of relevance to the pattern of Ca^{2+} oscillations in species such as the horse when they are fully characterized. However, further investigation is urgently required to test the validity of these theories, before any particular role is assigned to specific PLCζ populations in a sperm.

5. Variations on PLCζ activity

Although the presence of PLCζ is not a species-specific feature of mammalian sperm, it would seem that the intrinsic activity of PLCζ varies depending upon the species studied. For example, when tested for their ability to cause Ca^{2+} oscillations in mouse eggs, a rank order of potency for PLCζ RNA seems apparent, such that horse > human > monkey > mouse > rat, in terms of ability to induce repetitive Ca^{2+} release [31,53,65]. The most quantitative data on PLCζ protein levels between different species is from comparisons between human and mouse PLCζ. As little as ~1 fg of human PLCζ was capable of triggering an initial Ca^{2+} increase in mouse eggs, compared to the >30 fg for mouse PLCζ [29,66]. For the majority of species, it is not known how much PLCζ is contained within a single sperm. However, estimates in the mouse suggest ~50 fg per sperm [29,42], whereas in the bull PLCζ was reportedly present at 105–165 fg per sperm [39]. Thus, there may be variation in both levels and potency of sperm PLCζ depending upon the species studied. Perhaps an explanation for such physiological variance may be due to evolutionary constraints relating to relative size of the eggs being fertilized. Indeed, there seems to be a ~10-fold range in egg volume, going from the smaller rodent eggs to the larger eggs of domestic animals. Considering that the range of sperm size is not as significant, a human or horse sperm may need to deliver a more potent package of PLCζ to trigger Ca^{2+} release in eggs of their own species. However, it should be noted that there may also be differences in the sensitivity of eggs to PLCζ. For example, mouse PLCζ appears to be more active than rat PLCζ in mouse eggs, despite mouse and rat eggs being of similar size. We might expect the species' rank order of egg sensitivity to PLCζ should run in the opposite direction to PLCζ potency, i.e. rat > mouse > human > horse.

An apparent disparity between species has also been noted in the expression pattern of PLCζ during spermiogenesis. PLCζ protein is first detectable at the elongated spermatid stage, with round spermatids not exhibiting signs of PLCζ expression [37]. This correlates with the finding that mouse round spermatid injection does not result in egg activation [67]. However, round spermatids from humans are able to successfully elicit Ca^{2+} oscillations following injection into mouse oocytes [68,69], consistent with the assertion that some versions of PLCζ are more potent than others, in this case human PLCζ significantly more so compared to mouse PLCζ [66,68]. It is unfortunate that the PLCζ knockout male mice fail to make sperm, and their testes only contain round spermatids [70]. As there is no Ca^{2+} releasing activity at this stage in mice, this has precluded any definitive analysis of the role of PLCζ in fertilization or ICSI. Nevertheless, it might be valuable to test the effect of these PLCζ null mouse spermatids in rat eggs.

6. Conclusions

We have long known that all eggs studied to date are activated by Ca^{2+} transients at fertilization. The search for the causative factor in the sperm eliciting these Ca^{2+} increases in eggs has been a major and often unresolved issue, remaining one of the central problems of fertilization biology in all species. It is notable that in some species, such as sea urchins, there has been a proliferation of candidate sperm factors proposed to cause the single Ca^{2+} increase at fertilization. Since all these candidates cause a similar single Ca^{2+} increase, there is no clear way of distinguishing between them. We have a major advantage in studying mammalian eggs whereby the physiological process enforces strict requirements for a candidate factor that might be used by the sperm to trigger Ca^{2+} release in the egg. These can be enumerated as a set of stringent and independent criteria for the mouse; (1) the sperm factor is able to trigger a long-lasting series of Ca^{2+} oscillations when introduced into the egg. (2) The amount of the factor found in sperm should produce the same frequency of Ca^{2+} oscillations as occurs within fertilizing eggs. (3) The factor should generate IP_3 in eggs as demonstrated, for example, by its ability to cause down-regulation of IP_3Rs. (4) The factor should hydrolyze an endo-membrane PIP_2. (5) The factor should localize to the pronuclei following cessation of Ca^{2+} oscillations.

These criteria encapsulate fundamental features of the way the sperm-borne Ca^{2+} releasing sperm factor functions. All these criteria are satisfied by PLCζ, which is currently the only sperm factor candidate to satisfy these stringent criteria. The major remaining scientific issues include determining whether PLCζ is the only agent causing Ca^{2+} release in mammalian eggs and how it targets intracellular PIP_2. Answering these questions will help provide a complete molecular explanation of the way in which mammalian sperm triggers egg activation at fertilization.

Acknowledgments

We are grateful for support for our research from the Wellcome Trust, MRC, BBSRC and Cardiff University.

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