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Fertilization in echinoderms

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This article is dedicated to Professor William J. Lennarz

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ABSTRACT

For more than 150 years, echinoderm eggs have served as overly favored experimental model systems in which to study fertilization. Sea urchin and starfish belong to the same phylum and thus share many similarities in their fertilization patterns. However, several subtle but fundamental differences do exist in the fertilization of sea urchin and starfish, reflecting their phylogenetic bifurcation approximately 500 million years ago. In this article we review some of the seminal and recent findings that feature similarities and differences in sea urchin and starfish at fertilization.

1. Introduction

Owing to the availability of a large number of gametes that can be easily fertilized *in vitro*, echinoderm eggs have been widely used for the study of oocyte maturation and fertilization. At spawning, sea urchin eggs have already completed meiosis and contain a haploid nucleus. In contrast, starfish oocytes are fertilized at the diploid stage, and the meiotic division is completed after the sperm entry. Because of this readiness of the eggs in the gonad, sea urchin eggs have been favored in early studies showing the separation of the vitelline layer from the egg plasma membrane, which is the hallmark of echinoderm fertilization. On the other hand, starfish represent synchronized oocytes arrested at the first prophase of meiosis, which can be induced to undergo meiotic maturation. Thus, these larger and more transparent oocytes have provided an opportunity to study the regulation of meiotic cell cycle as well as fertilization. Starfish sperm extend a long and slender acrosomal process to reach the egg surface across the deep layer of the jelly coat. Much has been learned about the morphological and biochemical changes that happen in both gametes, and it has become clear that Ca^{2+} is an essential actor in the processes. However, relatively little is known about the precise mechanisms that regulate the Ca^{2+} release and the changes that lead to monospermic fertilization. This review will discuss the information presently available on the process of fertilization in sea urchin and starfish eggs.

2. Sperm activation

The chain of reactions culminating in fertilization initiates in the spermatozoon that undergoes physiological changes upon contacting the eggs. In the testis, sea urchin spermatozoa are immotile due to the high CO_2 pressure that keeps the intracellular pH (pHi) below the levels needed to activate dynein ATPase in the flagella. When exposed to seawater, protons are released and the spermatozoa begin to swim. Hyperpolarization of its plasma membrane induced by the low K^+ in the seawater contributes to the pHi rise and to the concomitant activation of adenylyl cyclase. This activates cAMP-dependent protein kinase (PKA) that phosphorylates proteins essential for flagella motility [1]. Small diffusible activating peptides (SAPs) present in the egg jelly induce changes in sperm motility and straighter swimming patterns towards the egg. Speract, a decapeptide first purified from *Strongylocentrotus purpuratus* eggs, cross-links to a 77 kDa membrane receptor which stimulates the activation of a membrane-bound guanylyl cyclase resulting in a transient increase of cGMP. This opens a novel tetrameric K^+-selective cyclic nucleotide-gated ion channel (Sp-tetra-KCNG) and induces hyperpolarization of the sperm plasma membrane, which in turn rapidly increases pHi and concomitantly decreases intracellular Ca^{2+} due to the concerted activity of two

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exchangers, a Na^+/H^+ and a flagellar K^+-dependent Na^+/Ca^{2+} exchanger [2]. The termination of the cGMP is ensured by a phosphodiesterase in sperm flagella (suPDE5) [3]. The sperm hyperpolarization is followed by a sustained depolarization. Ca^{2+} influx initiates at the onset of recovery from hyperpolarization then enhances itself during the depolarization phase due to the voltage-dependent Ca^{2+} channels and the production of cAMP which stimulates the opening of a Ca^{2+} channel. Thus, cycles of hyperpolarization and depolarization of the membrane potential induce changes in the curvature of the sperm swimming trajectory through the generation of trains of Ca^{2+} fluctuations with Ca^{2+} reuptake by way of Ca^{2+} ATPases in the sperm head and $Na^+/Ca^{2+}/K^+$ exchangers in the flagellum [4,5].

3. Acrosome reaction

The significance and the kinetics of the sperm acrosome reaction (AR) were discovered by Dan [6] in starfish sperm. She found that increased permeability to Ca^{2+} was the triggering event leading to exocytosis of the acrosomal vesicle and to actin polymerization at the tip of the sperm head to prolong a 25 μm long filament process (Fig. 1). In sea urchin, the acrosomal filament extends for only 1 μm from the head, and is covered with bindin, the adhesive protein responsible for the species-specific attachment of sperm to the egg's vitelline layer [7]. The K^+ and H^+ efflux accompanying the extension of the acrosomal filament results in changes in the membrane potential, and the Na^+-dependent increase in pHi by a Na^+/H^+ exchange correlates temporally with Ca^{2+} uptake [8]. The components of the jelly coat that induce AR in *S. purpuratus* are fucose sulfate polymers (FSP) [9]. Three receptors for the egg jelly (suREJ1, suREJ2 and suREJ3) present in sea urchin sperm are presumably involved in the calcium signaling pathways eliciting the AR [10]. Upon binding of FSP to suREJ1, the biphasic Ca^{2+} influx and the activation of $InsP_3Rs$ at the acrosomal membrane maintain a sustained intracellular Ca^{2+} increase in sperm [5].

Starfish spermatozoa undergo AR upon encountering three components of the jelly coat. Lillie [11] first suggested that the jelly coat may represent the egg receptor for sperm, which induces the AR in many echinoid species. A large sulphated proteoglycan-like molecule called ARIS (AR-inducing substance) works with Co-ARIS, a group of sulphated steroidal saponins and asterosap. The latter chemotactic agents bind to a guanylyl cyclase receptor in the flagellar plasma membrane and hyperpolarize the sperm plasma membrane to induce a transient increase of cGMP and Ca^{2+}. Asterosap transiently increases Ca^{2+} by activating the reverse-operation of a K^+-dependent Na^+/Ca^{2+} exchanger and decrease in pHi. The sustained increase of Ca^{2+} induced by the simultaneous treatment of sperm with ARIS and asterosap may involve a store operated Ca^{2+} channel [12,13].

4. Gametes interaction and fast block to polyspermy

Fertilization thus depends on the activation of spermatozoa that allows their binding and fusion to the egg plasma membrane. Species-specific gamete interaction is of particular importance to the species having external fertilization, and its molecular mechanism is relatively well-studied in sea urchin (see the review by Vacquier on bindin and sperm receptors in the same issue). On the other hand, a fertilized egg requires a mechanism to prevent the formation and development of the polyspermic zygotes. In echinoderm, while elevation of the fertilization envelope may serve as a mechanical block to polyspermy, whether or not a fast block mechanism exists remains controversial. As the rate of re-fertilization of sea urchin eggs is much lower than that of the initial fertilization, a block to polyspermy must be rapidly established (in about two seconds) after the interaction of the first successful sperm [14]. Intracellular recordings from sea urchin eggs have shown that the first detectable electrical event at fertilization is the step-like depolarization, which is accompanied by an increase in voltage noise about 13 s (the latent period) before the long depolarization of the membrane potential (fertilization potential). Jaffe [15] first suggested that this rapid shift of the membrane potential to a positive level causes the fast block to polyspermy, which renders the egg plasma membrane refractory to supernumerary spermatozoa. The hypothesis of electrical fast block to polyspermy has been reported in several other species, but was not supported unanimously [14]. No change in egg receptivity to sperm was observed in *S. purpuratus* during the latent period [16]. Electrophysiological measurements on eggs from two different sea urchin species at fertilization in high sperm density showed that creation of multiple

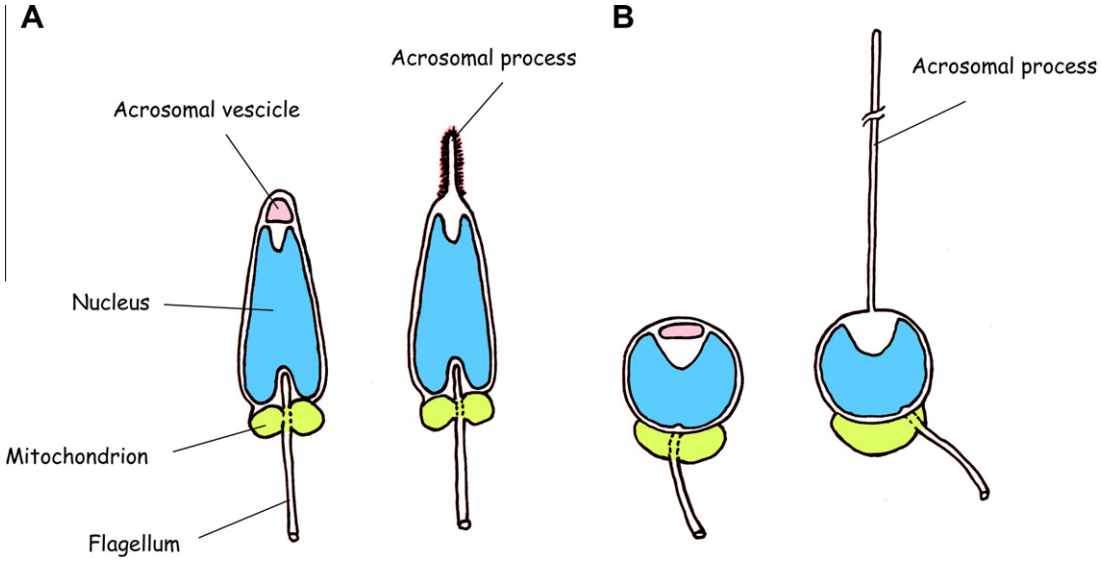

Fig. 1. Morphology of sea urchin and starfish sperm before and after acrosome reaction. While sea urchin spermatozoon (*S. purpuratus*) generally appears cone-shaped (A), starfish spermatozoon (*A. pectinifera*) bears more spherical outline (B). Acrosome-reacted sperm of starfish extends a long (ca 25 μm) rigid acrosomal process that is cored by actin filaments.

small step-depolarizations (polyspermy) were mainly dependent on sperm density, and not on the change of the membrane potential [17], which was rather interpreted as an electrical result of cortical granules exocytosis [14]. Thus, whether membrane depolarization is responsible for the fast block to polyspermy remains controversial [18].

5. Ca^{2+} signaling at fertilization of sea urchin eggs

A turning point in the studies on the egg activation was the pioneering observation of a Ca^{2+} increase in the eggs of sea urchin following fertilization [19]. However, it took almost 40 years to conclusively demonstrate that Ca^{2+} was indeed responsible for egg activation of *Lytechinus pictus* and *S. purpuratus* eggs with the use of Ca^{2+}-ionophore A23187 that elicited cortical granules exocytosis, membrane conductance changes, the respiratory burst, and the increases of protein and DNA synthesis, all hallmarks of egg activation [20]. The ionophore-induced Ca^{2+} increase and membrane elevation still occurred in Ca^{2+}-free seawater, indicating that liberation of Ca^{2+} from the intracellular stores is mainly accountable for egg activation. The Ca^{2+}-specific luminescent protein aequorin visualized the explosive rise of free Ca^{2+} during fertilization of medaka and sea urchin eggs [21,22]. The Ca^{2+} signals in fertilized eggs initiated at the point of sperm entry and spread over like a wave, reaching the opposite pole in about 20 s [23]. Two phases of Ca^{2+} increase at fertilization have been demonstrated in sea urchin eggs: (i) a synchronized Ca^{2+} increase at the egg periphery (cortical flash) due to a rapid influx, (ii) Ca^{2+} liberated from the intracellular stores starting from the sperm-interaction site [24].

The search for the molecular mechanisms by which the sperm-egg interaction liberates Ca^{2+} came across the contemporary finding that an increase in the Ca^{2+}-inducing second messenger 1,4,5 inositol trisphosphate ($InsP_3$) correlated with the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) in the plasma membrane by phospholipase C (PLC) [25]. The injection of $InsP_3$ into sea urchin eggs indeed caused a Ca^{2+} rise and the exocytosis of the cortical granules [26]. $InsP_3$ formed at the sperm interaction site thus appeared to bind to the receptor on the egg endoplasmic reticulum (ER), and the local release of Ca^{2+} may further propagate the Ca^{2+} wave by a Ca^{2+}-induced Ca^{2+} release (CICR, see below) [27].

Studies on three species of sea urchin showed that microinjected GTP-γS caused Ca^{2+} release and cortical granules exocytosis, indicating that G-protein-linked phospholipase C (PLCβ) may mediate egg activation [28]. Alternatively, the gametes interaction may activate SRC family kinases (SFKs), which in turn activates PLCγ to produce $InsP_3$ [29]. Indeed, echinoderm eggs contain both PLCβ and PLCγ [30], and tyrosine kinase activity becomes stimulated in sea urchin eggs before the initiation of Ca^{2+} increase [31]. Inhibiting the endogenous PLCγ by injecting the SH2 domain of PLCγ into sea urchin and starfish eggs have shown that fertilization Ca^{2+} wave may be mediated by a SFK-PLCγ signaling pathway [29].

Another line of thoughts regarding sperm-induced Ca^{2+} increase in fertilized egg is the conduit theory pioneered by Loeb in 1899 that has been elaborated in various model systems [32]. In this view, the Ca^{2+} increase in eggs at fertilization could be induced by sperm through a direct infusion of Ca^{2+} itself [8,33] or other 'sperm factors'. Among other candidates, $InsP_3$, nitric oxide (NO), nicotinic acid adenine dinucleotide phosphate (NAADP), and several proteins were considered [26,34,35], and in mammalian fertilization, the sperm-specific PLCζ has recently earned its support as a sperm factor [36].

6. cADPr and NAADP-mediated Ca^{2+} release at fertilization of sea urchin and starfish eggs

In analogy to the Ca^{2+} transients in other cell types, a CICR mechanism distinct from the $InsP_3$-mediated Ca^{2+} release has been shown in sea urchin eggs. cADPr, a metabolite of NAD^+, mobilized Ca^{2+} by a CICR mechanism acting on ryanodine receptors (RyRs) that were shown to be active in sea urchin eggs. Thus, this mode of Ca^{2+} release in concert with the $InsP_3$-mediated pathway may modulate the spatiotemporal pattern of the Ca^{2+} wave at fertilization [37]. Three ADP-ribosyl cyclase isoforms (ARCs) that produce cADPr have been characterized in sea urchin eggs. While ARCα has an extracellular localization, ARCβ and ARCγ are present in the acidic cortical granules that generate the fertilization envelope upon exocytosis. The active ARCβ and ARCγ might provide cADPr to induce Ca^{2+} rise to drive cortical granules exocytosis [38].

One report suggested NO as the initiator of the Ca^{2+} release at fertilization in sea urchin. NO production occurred in both acrosome-reacted sperm and in activated eggs. Binding of NO to a guanylyl cyclase could induce a cGMP-dependent activation of ARC and produce cADPr [34]. The level of cGMP increases at fertilization [31,39], and thus could stimulate the synthesis of cADPr, supporting the hypothesis that cADPr may control intracellular Ca^{2+} signaling in fertilized sea urchin eggs [40]. However, other investigators showed that the NO increase occurred after the intracellular Ca^{2+} rise. It was thus concluded that the NO production was dependent on the Ca^{2+} increase initiated by $InsP_3$ at fertilization, and that its role was to sustain the second phase of Ca^{2+} response in a cADPr-regulated RyRs pathway [41].

The sea urchin egg is responsive to yet another Ca^{2+}-linked second messenger: NAADP. In sea urchin eggs with centrifugation-stratified organelles, the patterns of Ca^{2+} release by uncaged NAADP was clearly distinct from those of cADPr and $InsP_3$, indicating the existence of a Ca^{2+} store that may be distinct from the ER. In sea urchin eggs, NAADP appeared to mobilize Ca^{2+} from a lysosome-related organelles, as judged by the loss of the Ca^{2+} signals in the eggs treated with Glycyl-L-phenylalanine-ß-naphthylamide (GPN) and bafilomycin A1 [42].

Recent findings have provided evidence that NAADP activates a two pore channel (TPC) located on the acidic Ca^{2+} stores in the endo-lysosomal system [43]. *S. purpuratus* eggs express three TPCs (*Sp*TPCs). Immunostaining of *Sp*TPC3 revealed a punctate cortical and cytoplasmic staining pattern. Heterologous expression of *Sp*TPCs in starfish oocytes has shown that all three isoforms have a cortical distribution [44], in line with the finding that the greatest NAADP sensitivity resides in the cortical region of starfish eggs [45–47]. As NAADP largely increases in sea urchin sperm at AR, a bolus of messenger might trigger the cortical flash preceding the Ca^{2+} wave [35]. Thus, the current hypothesis in sea urchin eggs is that NAADP, probably delivered into the egg by the sperm, induces the cortical flash and the regenerative Ca^{2+} wave by acting on NAADP receptors (TPCs) localized on cortical acidic stores that are functionally coupled to the ER Ca^{2+} channels through a CICR mechanism.

7. The role of Ca^{2+} in cortical granules exocytosis

The autocatalytic cortical reaction that spreads from the sperm–egg interaction site was first discovered by Derbès (1847) and has been most extensively studied in sea urchin eggs [48]. The fusion of the cortical granules with the plasma membrane extrudes their contents into the perivitelline space due to the intracellular Ca^{2+} rise [49]. Isolated cortical granules of sea urchin eggs (cortical 'lawns') could be induced to undergo exocytosis upon addition of Ca^{2+} [50], whereas the microinjection of EGTA prevented the eleva-

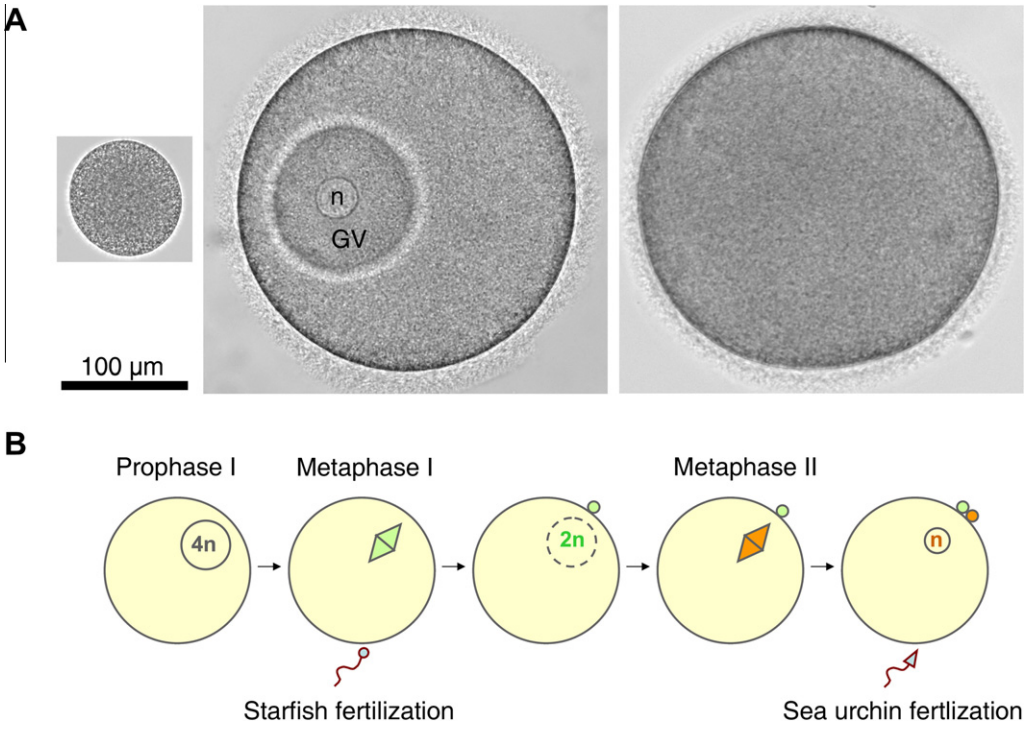

Fig. 2. Comparison of sea urchin and starfish eggs. (A) In photomicrographs displayed on the same scale, a sea urchin egg (*Paracentrotus lividus*) was apposed to the immature and mature eggs of starfish (*Astropecten aranciacus*). The average diameter of *P. lividus* eggs (80 µm) is about the size of the germinal vesicle (GV) of the starfish oocytes. Due to its large size (250–350 µm), even the nucleolus (n) is easily recognized in starfish oocytes. (B) Sea urchin and starfish eggs are fertilized at different meiotic stages (see the text). The first (2n) and second (n) polar bodies are depicted with small circles in green and orange, respectively.

tion of the fertilization envelope [51]. At fertilization, the elevation of the vitelline layer is initiated by a trypsin-like protease released from the cortical granules that frees the vitelline layer from the plasma membrane. This may help to prevent supernumerary sperm binding. The secreted material self-aggregates in the presence of Ca^{2+}, and this process is strongly inhibited by reducing agents, as the hardening of the fertilization envelope is a consequence of transglutaminase and peroxidise activities [52,53]. In addition, two bursts of microvillar elongation occur, which are essential for sperm incorporation and the subsequent cleavage [54].

8. Starfish oocytes as a model system for studying cortical and nuclear maturation at meiosis

The importance of the cytoplasmic maturation of the egg has been emphasized for more than a century [55]. Immature oocytes extracted from the starfish gonad are arrested at the first prophase of meiosis, and contain a large nucleus named the germinal vesicle (GV) (Fig. 2A). They are transparent and very large (about 300 µm), and thus suitable for imaging experiments. By adding the hormone 1-methyladenine (1-MA), the oocytes can be induced to resume meiosis and to undergo the physiological and morphological changes necessary for normal fertilization, the most prominent sign of which is the breakdown of the nuclear envelope (GVBD). Thus, starfish oocytes have provided an excellent system in which to study biochemical events of meiotic maturation, optimization of the Ca^{2+}-release systems, the establishment of polyspermy-preventing mechanisms, and the changes of the egg-dependent sperm pronucleus [56,57].

At variance with sea urchin eggs, the optimum period to fertilize starfish eggs is at the interval between GVBD and extrusion of the first polar body (Fig. 2B). When the immature oocytes at the GV stage are fertilized, several fertilization cones are formed and multiple sperm enter the oocytes (Fig. 3A). Thus, the exposure to 1-MA drastically decreases the ability of the mature eggs to be penetrated by supernumerary sperm (Fig. 3B). On the other hand, overmatured eggs beyond extrusion of the first polar body exhibit polyspermy and abnormal development [58]. It is noteworthy that overmatured eggs in human display regional loss of microvilli [59]. It is conceivable that the mature eggs at the optimal time frame for fertilization present the adequate structural organization of the cortex for ensuring both monospermic sperm entry and the normal elevation of the fertilization envelope [60–62]. Likewise, in sea urchin, immature oocytes also fail to undergo cortical granule exocytosis at fertilization, and lead to polyspermy. These oocytes exhibit the first electrical change about 5 s after sperm addition, as opposed to the 13 s period observed in the mature eggs. Each sperm entry is marked by a step depolarization and by the formation of large cytoplasmic protrusions on the oocyte surface [63].

Cortical maturation also includes cytoskeletal changes. Exposed to 1-MA, immature oocytes of starfish immediately reorganize microvilli on the surface, caused by the rapid assembly and disassembly of the filamentous actin (F-actin) [64]. An equally rapid change that takes place is the fast release of intracellular Ca^{2+} in the cytoplasm and nucleus [65]. The Ca^{2+} wave stimulated by 1-MA starts at the vegetal hemisphere and propagates to the opposite pole exclusively through the cortical layer. Interestingly, the dynamics of the 1-MA-induced Ca^{2+} signal is spatially and temporally influenced by the architecture of the cortical actin cytoskeleton [66,67]. The surface of the mature eggs, however, shows a decrease in microvillar length, along with F-actin-based relocation of the cortical granules which are now seen intimately apposed to the plasma membrane [60,68].

At meiotic maturation of starfish oocytes, the electrical properties of the plasma membrane abruptly shifts at GVBD due to the loss of K^+ permeability, and the resting potential switches from about −90 to −20 mV [17,69], and the transition process may re-

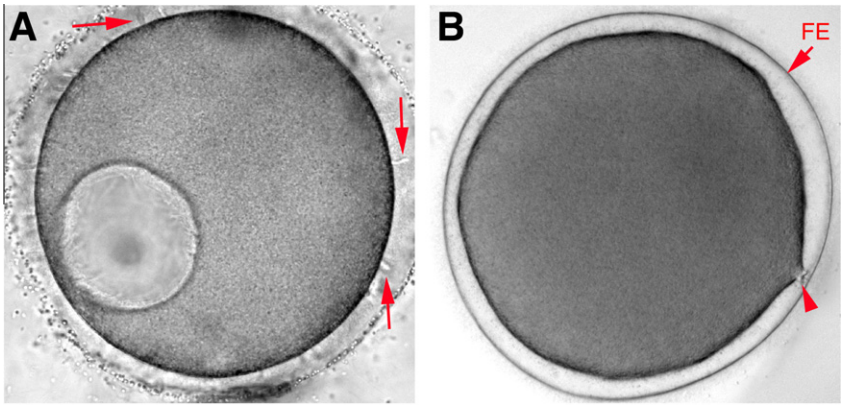

Fig. 3. Fertilization of the immature oocyte and mature eggs of starfish (*A. aranciacus*). (A) At fertilization, the GV-stage oocyte forms numerous cytoplasmic protrusions (arrows) (A), while the mature eggs form a single fertilization cone (arrowhead) and elevation of the fertilization envelope (FE) (B). These differences are attributed to the changes of the egg surface and the ectoplasm that take place during meiotic maturation.

quire contribution of nuclear components [70]. The mechanism by which 1-MA alters the electrical properties of the plasma membrane is unknown, but it may reflect the structural reorganization of the cortex and the changes in the nucleus. These changes collectively optimize the Ca^{2+} response and ensure monospermic fertilization [46,57,71].

9. Optimization of the intracellular Ca^{2+}-release system during maturation of starfish oocytes

Compared with the immature oocytes at GV stage, matured eggs of starfish respond to the same dose of $InsP_3$ with much higher Ca^{2+} release [61,62,71]. This might be in part due to the reorganization of the endoplasmic reticulum (ER) that takes place during the maturation process [72]. The increased sensitivity of the Ca^{2+} stores to $InsP_3$ starts at the animal hemisphere of the maturing oocyte and spreads along the animal-vegetal axis to the opposite side [62,73]. The development of the increased sensitivity to $InsP_3$ is linked to the nuclear activation of the maturation-promoting factor (MPF), a cyclin-dependent kinase controlling the entry of eukaryotic cells into the M phase [62,74]. The higher Ca^{2+} response to $InsP_3$ at the end of maturation was not linked to the redistribution or to the increase of $InsP_3$Rs, but rather correlated with the eggs' development of an unusual ability to respond to latrunculin-A (LAT-A) with a Ca^{2+} release [75]. The spatiotemporal modifications of the actin cytoskeleton during maturation might thus be important for optimizing the Ca^{2+} response to $InsP_3$ and fertilizing sperm.

10. Gametes interaction and activation in starfish

The fertilization of starfish eggs is of special interest because of the long and slender sperm acrosomal process (Fig. 1). The question of where it originates, i.e. from the egg or from the spermatozoon, has stimulated intense debates in the literature. The observed acrosomal filament was initially thought to be an extension of the egg's fertilization cone or the tubular protrusion of the ectoplasm reaching the outer edge of the jelly layer to capture the spermatozoon [76–78]. Just [79], rejected this early interpretation by stating that "the cone forms after the attachment of the spermatozoön to the vitelline membrane Moreover, this strand is a prolongation of the spermatozoön, the tip of which is fixed within the cone." Dan [6] finally settled the argument by demonstrating that sperm can form acrosomal process in the "egg water" without eggs. The sperm's functional contact on egg plasma membrane via acrosomal process was nicely demonstrated by electrophysiology [80] and calcium imaging coupled with digitally enhanced video camera [81]. Such contact via the tip of the acrosomal process was respectively manifested by the changes of the membrane potential and the generation of the cortical flash and the initial Ca^{2+} wave at the site of sperm interaction, and both electrical and Ca^{2+} changes took place while the sperm head was still on the rim of the jelly coat [80–82].

Starfish have another edge over sea urchin in studying the kinetics of Ca^{2+} rise. The starfish oocytes are much larger (Fig. 2A), and it take longer time for the Ca^{2+} wave to propagate to the opposite side of the egg (Fig. 4). However, the planar velocities of the Ca^{2+} waves in the two eggs are virtually the same, implying that the 'irritable matrix' of the starfish and sea urchin eggs might be the same. Nonetheless, the starfish and sea urchin eggs might have subtly different way of initiating the Ca^{2+} waves at fertilization. It was originally suggested that NAADP may have a triggering role in initiating the sperm-induced Ca^{2+} response at fertilization [83,84]. Injection of NAADP or photoliberation of the caged NAADP in the starfish eggs induced a Ca^{2+} response only in the cortical region. Unlike $InsP_3$, Ca^{2+} response to NAADP was not affected by the removal of the nucleus [45,46]. At variance with sea urchin eggs, the NAADP-induced Ca^{2+} response in starfish eggs required external Ca^{2+} and was selectively inhibited by blockers of L-type and store operated Ca^{2+} channels, which bears a striking difference from the pathway involving NAADP-responsive Ca^{2+} stores in the acidic organelles of sea urchin eggs [35]. In line with a role in initiating egg activation, NAADP mimicked the sperm-evoked depolarization by activating a Ca^{2+}-mediated inward current which was blocked by BAPTA but was not affected by the impairment of lysosomes with Bafilomycin A1 or GPN, nor by agents blocking

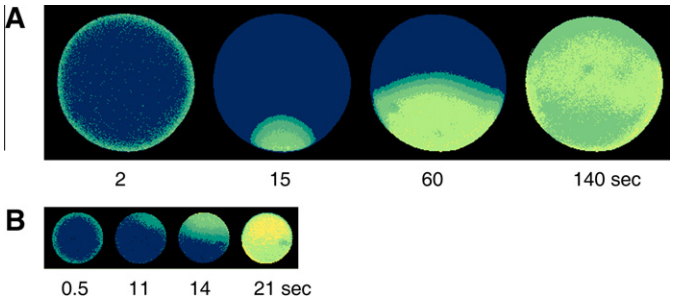

Fig. 4. Calcium signals in sea urchin and starfish eggs at fertilization. The propagation of the Ca^{2+} waves in the *A. aranciacus* and *P. lividus* eggs were visualized by Calcium Green and displayed in pseudocolors on the same scale. While it takes about 20 s for the wave front to reach the other end of the *P. lividus* eggs, the large eggs of *A. aranciacus* takes much longer time, proportional to its size.

RyRs and InsPRs [47,85,86]. To date, there is no evidence for an increase in NAADP in the activated starfish sperm nor in the egg at fertilization, and therefore it is still puzzling how NAADP could initiate the Ca^{2+} response in this species. The possibility that a cADPr-sensitive RyRs may sustain the fertilization Ca^{2+} response initiated by NAADP in starfish is less likely in view of the finding that the sperm-induced Ca^{2+} increase in *A. aranciacus* is not affected by the antagonist of cADPr at variance with sea urchin eggs [37,41,87]. Similarly, cADPr-mediated pathway may not be an absolute requirement in propagating fertilization Ca^{2+} signals in starfish, as the regenerative sperm-induced Ca^{2+} response takes place in the Japanese species *Asterina pectinifera* although these eggs are not responsive to cADPr [66].

11. Actin cytoskeleton and calcium signaling at fertilization of starfish eggs

Perturbation of the finely regulated actin cytoskeleton of starfish eggs by various methods leads to alteration of the Ca^{2+} signaling pattern at fertilization and in response to Ca^{2+}-releasing second messengers. This has been demonstrated by cofilin, latrunculin A, jasplakinolide, GDPβS, and the PIP2-binding domain of PLCδ1 [67,81,88,89]. Surprisingly, the classical inhibitor of $InsP_3Rs$, heparin, caused hyperpolymerization of cortical actin, and displayed significant reduction of cADPr-dependent Ca^{2+} release. When these eggs with deregulated cortical actin cytoskeleton were fertilized, there were signs of multiple sperm interactions and entries [81,89]. Thus, the fine regulation of the actin cytoskeleton and its associated reorganization at the egg surface might contribute to the prevention of polyspermy. Moreover, the coordinated movement of the cortical actin fibers at fertilization might play an important role in early development. Perturbation of the actin cytoskeleton by a brief exposure to ionomycin inhibited the orderly centripetal translocation of actin fibers that normally occurs at the fertilization of echinoderm eggs [81,90], and was associated with an alarming rate of failed development of the monospermic zygotes [91]. These results highlight the importance of the structural organization of the egg cortex in the modulation of the events associated with oocyte maturation, fertilization and embryonic development.

12. Conclusion

Fertilization comprises a series of interaction and mutual activation between the egg and spermatozoon. The changes that occur at distance or in direct physical contact exhibit some parallelism in the two gametes. The acrosomal reaction of the sperm and the elevation of the fertilization envelope in echinoderm eggs are both based on exocytosis. The two processes are triggered by intracellular increase of Ca^{2+}. The perturbation of the maturation process and surgical extraction of the GV have permitted to dissect the specific roles played by the Ca^{2+}-linked second messengers (NAADP and $InsP_3$) in shaping the essential cortical and cytoplasmic Ca^{2+} increase in starfish eggs. The results have revealed the importance of egg cortex and its structural changes that are finely regulated during maturation and fertilization. Behind these changes is the actin cytoskeleton that modulates events associated with fertilization, i.e., the cortical granules exocytosis, the Ca^{2+} response, the incorporation of the sperm, and the cleavage of the zygote. Perhaps these changes might be best summed by quoting Just's intuitive statement [92]: "Without the ectoplasm fertilization cannot take place, that in both fertilization and parthenogenesis the response of the ectoplasm to the inciting means for development is prognostic for the quality of the future development; that in cell-division the ectoplasm initiates the event by regulating the movements within the cytoplasm and that by redistribution of its structure and relocalization of its activity, it establishes new cell-surface; and that during differentiation the ectoplasm increases in amount and reveals a differential activity."

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