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Mini Review

Fertilization and the oocyte-to-embryo transition in *C. elegans*

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Fertilization is a complex process comprised of numerous steps. During fertilization, two highly specialized and differentiated cells (sperm and egg) fuse and subsequently trigger the development of an embryo from a quiescent, arrested oocyte. Molecular interactions between the sperm and egg are necessary for regulating the developmental potential of an oocyte, and precise coordination and regulation of gene expression and protein function are critical for proper embryonic development. The nematode *Caenorhabditis elegans* has emerged as a valuable model system for identifying genes involved in fertilization and the oocyte-to-embryo transition as well as for understanding the molecular mechanisms that govern these processes. In this review, we will address current knowledge of the molecular underpinnings of gamete interactions during fertilization and the oocyte-to-embryo transition in *C. elegans*. We will also compare our knowledge of these processes in *C. elegans* to what is known about similar processes in mammalian, specifically mouse, model systems. [BMB reports 2010; 43(6): 389-399]

INTRODUCTION

The transition from a quiescent, arrested oocyte to an embryo with tremendous developmental potential is tightly regulated by a variety of mechanisms until the time of fertilization. Fertilization triggers development by initiating multiple cell biological processes, including protein synthesis, RNA and protein degradation, organelle and cytoskeletal remodeling, and cell division (1). Identifying and understanding the genes and interactions of the gene products will aid in our knowledge of one of the most dynamic processes in biology. Understanding the genetics and molecular basis of fertilization and the oocyte-to-embryo transition has important clinical implications, as approximately 15% of couples are infertile and genetic causes may play a prominent factor in these cases (2, 3). Additionally, there is a need for novel contraceptive technologies, and understanding the genetics and molecular basis of fertilization will aid in the development of contraceptives (4). Finally, there is concern that the increased reliance on assisted reproductive technologies (ART) to treat clinical cases of infertility may result in future health issues for children conceived using these technologies (5, 6). Basic research that seeks to understand the fundamentals of fertilization, development, and causes of infertility will aid in more accurate diagnosis of clinical cases and reduce the need for ART.

Numerous model organisms have been used to understand fertilization and the oocyte-to-embryo transition. The mouse model system has been particularly important for understanding the genetics and molecular basis of fertilization because of the ability to create gene-specific knockout mice and to study gamete function and development in a defined *in vitro* system (7). More recently, the nematode *Caenorhabditis elegans* has emerged as a valuable model system to understand fertilization and early development (8). *C. elegans* is an ideal model organism for studying fertilization and the oocyte-to-embryo transition because it has a short reproductive cycle (~3.5 days), produces a large number of progeny, and is transparent, which allows for the direct observation of oocyte development, ovulation, fertilization, and early embryogenesis in intact animals (9). *C. elegans* also has a sequenced genome and its entire cell lineage is mapped, and it is amenable to genetic crosses, highly responsive to RNAi, and easily cultivated in the lab (10-12).

Fertilization in *C. elegans* shares many characteristics with mammalian fertilization, including the need for sperm activation in the female reproductive tract, internal fertilization and multiple egg activation events including a membrane block to polyspermy, cortical granule (CG) exocytosis, meiotic resumption, and activation and degradation of selected maternal mRNAs and proteins (Table 1) (1, 8, 13). In this review, we will discuss *C. elegans* as a model system for fertilization and the oocyte-to-embryo transition. We will highlight what is known about the molecular details of fertilization in *C. elegans* and compare that to the mouse model system. Additionally, we will discuss the transition of an oocyte to a developing embryo after fertilization in *C. elegans* and highlight what is known about the analogous process, egg activation, in the mouse. Finally, we will discuss some other aspects of fertilization, including cortical granule exocytosis and the membrane block to polyspermy, and how the use of *C. elegans* as a model system will provide insight into the control of these processes.

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Fertilization and the oocyte-to-embryo transition

Both gametes must undergo multiple developmental steps before making contact with the other and precise interactions and regulation are necessary for successful fertilization and embryonic development. The oocyte-to-embryo transition is comprised of two steps, meiotic maturation and egg activation (14). Meiotic maturation is the process by which an immature oocyte transitions to a fertilization-competent egg (15). In mammals, oocytes remain arrested for prolonged periods during meiotic prophase I until they are triggered by hormonal signals to enter meiotic M phase and initiate meiotic division (14). The oocytes are subsequently ovulated and reach a second arrest point (metaphase of meiosis II in mammals). The developed oocytes are now referred to as eggs and are competent for fertilization (13, 14). In mammals, sperm interact with the egg on multiple levels (16); here we will focus only on the interactions between the sperm and egg plasma membrane. After the sperm and egg come into contact with one another, they bind and fuse, which triggers activation of the egg and ultimately the development into a totipotent zygote (13, 14). In most animals, egg activation is triggered by fertilization by a single sperm, which thereby causes an increase in intracellular calcium (Ca^{2+}). This increase in calcium initiates the molecular events of egg activation, including the release of oocyte meiotic arrest, generation of female and male pronuclei, changes to maternal mRNAs and protein populations, cytoskeletal rearrangements, and the modification of the outer coverings of the egg to prevent polyspermy and to support embryogenesis (13).

Table 1. Comparison of *C. elegans* and mouse fertilization and oocyte-to-embryo transition and *C. elegans* genes important for sperm-egg fusion

Similar
Sperm activation
Internal fertilization
Calcium increase after fertilization
Sperm provide paternal contributions to the zygote
Cortical granule (CG) exocytosis
Membrane block to polyspermy
Meiotic resumption
Activation and degradation of selected maternal mRNAs and proteins
Different
Sexes - Hermaphrodite and male
Sperm have membranous organelles (MOs), but no acrosome
Sperm use a pseudopod for motility instead of flagella
Vitelline layer, no zona pellucida (ZP)
Fertilize at prophase I, instead of metaphase II
C. elegans genes important for sperm-egg fusion (putative functional protein domains)
spe-9 (EGF-repeats)
spe-38 (Unknown)
spe-41/trp-3 (Coiled-coil, Ankyrin)
spe-42 (DC-STAMP, C4C4-type RING finger)
egg-1 and *egg-2* (LDL receptor repeats)

C. elegans as a model organism

C. elegans has two sexes: males and hermaphrodites (Fig. 1) (12). Adult males continually produce sperm, while hermaphrodites produce several hundred sperm during their final larval stage and then switch to oocyte production as adults (17). Therefore, adult hermaphrodites are essentially females with stored sperm (18). *C. elegans* sperm are amoeboid and lack two recognizable features of mammalian sperm, a flagellum and an acrosome (a large secretory vesicle on the sperm head necessary for fertilization in other species). *C. elegans* oocytes have a vitelline layer but do not have a thick egg coat (referred to as the zona pellucida in other species) (19).

The transition of an immature round, non-motile *C. elegans* spermatid into a mature amoeboid, motile spermatozoon (sperm) is termed spermiogenesis (also referred to as sperm activation); this process occurs in differently in hermaphrodites and males. In hermaphrodites, spermiogenesis occurs in the spermatheca, and in males, it occurs when sperm mixes with seminal fluid during ejaculation into the hermaphrodite during copulation (20). Sperm activation appears to involve cation in-

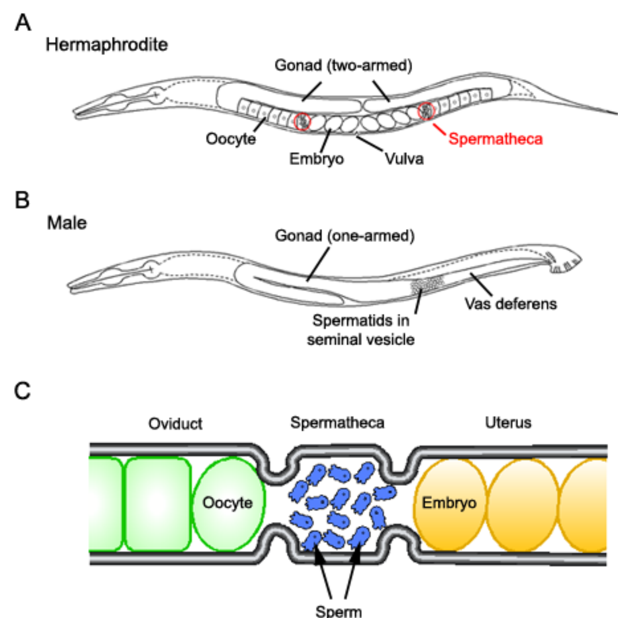

Fig. 1. Reproductive tracts of *C. elegans* sexes (hermaphrodite and male). (A) Hermaphrodite - The gonad is two-armed and converges on a single vulval opening. The spermatheca is outlined by red circles. (B) Male - The gonad is one-armed. Spermatids accumulate in the gonad and are stored in the vas deferens until ejaculation during mating. (C) A schematic of the *C. elegans* reproductive tract with a focus on the spermatheca.

flux, pH elevation, and intracellular Ca^{2+} release (20-24). Mature sperm must crawl towards the spermatheca and maintain their position in the reproductive tract while eggs are ovulated into the spermatheca. Oocytes produce a prostaglandin-like signal produced from polyunsaturated fatty acid precursors that signal sperm to migrate into the spermatheca and towards the oocytes (25).

In *C. elegans*, meiotic maturation of oocytes, ovulation, and fertilization are temporally coupled (14). During oogenesis, the developing oocyte begins to undergo the first meiotic division but then arrests in diakinesis, the final stage of the prophase (15). Traditionally, *C. elegans* oocytes are not referred to as eggs because they are never arrested at metaphase II like mammalian eggs. A secreted sperm protein hormone, MSP (major sperm protein), triggers the resumption of meiosis and gonadal sheath contraction (26). As a result, the maturing oocyte is ovulated into the spermatheca, where sperm storage and fertilization occurs (Fig. 1C) (17, 27). The pseudopod of the amoeboid *C. elegans* sperm makes the initial contact with the oocyte (28). The sperm then fuses with the oocyte. In the absence of males, hermaphrodites will self-fertilize utilizing their own sperm until the supply of sperm is exhausted; however, hermaphrodites that have the opportunity mate with males will produce outcross progeny, as male-derived sperm are competitively superior at oocyte fertilization when compared to hermaphrodite-derived sperm (9, 11, 17). Fertilization is highly efficient in *C. elegans*: in the unmated hermaphrodite, sperm are a limited resource and virtually every functional sperm will fertilize an egg (18). This remarkable efficiency is the result of the coordination of fertilization with oocyte maturation, ovulation, sperm signaling, and sperm migratory behavior.

Sperm entry promotes the establishment of embryonic polarity and triggers egg activation (29). Egg activation results in the completion of meiosis, polar body formation, cytoskeletal reorganization, and the secretion of chitinous eggshell (27). The egg then begins embryogenesis while passing through the uterus and is eventually laid and hatched (8).

As mentioned earlier, one of the advantages of *C. elegans* as a model system is the ability to isolate and propagate loss-of-function mutations that lead to sterility (methods to do so reviewed in Jorgensen and Mango (30)). Additionally, reverse genetic approaches, including RNAi treatment or gene knockout, are also viable options to study gene function. Unfortunately, many genes are refractory to RNAi and sperm genes are particularly insensitive to RNAi mediated knockdown (8, 31). A large number of "sterile" mutants have been isolated in *C. elegans* with the potential defects in gametogenesis, germline development and proliferation, or embryonic development (8, 31, 32).

Sperm-sterile mutants are referred to as *spe* for spermatogenesis defective or *fer* for fertilization defective, and egg-sterile mutants are referred to as *egg* (8, 31-33). *spe* and *fer* mutants are equivalent to paternal infertility and these mutants produce spermatocytes, spermatids, and/or sperm whose functions are aberrant during spermatogenesis, spermiogenesis, and/or fertilization. *spe* mutant hermaphrodites produce very few progeny and lay unfertilized oocytes; however, when *spe* mutant hermaphrodites are mated with wild- type males, outcross progeny are produced, indicating that defective sperm function is the cause of sterility in hermaphrodite *spe* mutants (31, 32). To date, approximately 60 *spe* genes have been isolated following treatment of hermaphrodites with chemical mutagens such as ethyl methanesulfonate (EMS) and trimethylpsoralen (TMP), and, based on allele frequency, many more sterile mutants should be isolated (8, 31). For purposes of this review, we will focus on the *spe* genes required for fertilization and embryogenesis (see Nishimura and L'Hernault for a full review of *spe* mutants (31)). *egg* mutants are equivalent to maternal infertility and produce oocytes that cannot be fertilized and/or activated by wild-type sperm. When *egg* hermaphrodites are crossed to wild-type males, no progeny are produced. Sperm-sterile and egg-sterile mutants can be distinguished by crossing wild-type males to mutant hermaphrodites and determining if progeny are produced (8, 33). The interactions between sperm and oocyte molecules necessary for fertilization are not well understood in *C. elegans*. This is partially because there is no *in vitro* fertilization (IVF) system that can be used to probe molecular function. However, the use of genetic screens allows for an unbiased approach to identifying important genes and can be especially powerful in identifying genes that were not previously thought to play a role in fertilization.

FERTILIZATION

Genetic basis of fertilization in *C. elegans*

spe-9 was the first member identified of a class of *spe* genes necessary for fertilization in *C. elegans* (32, 34). *spe-9* class mutants produce sperm of normal morphology and motility that are able to make contact with the oocyte in the spermatheca; however, sperm from the mutants are unable to fertilize oocytes, indicating that the gene is necessary for sperm-oocyte interactions but not the prior steps of spermatogenesis or spermiogenesis (34). The seven genes that make up the *spe-9* class mutants are *spe-9, spe-13, spe-36, spe-38, spe-41/trp-3, spe-42, and fer-14*. Little is understood about *spe-13, spe-36*, or *fert-14* mutants (see Singson *et al*. and Nishimura and L'Hernault for more information (8, 31)).

spe-9: SPE-9 is a single-pass transmembrane molecule with a relatively short cytoplasmic tail and an extracellular domain containing 10 epidermal growth factor (EGF)-like repeats. EGF-like repeats are involved in extracellular functions such as adhesive or ligand receptor interactions, and SPE-9 is most closely related to ligands of the Notch/LIN-12/GLP-1 family of receptors (34). In spermatids, SPE-9 localizes to the plasma membrane, and in mature sperm, SPE-9 localization is restricted to the pseudopod (28). The extracellular domain and EGF repeats of SPE-9 are critical for its function (35). The mo-

lecular nature of SPE-9 and its localization on the pseudopod support the hypothesis that SPE-9 functions as a sperm ligand or oocyte receptor; however, the molecular or biochemical nature of its interactions with the oocyte is not understood (34).

spe-38: The *spe-38* gene is predicted to encode a novel four-pass integral membrane protein. Structurally similar molecules have been implicated in gamete adhesion/fusion, membrane adhesion/fusion, and the formation of tight-junctions. In spermatids, SPE-38 is found in sperm vesicles called the membranous organelles (MOs) (36). The MOs fuse with the plasma membrane during spermiogenesis, and, upon fusion, SPE-38 is released to the cell surface and then redistributed to the pseudopod, resulting in a similar localization pattern as that of SPE-9 (22, 36-38).

spe-41/trp-3: SPE-41/TRP-3 is a member of the transient receptor potential-canonical (TRPC) family of channel proteins. The loss of TRP-3 in *C. elegans* is correlated with a defect in calcium influx in activated sperm, but the consequence of this loss is unknown (39). TRP channels are also known to play a role in regulating sperm motility, but *spe-41/trp-3* mutant sperm do not have any detectable defects in motility or migratory behavior (39, 40). In mammalian sperm, calcium influx is associated with the regulation of membrane fusion events (41). Therefore, the defect in calcium influx may result in a defect in sperm-oocyte fusion. Unlike other *spe-9* class mutants, null alleles of *spe-41/trp-3* are not completely sterile. Instead, unmated mutant hermaphrodites are capable of producing small broods that are approximately 5% the size of wild-type broods. This indicates that SPE-41/TRP-3 is not absolutely essential for fertilization and that there may be an additional partially redundant channel protein involved in fertilization. SPE-41/TRP-3 is located in the spermatid MOs and moves to the surface after MO fusion. However, SPE-41/TRP-3 does not accumulate in any particular region of the plasma membrane (39). It is possible that SPE-41/TRP-3 may use its ankyrin and coiled-coil domains, much like other members of TRP family to form a homo- or hetero-tetramer (42). One hypothesis for SPE-41/TRP-3 function in fertilization is that oocyte binding may trigger the opening of calcium channels, thereby ensuring proper calcium influx needed for gamete fusion (31). There is evidence that TRPC family proteins play roles in lipid-processing events that facilitate membrane-membrane fusion events (31, 43).

spe-42: *spe-42* is predicted to encode a 7-pass transmembrane protein and it contains a dendritic cell-specific transmembrane protein (DC-STAMP) motif (44). In mammals, DC-STAMP is required for osteoclast precursor cell fusion (45). SPE-42 contains a C4C4-type RING finger domain that generally mediates protein-protein interactions (44). The SPE-42 RING finger domain may also have ubiquitin E3 ligase activity similar to the human finger protein CNOT4 (46, 47). However, C3HC4-type fingers, not the C4C4-type, usually have this enzymatic activity (48). A possible hypothesis for SPE-42 function is that it regulates the localization of function of a sperm protein that acts in sperm-oocyte interactions via ubiquitination (31).

egg-1 and egg-2: *egg-1* and *egg-2* encode type II transmembrane molecules with extracellular domains containing eight low-density lipoprotein (LDL)-receptor-repeats (33). LDL-receptor related molecules are known to function as receptors for a variety of ligands and mediate multiple cellular responses (49). The loss of function of either *egg-1* or *egg-2* lead to severely decreased hermaphrodite fertility, and the loss of both *egg-1* and *egg-2* leads complete sterility. Sperm are able to make contact with *egg-1/2* deficient oocytes but are unable to fuse with them. Male worms lacking *egg-1* and *egg-2* function have normal sperm production and fertility (33).

Sperm-egg fusion in mice

In contrast to what is known in *C. elegans*, far more molecules have been implicated in sperm-egg interactions in mice. However, the molecular nature of these interactions is not well understood. Here we will focus on the current best candidate molecules to mediate sperm-egg fusion. See Ikawa, *et al*. and Vjugina and Evans for a full review of molecules implicated in this process (16, 50).

IZUMO1: Currently, the best candidate sperm protein to mediate sperm-egg fusion is IZUMO1. In the late 1970s and 1980s, a variety of function-blocking antibodies were generated to study cell surface molecules (50). At this time, an anti-mouse sperm monoclonal antibody, OBF13, was generated and found to inhibit fertilization (51, 52). The OBF13 antigen was eventually identified as a testis-specific protein containing a single immunoglobulin (Ig) domain, and, because of its role in fertilization, the protein was named Izumo (IZUMO1) after a Japanese Shinto shrine to marriage. Male mice deficient in IZUMO1 are infertile and their sperm are unable to fuse with ZP-free eggs *in vitro* (53). At this time, IZUMO1 is the only sperm protein known to be essential for sperm-egg fusion.

IZUMO1 is present in the sperm plasma membrane and contains a site of *N*-glycosylation that is necessary for it to be maintained in the plasma membrane (54). Before coming in contact with the egg plasma membrane, IZUMO1 undergoes a relocalization event during the acrosome reaction (51, 53, 55). The acrosome reaction is somewhat analogous to the MO fusion in *C. elegans* sperm and only acrosome-reacted sperm are able to fuse with the egg. During the acrosome exocytosis, there are multiple fusion events between the plasma membrane and outer acrosomal membranes at the anterior region of the sperm head (56). As a result, the contents of the acrosome are released and the inner acrosomal membrane is exposed (7, 56, 57). This results in a reorganization of the plasma membrane of the sperm head and a change in localization of multiple proteins (56). IZUMO1 is localized in the acrosome prior to the acrosome reaction and relocalizes to the equatorial region after the acrosome reaction (51, 53, 55). The redistribution of IZUMO1 after the acrosome reaction requires a testis-specific serine kinase, TSSK6. Mice deficient in TSSK6 are infertile and sperm from these mice are unable to fuse with

eggs (55).

Recently, using amino acid sequence alignments of the unique N-terminal region of IZUMO1, three new proteins, IZUMO2, IZUMO3, and IZUMO4, have been identified. To date, the localization and complex formation of only IZUMO3 and 4 have been studied. IZUMO3 is a membrane protein present in the post-acrosomal region of the sperm head, while IZUMO4 is speculated to be an intra-acrosomal protein that is lost after the acrosome reaction. IZUMO 1, 3, and 4 have been shown form complexes; however the components of the complexes have not been indentified. It is of note that expression of IZUMO 3 or 4 is not disturbed in *Izumo1*-null sperm (58).

At present, IZUMO1 appears to be one of the primary sperm surface molecules responsible for mediating sperm-egg fusion. Because the molecular mechanism of IZUMO1- mediated fusion is largely unknown, information that sheds light on the process of glycosylation of IZUMO1 and IZUMO complexes will aid in understanding if IZUMO1 is in fact responsible for directly mediating fusion with the egg plasma membrane.

CD9: CD9 is a membrane protein expressed in many cell types in the body, but the genetic disruption of *Cd9* only affects the function of eggs (50, 59, 60). CD9 is member of the tetraspanin family of membrane proteins that play roles in cell adhesion, cell motility, and tumor cell metastasis. Tetraspanins contain four transmembrane domains with one large extracellular loop and another smaller loop. They interact with other molecules in the plasma membrane including other tetraspanins, integrins, growth factor receptors, IgSF proteins, complement regulatory proteins, and proteoglycans, and they are thought to regulate plasma membrane functions by creating networks of proteins through their cis interactions (50). *Cd9*$^{-/-}$ female mice are severely subfertile because of defective sperm-egg interactions (59, 60). Mating trials show that 60% of *Cd9*$^{-/-}$ females became pregnant, but it took four times longer than it did for control females and their litter sizes were 25% those of normal females (59). IVF assays show that sperm can bind to *Cd9*-null eggs, but they rarely fuse. *Cd9*-null eggs can be fertilized and undergo normal embryonic development when sperm-egg membrane interactions are bypassed by intracytoplasmic sperm injection (ICSI) (60, 61). Interestingly, CD9 interacts *in vitro* with pregnancy-specific glycoprotein PSG17, a member of the carcinoembryoinc antigen subfamily of IgSFs that has not been detected on sperm (50). IZUMO1 is also an IgSF member, leading to the hypothesis that CD9 and IZUMO1 interact, but currently there is no evidence to support this (16, 50).

Recently, CD9-containing vesicles in the perivitelline space in eggs extracted from mice carrying an EGFP-tagged CD9 transgene have been identified, and CD9 has also been shown to be transferred to the sperm head prior to fertilization (62, 63). Interestingly, co-incubating *Cd9*-null eggs with *Cd9*-expressing eggs rescued the fusion defect observed with *Cd9*-null eggs, thereby allowing sperm to fuse with the eggs (62). However, neither the data for the release of CD9-containing vesicles from eggs nor the CD9 rescue experiment could be reproduced by another set of investigators (64). CD9 has also been observed on the inner acrosomal membrane and on the equatorial segment of acrosome reacted sperm; however, CD9 is not essential for sperm to fuse with eggs because CD9-deficient sperm are still capable of fertilization (65). The function of CD9 on sperm and the uptake of CD9 by sperm from egg-released vesicles are not understood, but the presence of CD9 on both the sperm and egg would represent a novel mechanism for CD9 in mediating sperm-egg fusion.

THE OOCYTE-TO-EMBRYO TRANSITION

The oocyte-to-embryo transition in *C. elegans*

In *C. elegans*, within 30 minutes after fertilization, two meiotic divisions take place to segregate the maternal chromosomes, at which point the cell has been converted from an oocyte to a mitotically active embryo (1, 15). Fertilization results in an increase in cytoplasmic calcium in the egg that is thought to trigger egg activation but the functional consequence is unknown (27). Additionally, at the time of fertilization, but independent of fertilization, the anaphase promoting complex (APC/C) is activated and further promotes egg activation events (66, 67).

Sperm genes required for egg activation and embryogenesis: Two components of *C. elegans* sperm are known to play a role in supporting egg activation and subsequent steps: SPE-11 and CYK-4. The *spe-11* gene is predicted to encode a sperm specific novel cytoplasmic protein (68). Sperm produced by *spe-11* mutants enter oocytes but are unable to activate them, and oocytes fertilized by *spe-11* mutants have defects in meiosis and embryogenesis, do not produce polar bodies, or secrete an eggshell (66, 69). Embryos produced by *spe-11* mutant oocytes and wild-type sperm develop normally (69). In addition, transgenic animals that ectopically express SPE-11 in oocytes can be fertilized by sperm lacking SPE-11 and are able to produce viable progeny (68).

CYK-4 is a Rho GTPase-activating protein (GAP). CYK-4/GAP targets GTPase RHO-1, a homolog of RhoA, and, along with the kinesin-like protein ZEN-4, is required for assembly of the central spindle in embryos (70). After fertilization, CYK-4/GAP, RHO-1/RhoA, and ECT-2 (a guanine nucelotide-exhange factor) act together to regulate an actomyosin network to produce a gradient within a one-cell embryo that results in cell-polarity (71).

Control of oocyte-to-embryo transition: In order to control the developmental potential of the oocyte, the downstream events after egg activation must be tightly regulated, both spatially and temporally. Recent work has identified multiple egg genes that comprise a novel regulatory complex responsible for controlling these events.

EGG-3, EGG-4, and EGG-5 are pseudo-phosphatases in the oocyte that regulate MBK-2 activity, a kinase known to regu-

late the oocyte-to-embryo transition (72-75). EGG-3, EGG-4, and EGG-5 are considered pseudo-phosphatases because they contain an inactive protein tyrosine phosphatase (PTP) domain that lacks a key residue in the catalytic active site (72, 73, 76). 62% of all *C. elegans* and 40% of human PTP-domain proteins are thought to be pseudo-phosphatases (77). Though their function is not completely understood, the PTP-domain proteins are thought to bind to phosphotyrosine residues and thereby prevent the activity of phosphatases (8, 76). Alternatively, PTP-domains have been hypothesized to act as a scaffold for the assembly of signaling complexes, where PTP-domain proteins bind to phosphorylated proteins and act as a base for multiprotein complexes (8, 76, 78).

EGG-3, which is required for egg activation and proper timing of early regulatory steps during the oocyte-to-embryo transition, has provided insight into the interdependence of the regulation of the cell cycle and polarity generation during early embryonic development (72, 74). EGG-3 controls these events regulating cell surface and cortex rearrangements during egg activation in response to sperm entry. In *egg-3* mutants, fertilization occurs normally, but the polarized dispersal of F-actin is altered, a chitin eggshell is not formed, and no polar bodies are produced (72).

EGG-3 associates with the oocyte plasma membrane in a pattern that is similar to that of MBK-2 as well as that of another regulator of the oocyte-to-embryo transition, CHS-1 (72, 74). CHS-1 is a chitin synthase that catalyzes the polymeriztion of UDP-N-acetyl-glucosamine, which is necessary to produce chitin (79-81). CHS-1 is required for eggshell deposition and formation, chromosome segregation, and polar body extrusion (81). The localization of CHS-1 and EGG-3 are interdependent and both genes are required for the proper localization of MBK-2 in oocytes (72, 74). EGG-3 binds MBK-2 and, after meiosis, co-localizes with it in the cortex of oocytes and in cytoplasmic puncta (72, 74, 75). EGG-3 plays a central role in spatial localization and temporal regulation of molecules that are directly involved in executing the oocyte-to-embryo transition (72-74).

egg-4 and *egg-5* are functionally redundant genes with 99.2% homology; for this reason, this pair of genes and their products are referred to as *egg-4/5* and EGG-4/5. Like EGG-3 and MBK-2, EGG-4/5 localizes to the oocyte cortex. EGG-4/5 also requires EGG-3 for proper localization. Without EGG-4/5, MBK-2 is not localized to the cortex and embryos lacking EGG-4/5, which have defects in meiosis, polar body formation, F-actin dynamics, and eggshell deposition (73).

The oocyte cortex plays a critical role in regulating fertilization and establishing embryonic polarity (14, 72, 73). Our current understanding is that EGG-3, EGG-4/5, and MBK-2 bind to each other and form a complex at the oocyte cortex (Fig. 2) (8). CHS-1 is an integral membrane protein that is also present at the cortex and is necessary for EGG-3 cortex localization (72, 79-81). EGG-3 binds to both MBK-2 and EGG-4/5. Like EGG-3, EGG-4/5 binds MBK-2 and the combination of EGG-3 and EGG-4/5 localize MBK-2 to the cortex (73). EGG-4/5 uses its PTP domain to bind the phosphotyrosine in the activation loop of MBK-2, thereby inhibiting MBK-2 by blocking access to substrates and reducing catalytic activity (75). The complex begins to dissociate when EGG-3 is targeted for degradation by the ubiquitin ligase containing APC/C that is activated at meiotic anaphase I (74). Once MBK-2 is released from the regulatory complex, its substrates include a variety of major regulators of the oocyte-to-embryo transition, including MEI-1, OMA-1, OMA-2, MEX-5, and MEX-6 (82-86). MEI-1 is necessary for the proper mitotic spindle formation, OMA-1 and OMA-2 are necessary for sequestration of a general transcription factor component, and MEX-5 and MEX-6 are necessary for regulating polarity (82, 83, 87). MBK-2 substrates are central in regulating the resumption of the first meiotic division after fertilization and triggering the transition from a quiescent developmental state to one of rapid development and division. The spatial and temporal regulation of MBK-2 is just beginning to be elucidated and the pseudo-phosphatases EGG-3, EGG-4, and EGG-5 are central to this regulation. While EGG-4 and EGG-5 are partially responsible for regulating MBK-2, it is evident that there are other regulating factors that have yet to be identified (73, 75).

Egg activation in mammals

Calcium wave transients play an essential role in egg activation in mammals and the downstream effects of these transients are better understood in mammals than in *C. elegans*. In the mouse model, the current accepted paradigm is that sperm introduces active PLCζ (PLCzeta) into the oocyte, which hydrolyzes the phospholipid phosphatidylinositol-bisphosphate (PIP_2) to generate diacylglycerol (DAG) and inositol 1,4,5-tri-

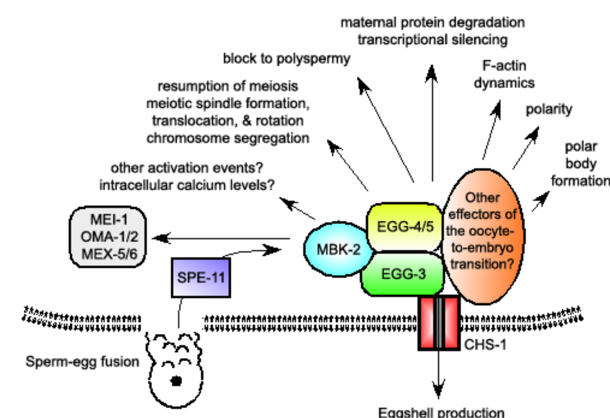

Fig. 2. Oocyte-to-embryo regulatory complex model. After sperm-egg fusion, *C. elegans* sperm provide SPE-11 for proper egg activation. The complex containing EGG-3, EGG-4, EGG-5, MBK-2, and CHS-1 is then triggered to coordinate and regulate multiple aspects of egg activation events.

phosphate (IP_3) (88, 89). IP_3 then binds to its receptor on the endoplasmic reticulum (ER), causing Ca^{2+} to be released into the cytosol. The temporal and spatial release of intracellular calcium is important for its regulatory activity and the release of Ca^{2+} regulates multiple calcium sensitive activities (13, 90). One of the most well-understood effects of calcium release is on the Ca^{2+}/ calmodulin-dependent protein kinase II (CaMKII). Ca^{2+} binds and activates CaMKII, resulting in the phosphorylation of the early mitotic inhibitor (Emi2). The phosphophorylated form of Emi2 allows another protein kinase, Plx1, to bind to Emi2 and phosphorylate it at another site (91-93). The phosphorylation by Plx1 leads to the destruction of Emi2 and relieves the inhibition of APC/C. APC/C then downregulates the MPF (maturation or M-phase promoting factor) heterodimer by degrading the regulatory subunit of MPF cyclin B and subsequent inactivates of the catalytic subunit of MPF Cdc2. The inactivation of MPF results in the exit from metaphase II. The increase in calcium also regulates multiple other protein kinases (i.e. PKC, MLCK, and MAPK) involved in downstream events of egg activation, including cytoskeletal rearrangements (90). Despite the breadth of knowledge about egg activation in eggs, indentifying the full complement of substrates from CaMKII and other protein kinases will allow for full understanding of the relationship between Ca^{2+} release and cell cycle progression as well as other egg activation events. A knockout mouse lacking PLCζ has yet to be generated, leaving open the possibility that PLCζ is not the sole sperm factor contributing to egg activation and that there are additional levels of regulation yet to be identified.

OTHER ASPECTS OF FERTILIZATION

Cortical granule exocytosis

Both *C. elegans* and mouse zygotes release cortical granules (CGs) after fertilization, but the CGs seem to have different functions. CGs are specialized secretory vesicles that are generated at the Golgi and stored in the cortex of the oocyte; their cargo is necessary for post-fertilization events (13). In mice, cortical granules contain proteases, glycosidases, and crosslinking enzymes that are necessary for the zona pellucida (ZP) block to polyspermy by altering the ZP and rendering it unreceptive to sperm (94). In the mouse, CG release is triggered by the increase in calcium during fertilization, which activates myosin light chain kinase (MLCK) to phosphorylate and activate myosin II to translocate CGs to the membrane (90, 95). The mechanism by which CGs fuse with the plasma membrane is unknown.

In *C. elegans*, CGs can be stained with markers similar to those used for mammalian CGs, but the function of *C. elegans* CGs differs. *C. elegans* do not contain a ZP, and CG exocytosis is actually a response to APC/C activation, not fertilization. Furthermore, CG exocytosis in *C. elegans* occurs too late in fertilization to be responsible for the membrane block and is more likely to be involved in the synthesis of the chitin eggshell. The exocytosis of CGs requires a number of cell cycle components, including the *C. elegans* ortholog separase-1 *sep-1*, as well as the small GTPase RAB-11 and the target-SNARE SYN-4 (19, 96). Despite the different functional roles that CGs play in mammals and *C. elegans*, understanding the regulation of CG exocytosis in both systems will provide insight into this cell biological process.

Membrane block to polyspermy

Little is known about the molecular basis of the membrane block to polyspermy in mammals, but recent studies have provided some insight into the process. Similar to other egg activation events, calcium plays an important role in the membrane block to polyspermy in mice (97). Ca^{2+} signaling is required for proper membrane block establishment and neither parthenogenetically activated eggs or eggs inseminated by ICSI mount a membrane block to polyspermy (98, 99). There is also some data that suggests that changes to the plasma membrane after fertilization also play a role in the membrane block (100). *C. elegans* oocytes do not have a ZP, but they do mount the membrane block to polyspermy, and, recently, the first genes known to regulate the membrane block to polyspermy in *C. elegans* were identified. In addition to playing a role in meiosis, polar body formation, F-actin dynamics, and eggshell deposition, EGG-4 and EGG-5 also seem to play a role in mounting the membrane block to polyspermy. *egg-4/5* double mutant hermaphrodites or hermaphrodites treated with *egg-4/5* RNAi resulted in polyspermy rates of 25% and 16%, respectively (73). *C. elegans* can provide a useful tool for understanding this long known, but little understood, aspect of reproduction.

CONCLUSION

Although the reproductive strategies of *C. elegans* and mammals differ significantly, there are still some common processes (Table 1). In both systems, sperm must acquire forward motility, dynamically reorganize molecules in the plasma membrane, locate and move toward an oocyte, bind to and fuse with the oocyte, and provide paternal contributions to the zygote. Furthermore, oocytes of both organisms must be able to undergo egg activation events after fertilization, including a membrane block to polyspermy, cortical granule (CG) exocytosis, meiotic resumption, and activation and degradation of selected maternal mRNAs and proteins, as well provide most of the material to support embryogenesis. Moreover, both organisms fertilize their eggs and begin development internally.

Increased understanding of fertilization and the oocyte-to-embryo transition in model systems will increase our understanding of the molecular mechanisms that govern human fertilization. The ability to identify the genes that control these processes is an important step in understanding fertilization. *C. elegans* has proven to be a valuable tool for gene identification and, along with mouse studies, can provide a more compre-

hensive list of genes involved in fertilization. The functionally homologous genes discovered in *C. elegans* need to be investigated in mammalian systems and vice versa. This will help close the gap in knowledge between the two systems and provide a more complete view of reproductive strategies. Though homologous genes may not be evident in *C. elegans* or mice, or even humans, these genes may regulate similar cell biological processes, which are important for understanding reproduction and general biological processes and cell functions.

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