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Calcium signaling surrounding fertilization in the nematode *Caenorhabditis elegans*

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Summary

Calcium plays a prominent role during fertilization in many animals. This review focuses on roles of Ca²⁺ during the events around fertilization in the model organism, *Caenorhabditis elegans*. Specifically, the role of Ca²⁺ in sperm, oocytes and the surrounding somatic tissues during fertilization will be discussed, with the focus on sperm activation, meiotic maturation of oocytes, ovulation, sperm-egg interaction and fertilization.

1. Introduction

Calcium plays a ubiquitous role in biological pathways. Fertilization is one such process that exploits Ca²⁺ mediated signals in several phases and many species across the animal kingdom seem to rely extensively upon Ca²⁺ signal for this purpose [1].

Caenorhabditis elegans is an attractive model system to study the biology of fertilization [2]. The transparent nature of the body permits the live examination of several key events of fertilization [3]. Especially, monitoring the influx of Ca²⁺ ions into oocytes enables one to directly correlate the Ca²⁺ signaling with the hall marks of fertilization in an intact animal.

C. elegans exist as sexually dimorphic males and hermaphrodites. Hermaphrodites are predominantly found in natural population and under normal culture conditions in the lab. Hermaphrodites produce both sperm and oocytes, which enable the animal to self-fertilize to produce self-progeny. Males are found very rarely in nature and produce only sperm. Males can mate with hermaphrodite to sire cross-progeny [4].

The reproductive system in *C. elegans* is consists of a simple U shaped tube (figure 1) [5]. At the distal most part of the gonad reside germ cells, which undergo mitotic proliferation, followed by meiotic division to give rise to gametes. During the initial phase of gametogenesis, hermaphrodites produce a limited number of sperm. Thereafter, it continues to produce oocytes only [6].

The diploid spermatocytes undergo complete meiotic division to give rise to haploid spermatids through a process called spermatogenesis [7–9]. The spermatids are round in shape, immotile and are incapable of fertilizing oocyte. Spermatids are rapidly activated to

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form mature spermatozoa through a process called spermiogenesis or sperm activation (figure 2) [8]. During the activation of sperm, pseudopods are formed from one side of the sperm, which enable the sperm to become motile and fertilization-competent. *C. elegans* sperm contain a specialized Golgi-derived vesicle called membranous organelle (MO). In spermatids, these organelles are sequestered inside the cytosol. During or following sperm activation, MOs fuse with plasma membrane and release their contents outside [9].

Oocytes undergo several cytological changes in preparation for fertilization and enter into spermatheca serially (see figure 1). The process of entry of oocyte into spermatheca is called ovulation [10]. Contraction of gonadal sheath cells accompanied by the dilation of distal spermatheca valve propels the oocyte into spermatheca [3]. Fertilization takes place in spermatheca, which can house both male derived and hermaphrodite derived sperm. Newly fertilized eggs then enter into the uterus, where early embryonic development takes place. Eventually eggs are pushed out on to the surrounding media through the vulva.

2. Roles of Ca^{2+} and Ca^{2+} binding proteins during sperm activation

Depletion of extracellular Ca^{2+} by treating spermatids with EDTA or EGTA does not compromise sperm activation, which suggests that Ca^{2+} from extracellular environment may not play a role in sperm activation [11]. In contrast, buffering intracellular Ca^{2+} by treating the spermatids with BAPTA-AM (a membrane permeable Ca^{2+} chelator) causes reduction in MO fusion, suggesting that intracellular Ca^{2+} is necessary for MO fusion and sperm activation [12].

The requirement of intracellular Ca^{2+} for the complete activation of *C. elegans* sperm raises an interesting question: is the intracellular pool of Ca^{2+} regulated in sperm? Most eukaryotic cells sequester Ca^{2+} in the endoplasmic reticulum (ER) in order to ensure the regulated delivery of Ca^{2+} into the cytosol [13]. However, most of the organelles, including ER, are discarded during spermatogenesis in *C. elegans* [14]. Therefore, it is less likely that ER could play a prominent role in regulating Ca^{2+} homeostasis in *C. elegans* sperm. Surprisingly, calreticulin, a Ca^{2+} buffering protein that resides in ER, is detected in *C. elegans* sperm and plays a role in fertility in *C. elegans* [15]. Since calreticulin has the propensity to retrotranslocate from ER to cytosol [16], it is likely that the observed CRT-1 staining in *C. elegans* sperm corresponds to the pool of cytosol-localized CRT-1 [15]. *In vitro* activated *crt-1* mutant spermatozoa are smaller than wild-type sperm, display shorter pseudopods and the nuclei are positioned off center [15]. Since larger sperm outcompete smaller sperm in *C. elegans* [17], the function of CRT-1 might be critical for the reproductive success of the worm.

In addition to ER, other organelles such as mitochondria, Golgi apparatus, lysosome and nucleus also stores Ca^{2+} [18]. Since sperm are enriched with mitochondria [19] it would be interesting to investigate the potential role of mitochondria in regulating Ca^{2+} homeostasis in *C. elegans* sperm. In addition to mitochondria, sperm are also packed with many MOs. Given that MOs are Golgi-derived organelle and possibly related to lysosome [20], it would be interesting to test the hypothesis that MOs function as a Ca^{2+} store in *C. elegans* sperm. Since sperm devoid of nucleus can successfully undergo spermiogenesis and fertilize oocyte [21], the contribution of Ca^{2+} mobilized from nucleus, if any, should be insignificant for regulating spermiogenesis in *C. elegans*.

Although intracellular Ca^{2+} is necessary, it's not sufficient for the sperm activation, since artificial increment of intracellular Ca^{2+} level by treating spermatids with Ca^{2+} ionophores does not initiate spermiogenesis [11]. However, it should be noted that the toxicity associated with ionophore treatment could also prevented spermiogenesis.

How is the Ca^{2+} signal coupled with sperm activation? Calmodulin is one of the Ca^{2+} binding proteins that are involved in variety of Ca^{2+} initiated signaling pathways. Surprisingly inhibition, rather than activation, of calmodulin induces spermiogenesis. A variety of calmodulin-inhibitors, such as trifluoperazine (TFP), chlorpromazine (CPZ), naphthalenesulfonamide (W7) induces spermiogenesis *in vitro* [22]. Although spermiogenesis is initiated by the calmodulin-inhibitor TFP, the resultant sperm do not fully undergo activation, but rather arrest as spermiogenesis intermediates. TFP treated spermatids do not fully form villar projection and are immotile. Upon flushing TFP, the morphology of the sperm become normal spermatozoa and become motile. Conversely, treatment of motile spermatozoa with TFP renders the sperm round and immotile, which suggests the plausible role of calmodulin in sperm-motility.

Formation of pseudopod and acquisition of motility is dependent on the assembly of specialized filaments of Major Sperm Protein (MSP). Given the involvement of kinases and phosphatases in modulating the assembly and disassembly of MSP filaments in nematodes [23–26], it is tempting to speculate that Ca^{2+} /calmodulin kinase – one of the main downstream target proteins of calmodulin - could potentially play a role in regulating the dynamics of MSP filament formation by altering the phosphorylation status of target proteins.

Calcineurin (a Ser/Thr phosphatase) is one of the downstream target enzymes activated by Ca^{2+} /calmodulin. Interestingly, the pseudopods of calcineurin mutant sperm are smaller in size. Since calcineurin is activated by calmodulin and the full activation of sperm likely depends on calmodulin, it's possible that Ca^{2+} /calmodulin dependent activation of calcineurin might be essential for the complete execution of spermiogenesis in *C. elegans*. Epistatic and/or pharmacological analyses using mutants of potential players would give us more details on the signal transduction pathway culminating in spermiogenesis.

All these observations suggest the possible role of calmodulin in blocking the initial onset of spermiogenesis and enabling the sperm to become motility-competent. However, the potential role of calmodulin in regulating sperm activation is unclear. First, calmodulin inhibitors elicit many biological effects that are not dependent on calmodulin [22]. Second, knock down of calmodulin homolog, *cmd-1*, leads to embryonic lethality [27], meaning that fertilization do occur normally and embryo do form (and then die) following *cmd-1* RNAi treatment. Since RNAi treatment does not guarantee complete elimination of transcripts, analysis of *cmd-1* null mutant would clear the ambiguity surrounding the *in vivo* relevance of calmodulin in fertility.

3. Fusion of membranous organelles (MO) with plasma membrane

During the activation of wild-type sperm, membranous organelles (MOs) move toward plasma membrane and fuse with it to form a fusion pore (see figure 2). The *fer-1* mutant sperm undergo activation, but the pseudopods are short and stubby [9]. Electron microscopy reveals that although the MOs abut plasma membrane, they fail to fuse with it, suggesting that FER-1 protein is essential for fusion of MOs with plasma membrane.

The FER-1 is a membrane protein that has C2 domains, which commonly mediate Ca^{2+} assisted events [12, 28]. Three isoforms of FER-1 were recognized by probing the extract of males with polyclonal antibody raised against part of FER-1 protein. The three isoforms are likely arising from proteolytic cleavage [12].

Probing the localization of FER-1 with anti-FER-1 antibody revealed that FER-1 is initially found in the MOs of spermatids. However, in mature spermatozoa, the FER-1 is found all over the plasma membrane, suggesting that after MOs fuse with plasma membrane, FER-1

exit MOs and gets distributed all over the plasma membrane. Quantitative immunoelectron microscopy using anti-FER-1 antibody also shows identical localization dynamics. This localization pattern is similar to one other protein that functions in sperm: TRP-3/SPE-41 [29]. However, it is not clear whether TRP-3/SPE-41 plays any role in the fusion of MOs or providing Ca^{2+} for FER-1 activation.

The *fer-1* mutants are hypersensitive to the depletion of intracellular Ca^{2+} level. Depleting the intracellular Ca^{2+} with 1mM of BAPTA-AM (a membrane permeable Ca^{2+} chelator) doesn't prevent the wild-type spermatids from fusion of MOs with plasma membrane. However, in specific *fer-1* mutant alleles, the same concentration of BAPTA-AM caused the reduction in the MOs fusion, corroborating the idea that Ca^{2+} might regulate FER-1 function, presumably through C2 domain [12]. FER-1 seems to link the Ca^{2+} signal with the fusion between MOs and the plasma membrane. The positioning of MOs abutting plasma membrane is independent of FER-1 function.

4. Meiotic maturation of oocytes

In *C. elegans*, oocytes are arrested in meiotic prophase I before fertilization. Sperm secrete MSP [30], which then bind with MSP receptors (VAB-1 and others) found on the surface of oocyte and initiate signal transduction pathway culminating in the resumption of meiosis or 'meiotic maturation' of oocyte [31]. Calcium plays important role during the meiotic maturation of oocytes. At least three proteins linked to the Ca^{2+} signaling coordinate the meiotic maturation of *C. elegans* oocytes: inositol 1,4,5-triphosphate receptor (ITR-1), N-methyl D-aspartate type glutamate receptor subunit (NMR-1) and Ca^{2+} /calmodulin dependent protein kinase II (UNC-43) [32]. In response to MSP signaling, these three proteins function in oocytes to regulate meiotic maturation [32].

Endoplasmic reticulum is one of the major warehouses of Ca^{2+} in most of the eukaryotic cells. Activated phospholipase C generates IP_3 from the plasma membrane, which then binds with IP_3 receptor found on the ER membrane to mobilize Ca^{2+} into the cytosol. The gene *itr-1* encodes IP_3 receptor homolog in *C. elegans* [33]. ITR-1 represses meiotic maturation in the absence of sperm [32]. The *itr-1* reduction-of-function and *itr-1* gain-of-function mutant females exhibits increased and decreased rate of oocyte maturation, respectively. Epistatic analyses indicate that *itr-1* acts downstream of *vab-1* to inhibit meiotic maturation [32].

Similarly, a mutation eliminating Ca^{2+} channel component NMR-1, results in the increased rate of oocyte maturation in females, which suggest that like ITR-1, NMR-1 also represses meiotic maturation of oocyte in the absence of sperm. Complex genetic analyses suggest that NMR-1 cross-talk with VAB-1 in coupling the MSP signal with meiotic maturation [32].

Increased cytosolic Ca^{2+} activates calmodulin, which in turn activates many downstream target proteins and UNC-43 is one such Ca^{2+} /calmodulin activated protein kinase. The *unc-43* loss-of-function mutant exhibits slower oocyte maturation rates, whereas *unc-43* gain-of-function mutant exhibits faster maturation rates, indicating that UNC-43 positively regulates meiotic maturation in *C. elegans* [32]. Epistatic analysis suggests that in the absence of sperm, NMR-1 negatively regulates UNC-43. Binding of MSP with VAB-1 triggers NMR-1 mediated activation of UNC-43 [32].

5. Propelling oocyte into spermatheca

Contraction of gonadal sheath cells, accompanied by dilation of distal spermathecal valve, push the oocyte into spermatheca. IP_3 signaling plays an important role in both contraction

of sheath cells and dilation of spermatheca valve (see figure 3). Sheath cell contraction requires extracellular Ca^{2+} , since removal of extracellular Ca^{2+} inhibits contractility of isolated gonads [34]. Oocytes secrete LIN-3/EGF ligand which binds with LET-23/EGF receptor found on the gonadal sheath cells to activate PLC-3/phospholipase C γ . PLC-3 converts PIP_2 into IP_3 from membrane which in turn binds with IP_3 receptor (ITR-1) on the ER membrane to mobilize Ca^{2+} . Several lines of evidence support this model: LET-23, PLC-3 and ITR-1 are expressed in sheath cells [34, 35]. Loss-of-function mutants and/or knock-down of *let-23*, *plc-3* and *itr-1* causes reduced sheath contractility and ovulation [34].

The dilation of distal spermatheca valve also depends on LIN-3/LET-23 mediated generation of IP_3 [36]. The transcription factors FOS-1 and JUN-1 promote the expression of phospholipase C (*plc-1*) in spermatheca [37]. PLC-1 generates IP_3 which in turn binds with ITR-1 to mobilize Ca^{2+} from ER. The level of IP_3 is regulated by altering its phosphorylation status. The enzymes IP_3 kinase (LFE-2) and type I 5 phosphatase (IPP-5) converts IP_3 into IP_4 and IP_2 , respectively [36, 38]. Tight regulation of IP_3 may be critical in order to ensure that only single oocyte will enter spermatheca per cycle. For example, the spermatheca of *ipp-5* mutant engulfs two oocytes per cycle, presumably due to the presence of excessive IP_3 [38].

LET-413 (*Drosophila* Scribble homolog) and DLG-1 (*Drosophila* Discs large homolog) maintain polarity in spermatheca and positively regulate ovulation through regulating the IP_3 pathway. Depletion of *let-413* or *dlg-1* causes ovulation-failure and sterility [39]. The *ipp-5* loss-of-function mutation and *itr-1* gain-of-function mutation can partially suppress the sterility observed in *let-413* or *dlg-1* knock-down animals, suggesting that the LET-413 and DLG-1 positively regulates the IP_3 pathway [39]. The extracellular matrix (ECM) surrounding the spermatheca also contributes to ovulatory signal. The ECM components EPI-1 (laminin- α) and EMB-9 (Collagen IV, $\alpha 1$) likely activate integrins (PAT-3) to generate IP_3 [40]. Knock down of *epi-1*, *emb-9* or *pat-3* causes sterility, which can be rescued by *ipp-5* loss-of-function mutation or *itr-1* gain-of-function mutation, implicating the link between ECM components and the IP_3 pathway [40].

Depletion of Ca^{2+} in the ER is sensed by a Ca^{2+} sensor which then activates a Ca^{2+} release - activated Ca^{2+} (CRAC) channel present on the plasma membrane to replenish the internal Ca^{2+} store [41]. *C. elegans* proteome has an ER Ca^{2+} sensor, STIM-1 and CRAC channel ORAI-1. Both STIM-1 and ORAI-1 are found in the gonadal sheath cells and spermatheca [42–44]. Orai1 first maintains the sustained response and at the termination of the stimulus it functions to replenish the stores. Maintaining the sustained response is an important function of the Orai1-mediated Ca^{2+} influx. Knock down of *stim-1* or *orai-1* causes identical phenotypes: sheath cells do not contract well and distal spermatheca fail to open during ovulation, which indicates that CRAC channel activity positively regulates ovulation in *C. elegans* (see figure 3) [42–44].

6. Cell surface localization of TRP-3/SPE-41 channel by SPE-38

TRP-3/SPE-41 is a transient receptor potential Ca^{2+} permeable channel that plays a role in the terminal phase of fertilization [29]. *trp-3/spe-41* mutants are nearly sterile. Several lines of evidences suggest that SPE-41/TRP-3 functions in the sperm. First, TRP-3/SPE-41 is expressed in sperm, not in oocyte. Second, supplying wild-type sperm to *trp-3/spe-41* mutant hermaphrodites (by crossing with wild-type males) rescues the sterile phenotype. Third, *trp-3/spe-41* mutant males do not sire progeny. Like the rest of the members of a similar class of mutants [19, 45], all aspects of sperm development and differentiation occur normally in *trp-3/spe-41* mutants [29]. The *trp-3/spe-41* spermatozoa are morphologically

indistinguishable from the wild-type spermatozoa; the sperm comes in direct contact with oocyte but fails to fertilize it [29].

Xu and Sternberg developed a protocol to measure changes in intracellular Ca^{2+} level in *C. elegans* sperm [29]. Two types of Ca^{2+} channels are found in *C. elegans* sperm: constitutively active Ca^{2+} -permeable channel (CAC) and store-operated channel (SOC). As the name suggests, CAC is constitutively active, therefore, Ca^{2+} will immediately enter through this class of channel upon increasing the concentration of extracellular Ca^{2+} . On the other hand, the SOC is activated in response to the status of intracellular Ca^{2+} store. Intracellular Ca^{2+} store can be depleted by the application of ionomycin and thapsigargin. When the sperm is subsequently exposed to Ca^{2+} , a larger influx of Ca^{2+} occurs through SOC. This phenomenon is called Store Operated Calcium Entry (SOCE).

The *C. elegans* spermatids exhibit very little CAC and SOCE activities. The mature spermatozoa, however, exhibit prominent SOCE activity. The SOCE activity is dramatically reduced in *trp-3/spe-41* mutant spermatozoa, suggesting that TRP-3/SPE-41 channel contributes to the SOCE in *C. elegans* spermatozoa [29].

TRP-3/SPE-41 is initially localized to the MOs in spermatids. In mature spermatozoa, TRP-3/SPE-41 exit MOs and gets distributed all over the plasma membrane. Since the TRP-3/SPE-41 channels are sequestered in MOs in spermatids - and therefore unexposed to extracellular milieu - barely any SOCE is detected in spermatids. The fusion of MOs with the plasma membrane redistributes TRP-3/SPE-41 channels on to the plasma membrane, which now are directly exposed to extracellular milieu, thereby contributing SOCE activity.

The localization of TRP-3/SPE-41 is dependent on another protein, SPE-38, which is a novel four-pass transmembrane protein that functions in fertilization [46]. Like *trp-3/spe-41* mutant, *spe-38* mutants undergo normal spermatogenesis and spermiogenesis, come in direct contact with the oocyte, but fail to fertilize them. SPE-38 is initially localized to MOs in spermatids. In mature spermatozoa, SPE-38 segregates to pseudopod.

In *spe-38* mutant spermatozoa, TRP-3/SPE-41 is trapped within the MOs, indicating that SPE-38 regulates the localization of TRP-3/SPE-41 (see figure 4) [47]. Considering the fact that fusion of MOs with plasma membrane occurs normally in *spe-38* spermatozoa [46], the inability of TRP-3/SPE-41 to exit MOs suggests that distribution of proteins from fused MOs to plasma membrane is regulated. SPE-38 and TRP-3/SPE-41 physically interact with each other and this interaction is regulated by their cytoplasmic regions [47].

Following the fusion of MOs with plasma membrane, MO resident proteins seem to be selectively permitted past fused MOs. An epitope recognized by the monoclonal antibody 1CB4 continues to reside in MOs even after the fusion of MOs with plasma membrane [12, 48]. Similarly, TRP-3/SPE-41 resides in the fused MOs of *spe-38* mutant spermatozoa, suggesting that diffusion of proteins from the fused MOs to plasma membrane is regulated by SPE-38.

Interestingly, transmission electron microscopy of mature spermatozoa shows electron dense 'collar' at the site of fusion pore [9]. This observation raises the possibility that the collar might act as a barrier for the free diffusion of proteins from MOs to plasma membrane and selective trafficking of proteins across the collar region is facilitated by proteins such as SPE-38 (refer to figure 4). At present, the identities of the individual constituents of the electron dense collar region are unknown. Isolating mutants defective in the MO collar would help us better understand the protein(s) that make or maintain the MO collar and illustrate the nature of the barrier between fused MOs and plasma membrane. Also, this would shine light on how SPE-38 assists in trafficking TRP-3/SPE-41 past the collar region.

Even though TRP-3/SPE-41 channels are stuck in MOs in the absence of SPE-38, the MOs are in direct contact with the extracellular milieu. As a result, TRP-3/SPE-41 is able to function from the fused MOs, as evidenced by the normal SOC activity in *spe-38* spermatozoa (figure 4) [47]. The observation that the TRP-3/SPE-41 mediated Ca^{2+} transients occur normally in *spe-38* spermatozoa, yet fail to fertilize oocyte, raises two possibilities. First, it is possible that the channel activity of TRP-3/SPE-41 is separable from its ability to serve as a sperm-egg recognition molecule. Second, unlike wild-type sperm, where TRP-3/SPE-41 channels are scattered all over the plasma membrane, the TRP-3/SPE-41 mediated Ca^{2+} influx is restricted only from the regions where MOs fuse with plasma membrane in *spe-38* mutants. Therefore, the spatial pattern of Ca^{2+} influx, and not the overall quantity of Ca^{2+} influx, might be decisive in enabling the sperm fertilization-competent.

What triggers TRP-3/SPE-41 channel activity? Following the co-expression of TRP-3/SPE-41 channel with a histamine receptor (H1R) in a mammalian cell line, treatment of histamine exhibited SOCE activity [29]. Similarly, STIM1 can bind with and activate TRPC in mammals [49]. Identifying endogenous protein(s) that trigger the activation of TRP-3/SPE-41 in *C. elegans* will be useful in delineating the mechanistic details of coupling the channel activity with fertilization.

7. Generation and propagation of Ca^{2+} wave following fertilization

Samuel *et al.* monitored the dynamics of intracellular Ca^{2+} during fertilization in *C. elegans* oocytes [50]. The distal region of the gonad is syncytial in nature, which permits the flow of an injected Ca^{2+} indicator dye (Calcium Green-1 dextran) into developing oocytes. When fully developed, the mature oocytes incorporate Ca^{2+} indicator dye in their cytoplasm, permitting the visualization of changes in intracellular Ca^{2+} levels [50]. The resting concentration of Ca^{2+} is estimated to be around 50–100nM in oocyte.

Fertilization triggers elevation of intracellular Ca^{2+} level to around 250nM [50]. The onset of Ca^{2+} elevation occurs at the leading edge of the oocyte – the edge of the oocyte that initially enters into the spermatheca [50]. Since the spermatheca is loaded with sperm, it means that the leading edge of the oocyte is the one that comes in direct contact with sperm at first [51]. Therefore, sperm usually enters at the leading edge of the oocyte. The point of sperm entry determines anterior-posterior axis in *C. elegans* [51]. Coincident with the sperm entry, the onset of Ca^{2+} elevation occurs at the leading edge of oocyte, which raises an interesting possibility that Ca^{2+} signaling might help specify anterior-posterior axis in *C. elegans* [50].

Recently, Jun Takayama and Shuichi Onami probed the fertilization induced Ca^{2+} transient in *C. elegans* oocyte using a more sensitive microscope (personal communication). They found that fertilization triggers biphasic Ca^{2+} waves in oocytes. First, a fast local wave appeared at the site of sperm-entry and disappeared shortly thereafter. Second, a slow global wave propagated from the site of ‘local wave’ to the opposite pole. The second response is reminiscent of the Ca^{2+} induced Ca^{2+} release activity (CICR) (Jun Takayama and Shuichi Onami, personal communication).

Two lines of evidence suggest that sperm triggers biphasic Ca^{2+} wave upon fertilization in *C. elegans*. First, mutant sperm that fails to enter oocyte (*spe-9* sperm) does not initiate Ca^{2+} wave in oocyte. Second, a mutant sperm that does enter oocyte, but fails to activate it (*spe-11* sperm), exhibits normal biphasic Ca^{2+} wave (Jun Takayama and Shuichi Onami, personal communication). TRP-3/SPE-41 channel dictates the onset of local wave. The *trp-3* mutants are nearly sterile and produce few progeny [29]. The initial fast ‘local wave’ was completely missing in those rarely fertilized *trp-3* mutant oocytes, indicating that TRP-3/

SPE-41 channel is essential for the onset of fast ‘local wave’. Although a nearly normal ‘global wave’ was generated in fertilized *trp-3* oocyte, there had been a significant delay in its onset, suggesting that TRP-3/SPE-41 is essential for regulating the temporal pattern of ‘global wave’ generation (Jun Takayama and Shuichi Onami, personal communication).

It would be interesting to investigate what channel(s) contributes towards the propagation of ‘global wave’ of Ca^{2+} in oocyte following fertilization. Many Ca^{2+} channels are enriched in oocytes [52]. Unlike sperm, oocytes are amenable to RNAi. Therefore, systematic knock-down of Ca^{2+} channels enriched in oocyte can be conducted. Any deviation from the wild type pattern of ‘global wave’, following the knock-down of any of these genes might reveal the Ca^{2+} channels required for ‘global wave’ formation after fertilization. Since many of these channels might also function in somatic tissue, performing such screen in a genetic background [*rrf-1(pk1417)*] that would permit knockdown of genes selectively in germ line and spare the somatic tissue [53] would offer practical advantage.

8. Conclusions

Calcium plays important roles in orchestrating various events that prepares and facilitates gamete maturation, interaction and ultimately, fertilization, in *C. elegans*. Full activation of sperm requires intracellular Ca^{2+} . Oocytes are arrested in meiotic prophase I and sperm-derived hormone, MSP relieves the block by modulating the activity of at least three proteins that function in Ca^{2+} signaling. Propelling maturing oocyte into spermatheca requires sheath cell contraction and spermatheca valve dilation, for which IP_3 mediated mobilization of Ca^{2+} from ER plays an important role. Finally, fertilization requires TRP-3/SPE-41 Ca^{2+} permeable channel, whose localization is governed by SPE-38. Calcium waves occur immediately following fertilization in oocytes, which requires TRP-3/SPE-41 channels.

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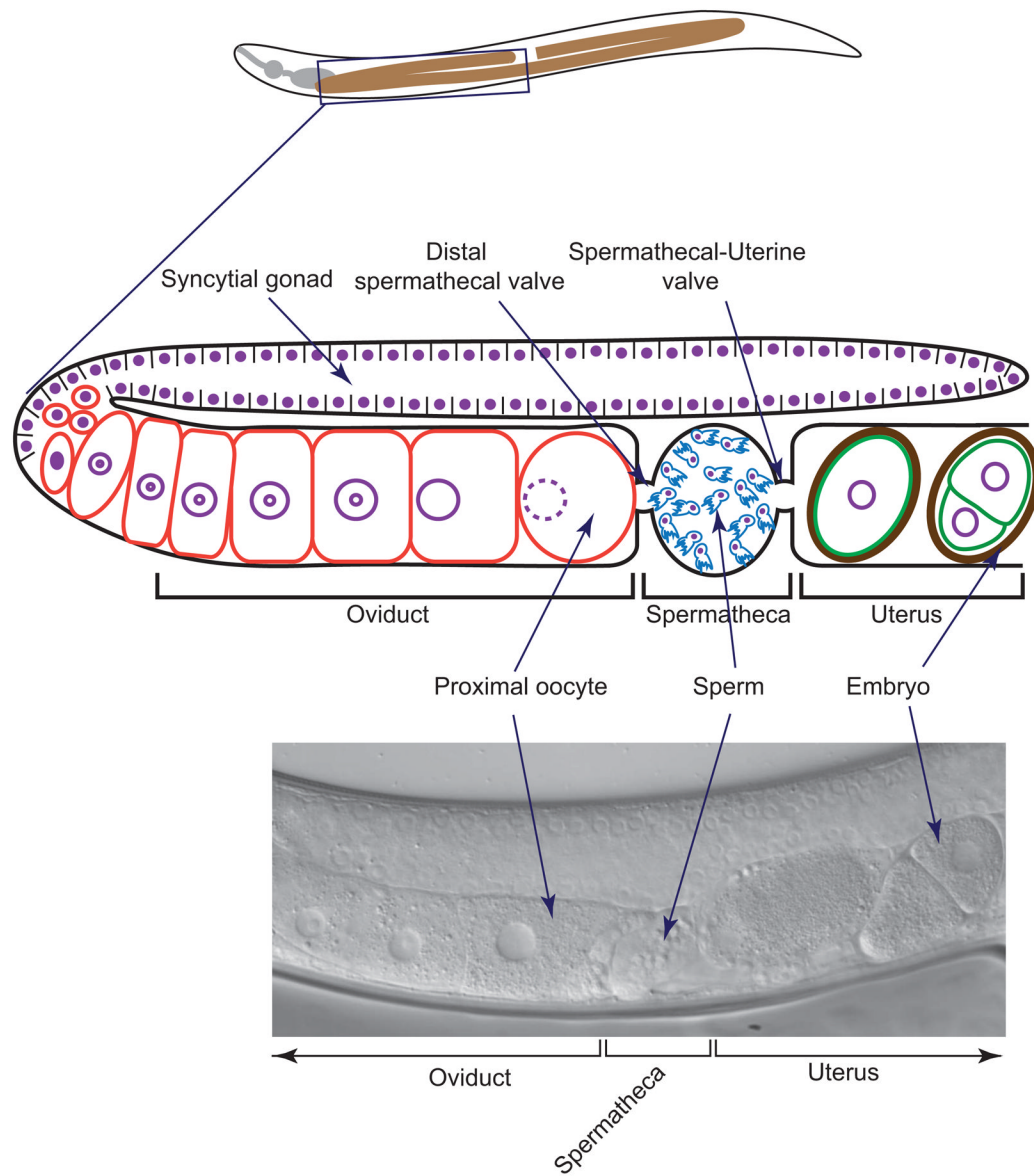


Figure 1. Schematic diagram showing the reproductive tract of adult hermaphrodite (top). Oocytes mature and enter one by one into spermatheca, which is the site of sperm storage and fertilization. Fertilized eggs enter into uterus where early embryonic development takes place. Eventually eggs are pushed out through vulva (not shown). DIC micrograph showing part of the adult gonad (bottom).

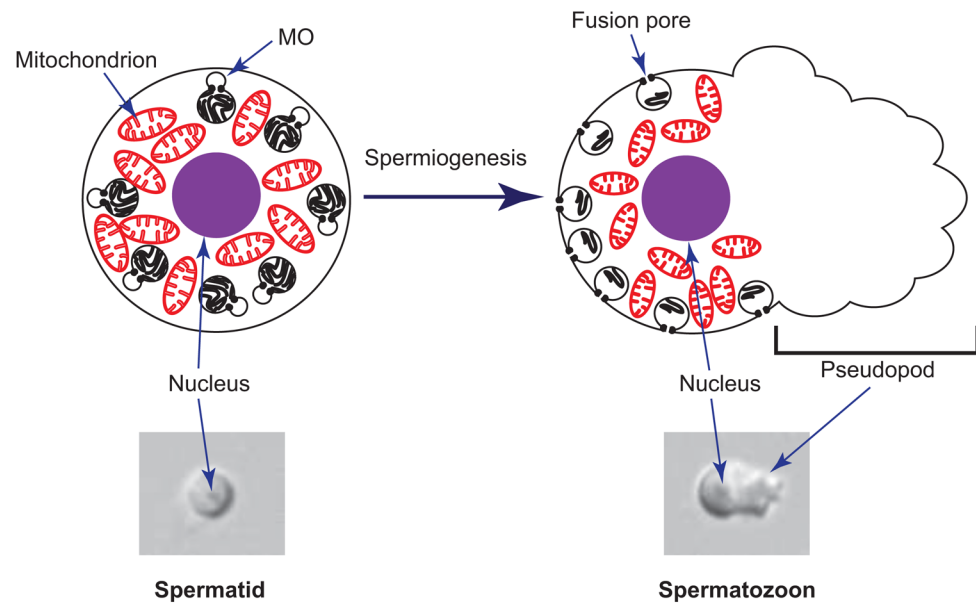


Figure 2. Schematic diagram illustrating spermiogenesis in *C. elegans* (top). Spermiogenesis refers to the process of converting round, immotile spermatids into polarized, motile spermatozoa. Sperm are packed with many mitochondria and Membranous Organelles (MOs). In spermatids, MOs reside inside the cell. During or following activation, MOs fuse with plasma membrane and release their contents outside. The spermatozoon extrudes pseudopod, which is the motility apparatus.

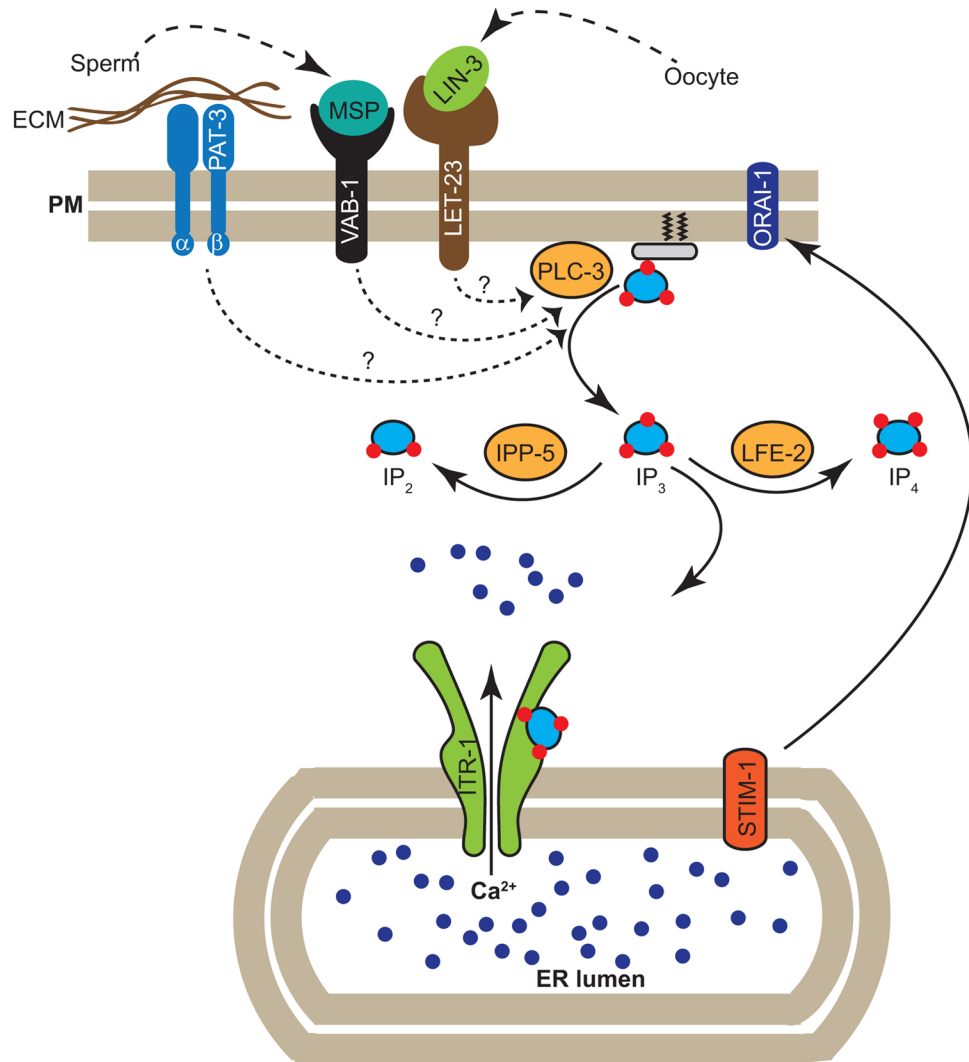


Figure 3. Cartoon showing the molecular components involved in modulating Ca^{2+} signal for regulating gonadal sheath cell contraction. Oocytes secrete EGF ligand (LIN-3), which binds with EGF receptor (LET-23). Sperm secrete MSP which bind with VAB-1/Ephrin receptor. Extracellular Matrix components surrounding sheath cell bind with integrin (PAT-3). Ligands-receptors interactions trigger signal transduction pathway that likely activates PLC- γ that results in the generation of Inositol triphosphate (IP₃). LFE-2 phosphorylates IP₃ to form IP₄. IPP-5 dephosphorylates IP₃ to form IP₂. IP₃ binds with IP₃ receptor (ITR-1) found on the endoplasmic reticulum to mobilize Ca^{2+} from ER. Depletion of Ca^{2+} from ER is sensed by ER Ca^{2+} sensor, STIM-1, which then activates Ca²⁺ release-activated Ca²⁺ channel (CRAC), ORAI-1 present on the plasma membrane to replenish the internal Ca²⁺ store.

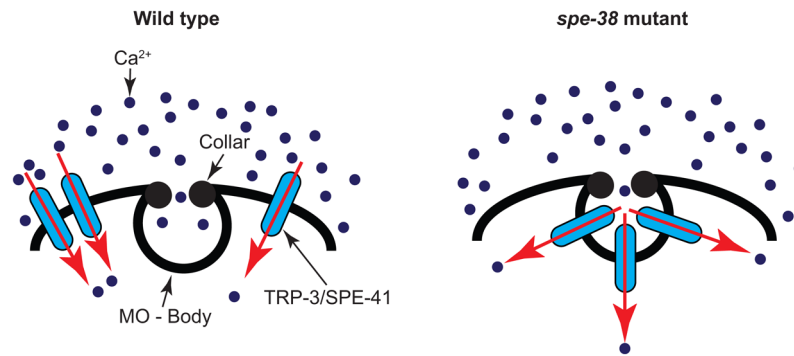


Figure 4. Ca^{2+} influx in wild-type and *spe-38* spermatozoa. In wild-type spermatozoa, TRP-3/SPE-41 channels are found all over the plasma membrane. In *spe-38* spermatozoa, TRP-3/SPE-41 channels are trapped within the fused MOs. However, TRP-3/SPE-41 channels are directly exposed to extracellular milieu and hence can function from fused MOs.