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Review

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

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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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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