
Control of oocyte meiotic maturation and fertilization*

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Abstract

Sexual reproduction depends upon meiosis for the generation of haploid gamete nuclei, which unite after fertilization to form the diploid zygote. The oocytes of most animal species arrest during meiotic prophase, and complete meiosis in response to intercellular signaling in a process called meiotic maturation. Oocyte meiotic maturation is defined by the transition between diakinesis and metaphase of meiosis I and is accompanied by nuclear envelope breakdown, rearrangement of the cortical cytoskeleton, and meiotic spindle assembly. Thus, the meiotic maturation process is essential for meiosis and prepares the oocyte for fertilization. In *C. elegans*, the processes of meiotic maturation, ovulation, and fertilization are temporally coupled: sperm utilize the major sperm protein as a hormone to trigger oocyte meiotic maturation, and in turn, the maturing oocyte signals its own ovulation thereby facilitating fertilization. This chapter highlights recent advances in understanding meiotic maturation signaling and gametic interactions required for fertilization.

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1. Overview

1.1. General features of meiotic maturation and fertilization

Sexual reproduction depends upon meiosis for the generation of haploid gamete nuclei, which unite after fertilization to form the diploid zygote. Despite this universal requirement, meiosis is regulated differently in oocytes and spermatocytes. Whereas spermatocytes proceed through the meiotic divisions uninterrupted, oocytes almost invariably arrest during one, and sometimes two stages following premeiotic DNA replication and meiotic recombination, depending on the species. This unique characteristic of oocyte meiosis, as well as its close temporal association with fertilization, was recognized early by developmental biologists (see Wilson, 1925), who coined the term meiotic maturation for the suite of physiological changes occurring in oocytes just before zygote formation. Oocyte meiotic maturation is defined by the transition between diakinesis and metaphase of meiosis I and is accompanied by nuclear envelope breakdown, rearrangement of the cortical cytoskeleton, and meiotic spindle assembly (Figure 1). Thus, the meiotic maturation process is essential for meiosis and may prepare the oocyte for fertilization.

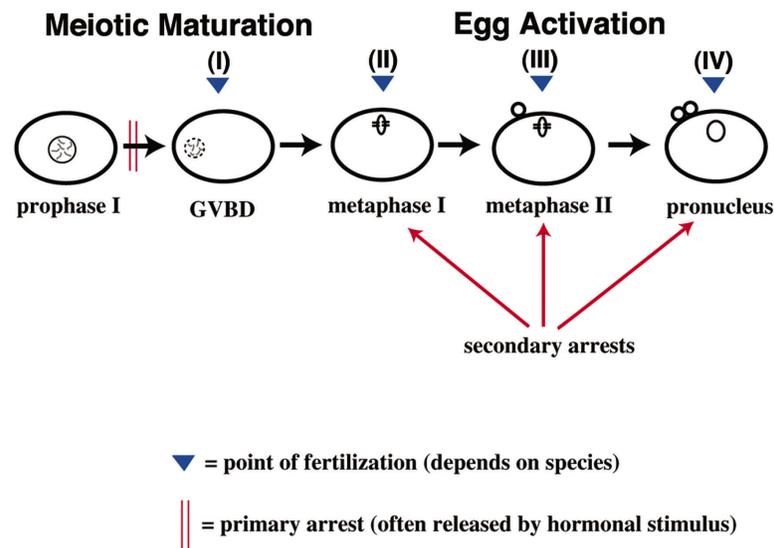


Figure 1. Oocyte Meiotic Maturation and Egg Activation. The oocytes of most animal species arrest in meiotic prophase I (reviewed by Masui and Clarke, 1979; Masui, 2001). In response to a hormonal stimulus (1-methyladenine in starfish; progesterone in *Xenopus*; MSP in *C. elegans*), oocytes begin meiotic maturation: the nuclear envelope breaks down (GVBD), as the oocyte enters M-phase from prophase. The point of fertilization is species-specific. In the case of *C. elegans* (I) fertilization occurs after maturation but prior to completion of meiosis I. In *C. elegans*, fertilization is required for completing both meiotic divisions. For most insects (II), fertilization occurs at metaphase or anaphase I. In most vertebrates (III), fertilization occurs at metaphase II. Sea urchins complete meiosis before fertilization (IV).

To ensure successful fertilization, oocyte meiotic maturation must be coordinated with other cellular events during oogenesis, including growth, meiotic chromosome reorganization, and ovulation. To achieve this coordination, intercellular signals regulate oocyte meiotic progression (reviewed by Ferrell, 1999; Masui, 2001). The timing of the meiotic divisions with respect to fertilization varies among species, likely reflecting the diversity of reproductive strategies observed in nature (Figure 1). Despite these differences in timing, extensive studies reveal striking conservation in the molecular underpinnings of oocyte meiotic maturation among different animals. It was classic studies of oocyte meiotic maturation in amphibian oocytes that led to the discovery of the Maturation Promoting Factor (MPF; Masui and Markert, 1971; reviewed by Masui, 2001). Genetic and biochemical analysis of the cell cycle, together with MPF purification, subsequently demonstrated that cyclin-dependent protein kinases are universal regulators of mitotic and meiotic cell cycle progression in eukaryotes (reviewed by Morgan, 1995).

1.2. Meiotic maturation and fertilization in *C. elegans*

C. elegans is an important system for addressing the fundamental events of oocyte meiotic maturation and fertilization, complementing studies in vertebrate systems (reviewed by Hubbard and Greenstein, 2000). Oocytes grow and develop in the proximal gonad arm of adult hermaphrodites (Figure 2A). Apoptotic cell death of germ cells is a conserved feature of oogenesis, occurring in adults near the gonad loop region, where the gonad tube bends ventrally. As germline nuclei transit through the loop region, they exit pachytene, become more fully enclosed by plasma membrane, and form a queue in the proximal gonad arm. The meiotic chromosomes condense as the

developing oocytes enter diplotene. In diakinesis, six discrete highly condensed bivalents, corresponding to the homologous chromosome pairs, are readily visualized (Figure 2B). Oocytes develop in close association with proximal gonadal sheath cells (Figure 2), smooth muscle-like cells that regulate meiotic maturation and contract to drive ovulation (McCarter et al., 1997; Hall et al., 1999; Miller et al., 2003). Meiotic maturation takes place in an assembly line fashion, such that the most proximal oocyte (often referred to as the -1 oocyte) matures, enters the spermatheca at ovulation, and is fertilized (Figure 2; Movie 1). In adult hermaphrodites, the cycle repeats every ~ 23 minutes (McCarter et al., 1999).

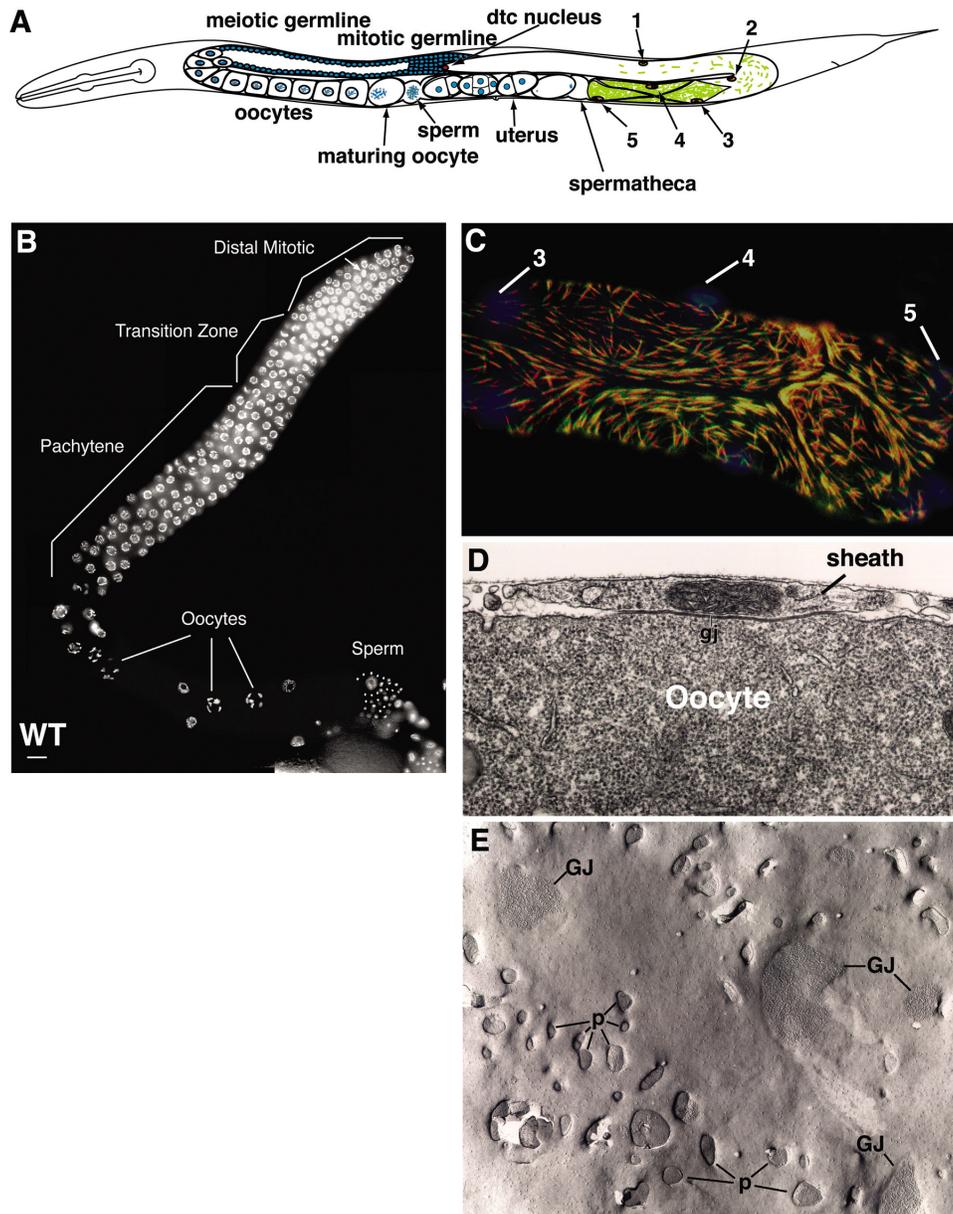
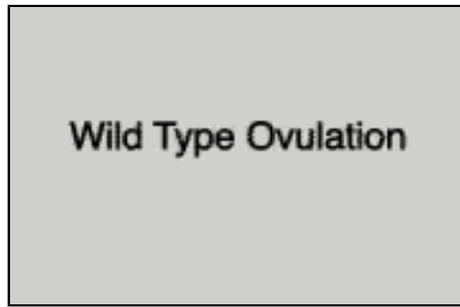


Figure 2. Anatomy Relating to the Control of Meiotic Maturation. (A) Representation of the adult hermaphrodite gonad. The anterior arm (on the left) shows the germ line and the posterior arm (on the right) shows the somatic cells of the gonad arm. Ten gonadal sheath cells cover the gonad arm. The position of one member of each pair is shown (1-5). The proximal sheath cells (pairs 3-5) contract to drive ovulation. (B) Dissected gonad from a wild-type young adult hermaphrodite illustrating oogenic meiotic progression. Bars, 10 μm . Photograph modified from Berry et al. (1997) with permission. (C) Immunofluorescent micrograph of the contractile proximal gonadal sheath cells (pairs 3-5). Nuclei detected by DAPI staining (blue) and myofilaments in red (MHCA) and green (MHCB). Reproduced from Hubbard and Greenstein (2000) with permission. (D) Gap junction (gj) between oocyte and proximal sheath cell. Photograph modified from Hall et al. (1999) with permission. (E) High magnification view of a freeze-fracture replica of sheath cell 4 covering the -2 oocyte. Clustered intra-membrane particles and pits correspond to gap junctional channels (GJ) between the oocyte and sheath cell. Irregular sheath pores (p) form fenestrae through which the oocyte surface is visible. Photograph modified from Hall et al. (1999) with permission.



Movie 1. Time-lapse Video of Oocyte Meiotic Maturation and Ovulation. The first ovulation in a young-adult hermaphrodite is shown. Events occur twelve times faster than real time.

In many animals, including many species of sponges, annelids, mollusks, and nematodes, sperm promote the resumption of meiosis in arrested oocytes (Masui, 1985; McCarter et al., 1999). In *C. elegans*, sperm utilize the major sperm protein (MSP) as a hormone to promote oocyte meiotic maturation and gonadal sheath cell contraction at a distance (Miller et al., 2001). MSP is also the key cytoskeletal element required for amoeboid locomotion of nematode spermatozoa (Italiano et al., 1996). MSP promotes oocyte meiotic maturation, in part by binding the VAB-1 Eph receptor protein-tyrosine kinase on oocytes, and in part by antagonizing an inhibitory somatic gonadal sheath cell pathway (Miller et al., 2003). Since hermaphrodites produce only a fixed number of sperm, meiotic maturation rates are initially high for the first two days of adulthood, but decline as sperm are used for fertilization. In gonochoristic species (e.g., *C. remanei*), oocytes arrest at diakinesis until the female is inseminated during mating. Likewise, in sex-determination mutants of *C. elegans*, which fully feminize the hermaphrodite gonad, oocytes also arrest until sperm are supplied. Thus, the MSP hormone functions as the linchpin of a sperm-sensing mechanism linking meiotic maturation and sperm availability, thereby ensuring fertilization.

2. Control of oocyte meiotic maturation

2.1. Oocyte growth, development, and differentiation

2.1.1. Pachytene progression and oogenesis

Progression through pachytene to diplotene requires the mitogen-activated protein kinase (MAPK) signaling pathway in the germ line (Church et al., 1995). MAPK activation is also involved in meiotic maturation (see below). Mutations in multiple RAS/MAPK pathway components cause a similar phenotype in which germline nuclei arrest in pachytene. The arrested germline nuclei are displaced from the surface of the gonadal tube and clump before the bend (Church et al., 1995; Hsu et al., 2002).

Whereas the RAS/MAPK pathway is required for fertility in both sexes, the *daz-1* gene, a homolog of the human Deleted in Azoospermia gene, is specifically required for progression through pachytene during oogenesis (Karashima et al., 2000). Since *daz-1* encodes a ribonucleoprotein-type RNA-binding protein, it may regulate translation during female meiotic progression. *skr-1* and *skr-2*, which encode two of the 21 Skp1-related genes in *C. elegans*, are also required for progression through pachytene during oogenesis (Nayak et al., 2002). SKP-1 and SKP-2 interact with the CUL-1 cullin and are likely to regulate ubiquitin-mediated protein degradation to promote progression through pachytene. The maxi-KH/STAR domain RNA-binding protein GLD-1 is also required for oocyte differentiation (Francis et al., 1995; Jones and Schedl, 1995). In the absence of *gld-1*, germ cells destined to become oocytes enter meiosis, proceed to pachytene, but then exit meiosis, reentering the mitotic cell cycle to form a germline tumor (Francis et al., 1995). GLD-1 functions as a translational repressor protein (Jan et al., 1999; Lee and Schedl, 2001) that also protects its mRNA targets from nonsense-mediated decay (Lee and Schedl, 2004). GLD-1 protein levels are highest during pachytene, drop sharply as germ cells progress to diplotene, and decrease to undetectable levels in growing oocytes (Jones et al., 1996). This observation suggested that *gld-1* may prevent the translation of proteins needed for oocyte growth and development in early meiotic prophase. The isolation of GLD-1 mRNA targets by immunoprecipitation identified candidate genes involved in oocyte development or function (Lee and Schedl, 2001).

2.1.2. Oocyte growth

Oocytes grow in the proximal gonad arm, and accumulate yolk lipoprotein particles. The vitellogenins, protein components of yolk particles, are synthesized in the intestine (Kimble and Sharrock, 1983) and secreted into the

pseudocoelom. Yolk lipoprotein particles appear to pass through the gonadal basal lamina, move through pores in the gonadal sheath cells (Figure 2E), are endocytosed, and stored in membrane-bounded granules within oocytes (Hall et al., 1999). Endocytosis, of yolk lipoprotein particles requires the RME-2 yolk receptor, a member of the low-density lipoprotein receptor superfamily (Grant and Hirsh, 1999). The spatial pattern of *rme-2* expression is controlled by *gld-1* (Lee and Schedl, 2001) and *atx-2* (Ciosk et al., 2004). The vitellogenins are cholesterol-binding proteins and RME-2 receptor-mediated endocytosis is required for normal transport of cholesterol into oocytes (Matyash et al., 2001). Since adult hermaphrodites turn over the entire volume of the gonad every 6.5 hours, (Hirsh et al., 1976), oocyte growth must be highly regulated. Understanding oocyte growth control is a frontier for the field. Signaling pathways that intersect with meiotic maturation and MAPK pathways appear to be involved (Gutch et al., 1998; T. Schedl, unpublished results).

2.2. Oocyte meiotic maturation

2.2.1. Description of anatomy and timing of events

Oocyte meiotic maturation can be viewed by time-lapse videomicroscopy of living animals (Ward and Carrel, 1979; Movie 1). The timing of landmark events during meiotic maturation (McCarter et al., 1999) and the ultrastructure of the proximal gonad (Hall et al., 1999) have been described. The nuclear envelope of the most proximal oocyte breaks down ~5 min prior to ovulation as it enters meiotic M-phase from prophase (McCarter et al., 1999). During maturation, the oocyte also undergoes a structural change termed cortical rearrangement (McCarter et al., 1999). These changes within the oocyte coincide with a reproducible sequence of somatic motor events mediated by the contractile proximal sheath cells (Figure 2A, Figure 2D) and the distal spermatheca resulting in ovulation. The gonadal sheath cells form gap junctions with oocytes (Figure 2D, Figure 2E) and regulate meiotic maturation and ovulation (Greenstein et al., 1994; McCarter et al., 1997; Rose et al., 1997; Miller et al., 2003).

2.2.2. The MSP signal

In a landmark study, McCarter et al., (1999) showed that a sperm-associated signal promotes oocyte meiotic maturation independent of fertilization. In females (genetically altered XX animals that produce no sperm), oocytes mature and are ovulated at low rates (< 0.1 maturations per gonad per hr). Mating to wild-type males, or fertilization incompetent sperm defective (*spe*) mutants restores the normal rate of oocyte maturation when sperm are plentiful (~2.5 maturations per gonad per hr). Until recently, a key unanswered question in the field concerned the nature of the sperm signal for oocyte meiotic maturation. Miller et al. (2001) demonstrated that the major sperm protein (MSP), the central cytoskeletal element required for the actin-independent motility of nematode spermatozoa, has a dual role in *C. elegans* reproduction, functioning as a hormone for oocyte meiotic maturation and gonadal sheath cell contraction. An *in vivo* bioassay was developed in which sperm-conditioned medium or sperm lysates were injected into the uterus of unmated *fog-2* female animals, and oocyte meiotic maturation and gonadal sheath cell contraction were monitored by time-lapse videomicroscopy. The bioactive factors were purified to homogeneity with reversed-phase high-performance liquid chromatography and shown to contain only MSP by mass spectrometry. Injection of MSP antibodies into the uterus of hermaphrodites results in a reduction in ovulation rates, consistent with the hypothesis that MSP is an endogenous signal. *C. elegans* MSP can signal in *C. remanei* (Miller et al., 2001) and *Ascaris* MSP can signal in *C. elegans* (M. Kosinski and D.G., unpublished results), consistent with observations of heterospecific matings in the genus *Caenorhabditis* (Hill and L'Hernault, 2001).

MAPK activation plays critical roles in regulating meiotic progression (Ferrell, 1999). In *C. elegans*, the MAPK pathway is required for pachytene progression (Church et al., 1995) and is important for meiotic maturation (M.-H. Lee and T. Schedl, pers. comm.). Proximal oocytes exhibit MAPK activation in the presence of sperm (Miller et al., 2001; Page et al., 2001), and MSP was shown to be sufficient to activate MAPK in oocytes (Miller et al., 2001). MSP begins as a cytoplasmic protein and does not possess a signal sequence. Recent results suggest that MSP is exported from spermatids and spermatozoa by a vesicle budding mechanism (M. Kosinski, K. McDonald, J. Schwartz, I. Yamamoto, and D. G., unpublished results).

2.2.3. MSP signaling and the sperm-sensing mechanism

The identification of MSP's signaling role posed the problem of how oocytes and sheath cells sense MSP. A key advance was the identification of the VAB-1 Eph receptor protein-tyrosine kinase as an MSP receptor (Miller et al., 2003; reviewed by Kuwabara, 2003). Recent results show that the VAB-1 ectodomain directly binds MSP *in vitro* (H. Cheng and D. G., unpublished results). The observation that *vab-1* null mutant oocytes respond to and bind MSP, suggests the involvement of additional MSP receptors. *vab-1* was shown to function in parallel to a somatic

gonadal pathway (Miller et al., 2003), defined by the POU-class homeobox gene *ceh-18* (Greenstein et al., 1994; Rose et al., 1997). These parallel pathways negatively regulate oocyte maturation and MAPK activation in hermaphrodite and female gonads. Eliminating *vab-1* and *ceh-18* function removes the dependence of meiotic maturation and ovulation on the presence of sperm. Therefore, this meiotic control mechanism resembles a cell cycle checkpoint (Hartwell and Weinert, 1989) and may confer a selective advantage in hermaphrodite and female species by conserving metabolically costly oocytes when sperm are unavailable for fertilization.

Positive effectors of oocyte meiotic maturation have been identified in *C. elegans*, including the cyclin-dependent kinase homolog CDK-1 (Boxem et al. 1999) and the polo-like kinase homolog PLK-1 (Chase et al. 2000). The zinc finger domain-containing proteins OMA-1 and OMA-2 are redundantly required for oocyte maturation and ovulation (Detwiler et al. 2001). In *oma-1; oma-2* double mutants, MAPK activation is not sustained and nuclear envelope breakdown does not occur. OMA-1 and OMA-2 may function upstream of two conserved cell cycle regulators, the MYT1-related kinase WEE-1.3 and CDK-1 (Detwiler et al. 2001). Since OMA-1 and OMA-2 contain zinc finger domains, they could function as regulators of translation or as regulators of proteolysis (DeRenzo et al., 2003).

3. Control of ovulation

The ovulatory process represents an attractive physiological model for how intercellular signaling affects smooth muscle function. Ovulation involves the coordinated function of the gonadal sheath cells, specialized smooth-muscle-like cells that surround oocytes (Figure 2). During ovulation, the proximal gonadal sheath cells contract rapidly, the distal constriction of the spermatheca dilates, and sheath cells pull the distal spermatheca over the mature oocyte (Movie 1). The maturing oocyte signals its own ovulation in two ways: it modulates sheath contractions, which includes an increase in contraction rate and intensity during ovulation, and it induces spermathecal dilation during ovulation (McCarter et al., 1999). Spermathecal dilation is regulated by a LIN-3(EGF)/LET-23(EGF-receptor) signal transduction pathway mediating the signal from oocyte to the distal spermatheca (Clandinin et al., 1998; T. Schedl, unpublished results). The *let-23* pathway required for spermathecal dilation during ovulation is *let-60(ras)*-independent and involves a downstream IP₃-mediated pathway (Clandinin et al., 1998). Dominant gain-of-function mutations in *let-60(ras)* also perturb ovulation, suggesting that ras-dependent pathways are also involved (T. Schedl, unpublished results). Mutations in two genes, *lfe-1/itr-1* and *lfe-2*, (gain-of-function and loss-of-function, respectively) were isolated in a genetic screen for suppressors of *let-23* sterility defects. *lfe-1/itr-1* and *lfe-2* genes encode an inositol (1, 4, 5) triphosphate receptor and an inositol (1, 4, 5) triphosphate-3-kinase, respectively (Clandinin et al., 1998). These results suggest that spermathecal dilation is likely to be dependent on calcium release regulated by IP₃. Consistent with this possibility, a mutant allele of *ipp-5*, which encodes a type I 5-phosphatase, predicted to lower IP₃ levels, exhibits a novel ovulation phenotype in which the spermatheca overextends, thereby ovulating two oocytes per cycle (Bui and Sternberg, 2002). Mutations in *plc-1*, which encodes a phospholipase C epsilon homolog, or *plc-1(RNAi)*, also result in spermathecal entry and exit defects (Kariya et al., 2004; Yin et al., 2004). *let-23*-mediated IP₃ signaling also plays a role in promoting the ovulatory gonadal sheath cell contractions (Yin et al., 2004).

Mutations that lead to defective ovulation cause an endomitotic oocyte (Emo) phenotype (Iwasaki et al., 1996). When oocytes are retained in the gonad arm due to defective ovulation, they undergo multiple rounds of nuclear envelope breakdown (M-phase entry) and S-phase, and become highly polyploid. A large class of genes can mutate to an Emo phenotype and these include *emo-1* (Iwasaki et al., 1996), *mup-2* (Myers et al., 1996), *mel-11* (Wissmann et al., 1999), tropomyosin, and troponin C (Ono and Ono, 2004) as well as genes involved in sheath or spermathecal development (Kostic et al., 2003; Aono et al., 2004). In the future, studies of the physiology of ovulation will no doubt benefit from the ability to measure calcium concentrations in the gonad (Samuel et al., 2001) and to record oocyte ion channels (Rutledge et al., 2001).

4. Control of fertilization

Successful fertilization depends on a series of gametic interactions in which spermatozoa and oocyte recognize each other, bind, and fuse (reviewed by Hardy, 2002). Egg activation (the process whereby the oocyte completes the meiotic divisions), a block to polyspermy, and activation of the embryonic program are dependent on fertilization. *C. elegans* provides a promising experimental system for examining fertilization because of the availability of fertilization-defective mutants (L'Hernault et al., 1988; reviewed by Singson, 2001). The central finding for the field was the phenotypic characterization and molecular cloning of *spe-9*, a gene whose function is required in sperm for fertilization (Singson et al., 1998). *spe-9* spermatozoa are fertilization defective, despite being able to crawl, signal

meiotic maturation, and contact ovulated oocytes. *spe-9* mutations do not affect spermiogenesis, and the mutant spermatozoa have a normal morphology at the EM level. *spe-9* encodes a transmembrane protein containing an extracellular domain with 10 EGF-like repeats. Based on these results, Singson et al. (1998) proposed that *spe-9* may function as a ligand for an oocyte receptor. Analysis of *spe-9*-deletion derivatives using a transgenic assay is consistent with this proposal and indicates that the EGF-like repeats and the transmembrane segment are required for function (Putiri et al., 2004). By contrast, the cytoplasmic domain was dispensable, ruling out the possibility that this region plays a signaling role within spermatozoa. *spe-9* localizes to the membrane of spermatids, but reorganizes to the pseudopod during spermiogenesis (Zannoni et al., 2003; Figure 3). Based on this result, Zannoni et al. (2003) proposed that the spermatozoan interacts with the oocyte cell surface in a “pseudopod first” manner. While there are no published ultrastructural observations of fertilization events in *C. elegans*, Foor (1968) reported a striking series of EM observations of fertilization in *Ascaris lumbricoides*, which provided evidence for initial contact of the pseudopod with the mature oocyte, fusion of cell membranes during fertilization, and first entry of the pseudopodal extension. The identification of oocyte cell surface molecules that mediate fertilization interactions is an exciting area for future work.

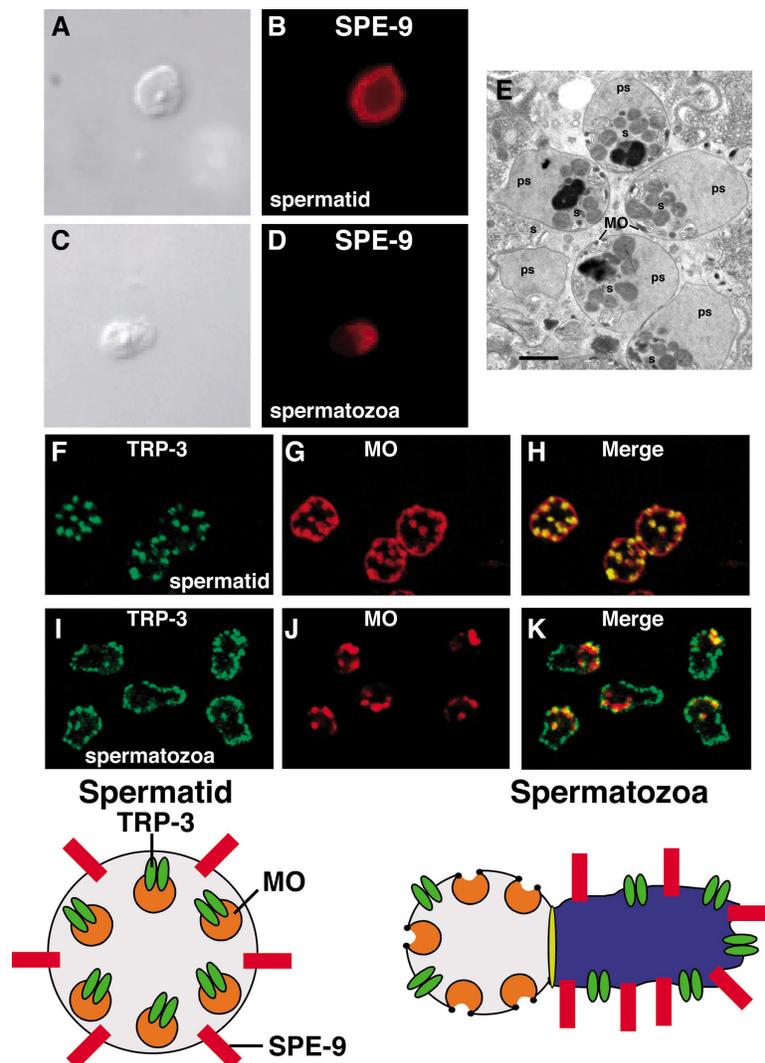


Figure 3. Dynamic Localization of Two Membrane Proteins Required for Fertilization. (A-D) Localization of SPE-9 in spermatids (A and B) and spermatozoa (C and D). SPE-9 localizes to the plasma membrane of spermatids. After spermiogenesis, SPE-9 is enriched at the plasma membrane of the pseudopod. Photographs reproduced from Zannoni et al. (2004), and used with permission. (E) High pressure freezing transmission electron microscopy of spermatozoa (s) in the spermatheca. Membranous organelles (MO) and pseudopods (ps) are indicated. During spermiogenesis (not shown) the MOs fuse with the plasma membrane and maintain a persistent fusion pore, flanked by an electron-dense collar. Electron micrograph courtesy of Kent McDonald. Bar, 1 μ m.

Further studies of fertilization-defective mutations of the *spe-9* class promise essential information on gametic interactions needed for fertilization. These mutations include *spe-13*, *spe-38*, *spe-42*, *fer-14*, and *trp-3* (Singson et al., 1998; Xu and Sternberg, 2003; A. Singson, T. Kroft, and S. L'Hernault, unpublished results). Xu and Sternberg (2003) reported an extensive characterization of *trp-3* (aka *spe-41*), which encodes a member of the transient receptor potential (TRP) superfamily of cation channels. While *trp-3* plays a critical role in fertilization, it is not absolutely essential because hermaphrodites homozygous for likely *trp-3* null mutations have brood sizes that are approximately 5% of the wild type. TRP-3 displays a dynamic localization during spermiogenesis. TRP-3 localizes to the MOs in spermatids and translocates to the plasma membrane of the cell body and pseudopod during spermiogenesis (Figure 3). Calcium imaging studies provided evidence that TRP-3 may function as a store-operated calcium entry (SOCE) channel in spermatozoa. In addition to SOCE channels, spermatozoa contain constitutively active calcium-permeable (CAC) channels. While *trp-3* mutant spermatozoa exhibit lower SOCE channel activity than the wild type, the CAC channel activity is normal. These results strongly suggest that TRP cation channel activity plays an important role in fertilization. In mammals, TRP channels play roles in the acrosome reaction (Jungnickel et al., 2001) and sperm motility (Castellano et al., 2003). Additional members of this large channel family may have conserved functions in fertilization.

5. Conclusion

Meiotic maturation and fertilization are fundamental developmental events, which have been extensively studied for over a hundred years. Yet, the molecular mechanisms underlying these critical processes remain incompletely understood. Many exciting biological questions surrounding these events remain. For instance, it is unclear how meiotic maturation events actually prepare the oocyte for fertilization. The sequence of molecular events needed at fertilization and their interdependencies are uncharacterized. How fertilization results in egg activation and triggers the embryonic program is largely unknown. Recent progress described here gives confidence that *C. elegans* provides a fertile field for answering basic questions about the fundamental developmental mechanisms of oocyte meiotic maturation and fertilization.

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