Chapter 11
Fertilization

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Abstract Fertilization—the fusion of gametes to produce a new organism—is the culmination of a multitude of intricately regulated cellular processes. In *Caenorhabditis elegans*, fertilization is highly efficient. Sperm become fertilization competent after undergoing a maturation process during which they become motile, and the plasma membrane protein composition is reorganized in preparation for interaction with the oocyte. The highly specialized gametes begin their interactions by signaling to one another to ensure that fertilization occurs when they meet. The oocyte releases prostaglandin signals to help guide the sperm to the site of fertilization, and sperm secrete a protein called major sperm protein (MSP) to trigger oocyte maturation and ovulation. Upon meeting one another in the spermatheca, the sperm and oocyte fuse in a specific and tightly regulated process. Recent studies are providing new insights into the molecular basis of this fusion process. After fertilization, the oocyte must quickly transition from the relative quiescence of oogenesis to a phase of rapid development during the cleavage divisions of early embryogenesis. In addition, the fertilized oocyte must prevent other sperm from fusing with it as

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T. Schedl (ed.), *Germ Cell Development in C. elegans*, Advances in Experimental Medicine and Biology 757, DOI 10.1007/978-1-4614-4015-4_11,

well as produce an eggshell for protection during external development. This chapter will review the nature and regulation of the various cellular processes of fertilization, including the development of fertilization competence, gamete signaling, sperm–oocyte fusion, the oocyte to embryo transition, and production of an eggshell to protect the developing embryo.

Keywords Fertilization • Oocyte-to-embryo transition • Egg activation • Spermiogenesis • Sperm activation • Eggshell • Polyspermy • Sperm migration • PUFA-derived signals • Major sperm protein (MSP)

11.1 Overview of Fertilization in *Caenorhabditis elegans*

Fertilization is the central molecular process of sexual reproduction. Despite this, the cellular and molecular mechanisms that govern sperm–egg fusion are not well understood. *C. elegans* serves as an excellent model system for dissecting the molecular and genetic processes of fertilization, as well as those that prepare the gametes for fertilization and ensure proper embryogenesis. Importantly, fertilization in *C. elegans* shares many characteristics with fertilization in other species, including mammals. In both *C. elegans* and mammalian fertilization, sperm activation occurs within the reproductive tract, fertilization occurs internally, and egg activation includes a polyspermy block, cortical granule exocytosis, meiotic resumption, and the activation and degradation of selected maternal mRNAs and proteins (Singson et al. 2008; Horner and Wolfner 2008; Marcello and Singson 2010; Stitzel and Seydoux 2007).

C. elegans has two sexes: hermaphrodite and male (Nigon and Dougherty 1949; Madl and Herman 1979). Hermaphrodite *C. elegans* are essentially females that produce and store sperm early in their life cycle before producing oocytes (Ward and Carrel 1979; Hirsh et al. 1976). Hermaphrodites produce approximately 100–175 immature sperm, or spermatids, per gonad arm during the final larval stage (L4) and then switch strictly to oogenesis for the remainder of their life (see Chap. 3, Zanetti and Puoti 2012; Ward and Carrel 1979; Hirsh et al. 1976).

Male-derived sperm are introduced to the hermaphrodite reproductive tract through the uterus during copulation (L'Hernault 2006; Stanfield and Villeneuve 2006). Immature sperm, or spermatids, are stored in the seminal vesicle of males and undergo a final maturation process, called sperm activation or spermiogenesis, as they are ejaculated into the hermaphrodite (Hirsh et al. 1976; Klass et al. 1976; Kimble and Hirsh 1979; Shakes and Ward 1989). During sperm activation, the spermatids mature to a motile, amoeboid sperm capable of fertilizing the oocyte (see Chap. 7, Chu and Shakes 2012). Both the sperm and oocyte send signals to increase the efficiency of fertilization. Oocytes release polyunsaturated fatty acid-derived signals to attract sperm to the oocytes and sperm then migrate to the spermatheca, the site of fertilization (Fig. 11.1) (Kubagawa et al. 2006). *C. elegans* sperm do not contain any actin and instead employ another cytoskeletal protein, major sperm

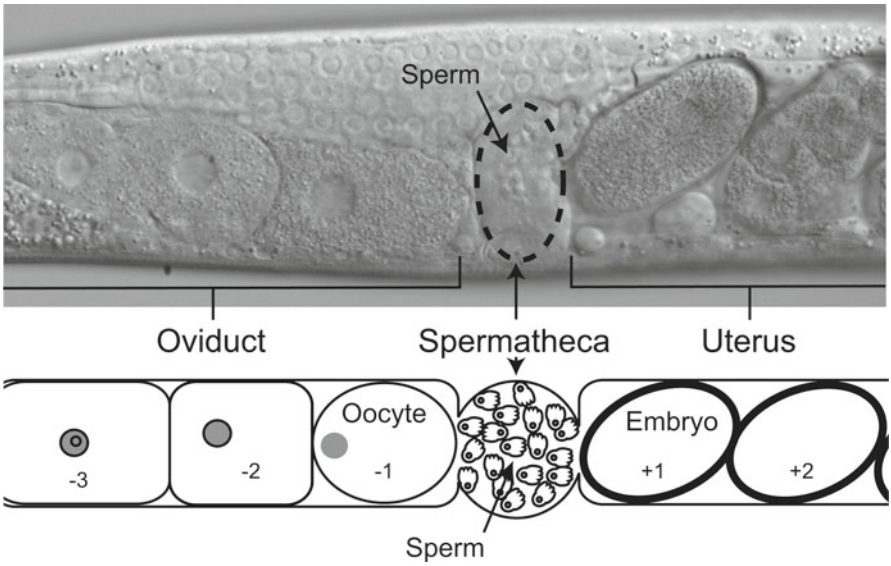

Fig. 11.1 The *C. elegans* reproductive tract. The *top image* shows a light micrograph of the *C. elegans* reproductive tract with focus on the spermatheca. The *bottom image* is a schematic of the same region. The proximal oocyte (−1) is ovulated from the oviduct into the spermatheca. Sperm are stored in the spermatheca as they await the ovulating oocyte. After fertilization, the embryo is pushed into the uterus (+1)

protein (MSP), to mediate motility (Roberts and Ward 1982). In addition to serving as the cytoskeletal protein that is responsible for sperm locomotion, MSP is also released from the sperm and stimulates meiotic maturation and ovulation of the oocyte into the spermatheca (Miller et al. 2001).

Fertilization triggers a number of rapid changes in the oocyte. The first detectable change in the oocyte after fertilization is an increase in intracellular calcium (Samuel et al. 2001). The oocyte then forms an actin-rich cap over the site of fertilization, completes meiosis, extrudes an eggshell, and initiates embryonic development (Parry et al. 2009; Maruyama et al. 2007). If the oocyte-to-embryo transition occurs normally, the embryo will divide in the uterus until approximately the 30-cell stage and then will be laid out of the vulva (egg-laying) (Hirsh et al. 1976).

Hermaphrodites are able to self-fertilize when males are not present in the population (L'Hernault 2006; Ward and Carrel 1979; Hirsh et al. 1976). The hermaphrodite-derived spermatids that are produced during L4 are stored in the proximal gonad and are also pushed into the spermatheca when the first oocytes are ovulated (McCarter et al. 1999; Singson 2001). In contrast to sperm activation of male-derived sperm, hermaphrodite-derived spermatids undergo sperm activation when they are deposited into the spermatheca (L'Hernault 2006). Fertilization in *C. elegans* is very efficient and almost all of the hermaphrodite-derived sperm fertilize the egg (Singson 2001).

If both male- and hermaphrodite-derived sperm are present in the spermatheca, the male-derived sperm are competitively superior to hermaphrodite-derived sperm and will preferentially fertilize the oocytes (L'Hernault 2006; Ward and Carrel 1979). It is not completely understood how male-derived sperm outcompete hermaphrodite-derived sperm (LaMunyon and Ward 1998). One hypothesis is that male-derived sperm are larger than hermaphrodite-derived sperm because of the differences in the physiological environment of male gonad; this size difference may aid in gaining access to the oocytes (Baldi et al. 2011; LaMunyon and Ward 1998). Interestingly, males preferentially mate with older hermaphrodites that have depleted their own sperm populations, and it is hypothesized that hermaphrodites produce a mating cue that attracts males when no activated sperm are present (Morsci et al. 2011).

The experimental techniques available to study the multitude of cellular processes that occur during fertilization in *C. elegans* make it an attractive model system (see Geldziler et al. 2011 for an extensive review of techniques). The most widely used method for understanding the mechanism of fertilization in *C. elegans* is genetic analysis (Geldziler et al. 2011). Forward genetic screens for fertility mutants in *C. elegans* are very powerful because it is relatively easy to isolate mutations in sperm-specific genes, and mutants can be isolated that have defects only in fertilization and/or egg activation, not spermatogenesis, spermiogenesis, or oogenesis (L'Hernault et al. 1988; Singson et al. 1998). RNAi has been helpful in identifying genes in the oocyte that are necessary for fertilization (Geldziler et al. 2004). However, many questions still remain about molecular basis of *C. elegans* fertilization. This chapter will outline the current status of the field of fertilization *C. elegans* and provide some perspective on the future directions of the field.

11.2 Preparing Sperm for Interaction with the Oocyte

11.2.1 Sperm Activation

C. elegans sperm must undergo a final maturation process, called sperm activation (spermiogenesis), to be fertilization competent. Sperm activation is a rapid post-meiotic differentiation that converts round immotile spermatids into mature amoeboid sperm (see Chap. 7, Chu and Shakes 2012; Shakes and Ward 1989) (Fig. 11.2). Hermaphrodite-derived spermatids undergo sperm activation when they are pushed into the spermatheca during the first ovulations and male-derived spermatids undergo sperm activation when they mix with the seminal fluid during ejaculation (L'Hernault 2006; Stanfield and Villeneuve 2006). Regardless of the origin of the sperm, two events take place that are critical to render the sperm fertilization competent: (1) membranous organelles (MO), which are specialized secretory vesicles derived from the Golgi, must fuse with the plasma membrane to release their glycoprotein contents and add critical transmembrane proteins to the sperm plasma membrane, and (2) sperm must gain the ability to crawl by extending a pseudopod from one side of the cell body (Fig. 11.2) (Shakes and Ward 1989; Roberts and

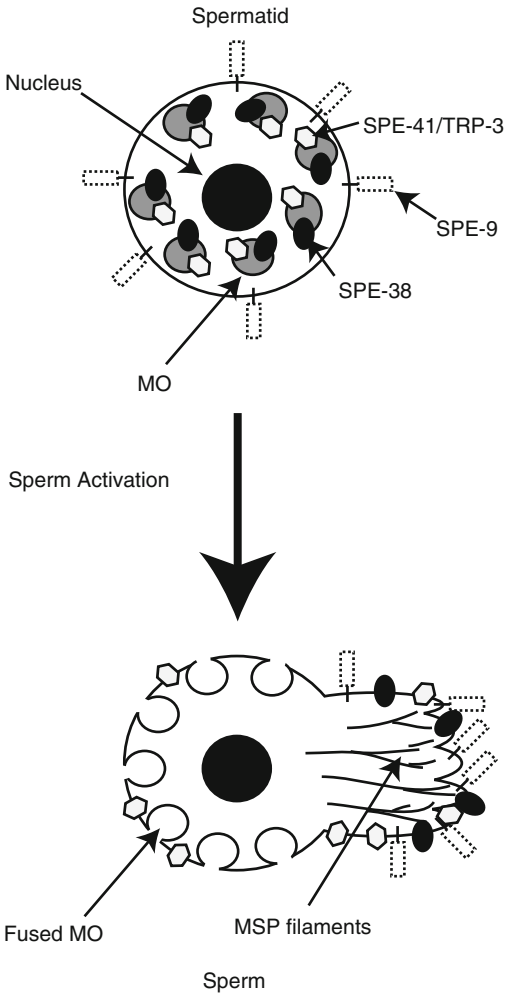

Fig. 11.2 Sperm activation in *C. elegans*. In order for sperm to be fertilization competent, they must undergo a maturation process called sperm activation (spermiogenesis). During sperm activation, membranous organelles (MO) in the immature spermatid fuse with the plasma membrane, resulting in membrane reorganization. The sperm membrane protein SPE-9 is always present in the plasma membrane but relocalizes exclusively to the pseudopod after sperm activation. SPE-38 and SPE-41/TRP-3 are both present in MOs in spermatids and change localization after MO fusion. After MO fusion, SPE-38 is present only on the pseudopod of the sperm while SPE-41/TRP-3 is present throughout the plasma membrane. In addition to MO fusion, the spermatid also extrudes a pseudopod on one side of the cell body during sperm activation. The pseudopod allows for sperm movement, which is necessary for locating the oocyte

Streitmatter 1984; Roberts and Ward 1982). Sperm activation (spermiogenesis) is covered extensively in Chap. 7 by D.S. Chu and D.C. Shakes. This chapter will focus on the mechanisms of sperm locomotion that are necessary for sperm to fertilize the ovulated oocyte in the spermatheca.

11.2.2 MSP Polymerization and Sperm Motility

Sperm motility is crucial for sperm to reach and fertilize the oocyte. The movement of the amoeboid sperm is made possible by a protrusion at its leading edge, adhesion to the substrate, and retraction near the cell body (Shimabukuro et al. 2011). Sperm from *C. elegans* and other nematodes accomplish this without actin or motor proteins (Roberts and Stewart 2000). Instead, nematodes employ an MSP-based motility system (King et al. 1994). In addition to signaling oocyte meiotic maturation and ovulation, MSP is also the structural component responsible for sperm movement (King et al. 1994; Smith 2006). MSP accounts for more than 40% of the cytosolic protein in nematode sperm (Smith 2006).

To better understand the mechanism of the MSP-based motility of nematode sperm, many studies were performed using the parasitic nematode *Ascaris suum* (Italiano et al. 2001). *A. suum* sperm are large and easy to isolate in large numbers, which is advantageous for imaging and allows for more convenient protein purification and biochemical analyses (L'Hernault and Roberts 1995). Moreover, the development of a cell-free filament assembly system from *A. suum* lysate has allowed for the reconstitution of purified motility factors *in vitro* (Italiano et al. 1996).

The three-dimensional structure of *C. elegans* MSP is very similar to that of *A. suum* MSP (Baker et al. 2002). X-ray crystallography and NMR have shown that MSP folds into a seven-stranded beta sandwich that resembles an immunoglobulin-like fold (Bullock et al. 1996a, b; Haaf et al. 1996). MSP monomers self-assemble to form dimers (Haaf et al. 1996). The MSP dimers are then polymerized to form helical subfilaments that are ultimately assembled into larger fibers (Haaf et al. 1996). In *A. suum,* the movement of the pseudopod is ATP dependent and is accomplished by the continual assembly of bundles of MSP filaments at the leading edge and concurrent disassembly of the filaments near the cell body (Miao et al. 2003).

Assembly of the MSP fibers at the leading edge is mediated by a crucial membrane protein and multiple phosphorylation events. A critical protein in MSP nucleation and assembly is MSP polymerization organizing protein (MPOP), a 48 kDa protein present on the inner leaflet of the plasma membrane (LeClaire et al. 2003). MPOP is selectively phosphorylated by an unknown tyrosine kinase at the leading edge, after which phospho-MPOP recruits MSP polymerization-activation kinase (MPAK) to the leading edge of the pseudopod, where MPAK then phosphorylates MFP2 (MSP fiber protein 2) (Yi et al. 2007). The phosphorylated form of MFP2 is present on the MSP fiber complexes in sperm and in the reconstituted MSP fibers *in vitro* (Buttery et al. 2003). MFP2 is thought to increase the rate of fiber formation by cross-linking MSP filaments or recruiting other proteins required for filament formation (Buttery et al. 2003; Grant et al. 2005). Together, these proteins regulate MSP assembly at the leading edge and are necessary for the pseudopod protrusions.

The disassembly of MSP filaments is as important as assembly in regulating pseudopod movement and is regulated by a putative protein phosphatase 2A homolog known as PP2A (Yi et al. 2009). PP2A is localized near the cell body,

which is the site of MSP depolymerization, and when PP2A is activated, it promotes the retraction of the MSP fiber (Yi et al. 2009). One of the substrates of PP2A is MFP3 (MSP fiber protein 3), which in its phosphorylated form binds with MSP filaments and stabilizes it (Yi et al. 2009). The dephosphorylation of MFP3 by PP2A causes MFP3 to detach from the MSP bundles, which causes the destabilization and depolymerization of MSP fibers (Yi et al. 2009). MFP1 (MSP fiber protein 1) also negatively regulates the elongation of MSP fibers in vitro but its relationship with PP2A and MFP3 is unknown (Buttery et al. 2003). The depolymerization of the MSP fibers is crucial for the forward movement of the cell body and amoeboid movement of sperm. Disassembly of MSP fibers leads to a gradual decrease in the optical density of filaments. Depolymerizing the MSP network produces sufficient force to propel the pseudopod (Shimabukuro et al. 2011).

An increasing number of studies have focused on understanding MSP dynamics in *C. elegans*. Batchelder et al. found that membrane tension plays an important role in motility, and they postulate that increasing membrane tension in the direction of movement streamlines MSP polymerization and optimizes motility (Batchelder et al. 2011). Phosphorylation of MSP has been observed in *C. elegans* and MSP dynamics seem to be the same, but the regulatory proteins between *C. elegans* and *A. suum* are not conserved (Fraire-Zamora et al. 2011). In *C. elegans*, homologs to proteins that regulate MSP assembly (MPAK, MPOP, or MFP2) have not been identified. Instead, SPE-6, a casein kinase I homolog, may play a role in MSP assembly (see Chap. 7, Chu and Shakes 2012; Varkey et al. 1993). MSP disassembly in *C. elegans* involves phosphatases, but not PP2A as in *A. suum*. As a potential alternative to PP2A, *C. elegans* employs two nearly identical sperm-specific PP1 phosphatases, GSP-3 and GSP-4 (GSP-3/4), for motility (Wu et al. 2011). GSP-3/4 are hypothesized to regulate the spatial disassembly of MSP and sperm from *C. elegans* lacking GSP-3/4 are immotile and have defects in pseudopod development (Wu et al. 2011). PP1 phosphatases are also necessary for sperm development and fertility in mice (Varmuza et al. 1999; Oppedisano et al. 2002; Chakrabarti et al. 2007; Huang and Vijayaraghavan 2004; Soler et al. 2009; Wu et al. 2011).

MSP plays diverse roles during fertilization and is necessary for generating the amoeboid movement of nematode sperm that is necessary for the sperm to migrate through the reproductive tract. Understanding MSP regulation will provide insight into how protein polymerization can generate and force and membrane protrusions (Smith 2006).

11.2.3 The Role of pH in Regulating Sperm Activation and MSP Polymerization

A change in intracellular pH can trigger sperm activation. Addition of weak bases, such as triethanolamine, is sufficient to activate *C. elegans* sperm in vitro (Ward et al. 1983). Similarly, the addition of vas deferens extract can activate *A. suum* sperm in vitro (Abbas and Cain 1979). Within 15 s of the addition of vas deferens

extract, the intracellular pH rises from 6.25 to 6.50 in *A. suum* sperm, suggesting that sperm activation is accompanied by an increase in intracellular pH (King et al. 1994). Understanding the mechanism by which pH triggers sperm activation might provide insights into how ion channel regulation can affect vesicle fusion events and morphology changes.

Alterations in intracellular pH also have effects on the polymerization of MSP. Treatment of *A. suum* sperm with a weak acid promotes the disassembly of MSP fibers and removal of the weak acid promotes the re-assembly of MSP fibers (King et al. 1994). Additionally, in activated *A. suum* sperm, a pH gradient is formed between the leading edge and the base of the pseudopod: the intracellular pH at the leading edge and base of the pseudopod are 6.35 and 6.17, respectively (King et al. 1994). In *A. suum* sperm, higher pH at the leading edge stimulates the phosphorylation of MPOP (see Sect. 11.2.2), which in turn assists in the nucleation of MSP fibers at the leading edge (Buttery et al. 2003).

11.3 Sperm and Oocyte Communication

11.3.1 Regulation of Sperm Guidance by Oocyte Polyunsaturated Fatty Acid-Derived Signals

For *C. elegans*, guiding sperm to and subsequently maintaining sperm within the spermatheca is crucial for maximal reproduction. Regardless of whether sperm originate from a hermaphrodite or male, sperm must be guided back into the spermatheca after they are pushed into the uterus by newly fertilized embryos on their way to the uterus (Ward and Carrel 1979).

In *C. elegans*, oocytes attract sperm to the spermatheca by releasing polyunsaturated fatty acid (PUFA)-derived prostaglandin signals produced by oocytes (Edmonds et al. 2010; Kubagawa et al. 2006). PUFAs are synthesized from dietary precursors by lipid anabolic enzymes. These enzymes, the so-called *fat* genes, include *fat-2*, a Δ12-desaturase that generates 18- and 20-carbon PUFAs (Watts and Browse 2002). In *C. elegans,* PUFAs produced within the intestine are initially incorporated into the yolk, which is then taken up by oocytes via receptor-mediated endocytosis using the low-density lipoprotein (LDL) receptor RME-2 (receptor mediated endocytosis-2) (Kubagawa et al. 2006; Grant and Hirsh 1999). Once the PUFAs enter the oocytes, they are converted into prostaglandins and eventually secreted into the reproductive tract (Edmonds et al. 2010). Notably, a number of signaling pathways in mammals are also regulated by prostaglandins, including many processes in mammalian reproduction such as ovulation and fertilization (Han et al. 2010).

Other *Caenorhabditis* species may also use prostaglandins for directional migration of sperm into the spermatheca (Singaravelu and Singson 2011). When males from one *Caenorhabditis* species were allowed to mate with hermaphrodites of

another closely related species, the ejaculated sperm successfully reached the spermatheca, implying that directed sperm migration within the *Caenorhabditis* genus employs an evolutionarily conserved signal (Hill and L'Hernault 2001; Singaravelu and Singson 2011).

11.3.2 Secretion of MSP by Sperm to Induce Oocyte Maturation and Gonadal Sheath Cell Contraction

In *C. elegans* sperm, the MSP provides the signal to induce oocyte meiotic maturation and ovulation (see Chap. 10, Kim et al. 2012). MSP has two separate signaling functions (Miller et al. 2001). The 20 C-terminal amino acids of MSP promote sheath cell contraction that leads to ovulation and MSP lacking these 20 amino acids promote oocyte maturation (Miller et al. 2001). Using sperm-derived signals to regulate ovulation rates increases the probability that fertilization will take place, as oocytes are less likely to enter an empty spermatheca (McCarter et al. 1999; Miller et al. 2001). MSP is secreted from sperm through an unconventional vesicle budding process (Kosinski et al. 2005). The MSP signal persists as long as sperm are present (Miller et al. 2001). The signal for the first ovulation in a hermaphrodite originates from the spermatids, which have MSP and can signal for oocyte maturation and sheath contraction (McCarter et al. 1999).

11.4 Sperm–Oocyte Fusion

11.4.1 spe and egg Genes

In order for sperm and oocytes to fuse, the gametes must be able to recognize one another and have the fusion machinery localized and assembled properly to mediate fertilization. Precise recognition is mediated by specific interactions between proteins present on the surfaces of the sperm and oocyte. Mutations disrupting the fusion machinery on either gamete will impair fertilization but development is expected to be otherwise normal. Genes that are essential for sperm development, differentiation, or function during fusion are classified as *spe* genes (also previously referred to as *fer* genes) (also see Chap. 7, Chu and Shakes 2012; Singson 2001; Parry and Singson 2011). *egg* genes are defined as genes that are essential for the oocyte's ability to promote sperm fusion or respond to fertilization but are not necessary for proper oogenesis (Singson 2001; Parry and Singson 2011). The number of genes known to be necessary for fertilization is low in all animal species and every gene that is identified and cloned will add a significant amount of information to our knowledge of how fertilization is mediated.

11.4.2 Sperm Genes Necessary for Fertilization

spe-9 was the first sperm gene necessary for fertilization to have been cloned (Singson et al. 1998). Sperm from animals with *spe-9* mutations undergo normal spermatogenesis and sperm activation and have normal morphology and motility, but are unable to fertilize oocytes (Singson et al. 1998). Subsequently, all sperm genes that are fertilization-defective but undergo normal sperm activation are members of the *spe-9* class of mutants. Currently, the *spe-9* class of mutants consists of seven mutants (*spe-9, spe-13, spe-36, spe-38, spe-41/trp-3, spe-42,* and *fer-14*) (Table 11.1) (Singson et al. 1998; Kroft et al. 2005; Chatterjee et al. 2005; Xu and Sternberg 2003; L'Hernault et al. 1988; Putiri et al. 2004; L'Hernault 2006; Nishimura and L'Hernault 2010). Four of the genes responsible for the *spe-9* class phenotype have been cloned (*spe-9, spe-38, spe-41/trp-3*, and *spe-42*), and we will focus on what is known about the function of these four genes.

spe-9: SPE-9 is a single-pass transmembrane protein with a large extracellular domain and a short cytoplasmic tail (Singson et al. 1998). The extracellular domain of SPE-9 has ten epidermal growth factor (EGF) repeats that are thought to mediate protein–protein interactions (Balzar et al. 2001; Singson et al. 1998). SPE-9 localization is dynamic: in spermatids, SPE-9 is localized uniformly over the entire plasma membrane, but after sperm activation, it is predominantly localized to the pseudopod (Fig. 11.2) (Zannoni et al. 2003).

spe-38: SPE-38 is a nematode specific, novel four-pass transmembrane protein that resembles many small tetraspanins which are associated with cell–cell interaction (Chatterjee et al. 2005). In spermatids, SPE-38 localizes to membranous organelles (MOs) (Chatterjee et al. 2005). In mature sperm, SPE-38 relocalizes to the pseudopod, similar to SPE-9 (Fig. 11.2) (Chatterjee et al. 2005).

spe-41/trp-3: SPE-41/TRP-3 is a transient receptor potential channel and is conserved from *C. elegans* to humans (Xu and Sternberg 2003). TRP-3 homologs have been implicated in a variety of pathologies, including cancer, heart disease, kidney disease, and pain (Dietrich et al. 2010; Kiselyov et al. 2007; Bodding 2007; Eder and Molkentin 2011; Chung et al. 2011). Like SPE-38, SPE-41/TRP-3 localizes to the MO in spermatids; however, SPE-41/TRP-3 localizes uniformly all over the plasma membrane in mature sperm and does not show pseudopod-only localization as do SPE-38 and SPE-9 (Fig. 11.2) (Xu and Sternberg 2003). In *Drosophila*, TRP channels have independent roles as a scaffold and calcium channel (Wang et al. 2005). Future areas of interest include determining whether the calcium channel activity of SPE-41/TRP-3 is separable from its role in fertility and whether SPE-41/TRP-3 plays a role in mediating the calcium increase observed during fertilization.

spe-42: SPE-42 is a novel six-pass transmembrane protein whose homologs are found in many metazoans, such as flies, mouse, and human (Kroft et al. 2005). SPE-42 contains a DC-STAMP (dendritic cell-specific transmembrane protein) domain, which is required for cell fusion of osteoclasts in mammals (Mensah et al. 2010; Miyamoto 2006). It has been hypothesized that the DC-STAMP domain of SPE-42

Table 11.1 Genes necessary for fertilization and the oocyte-to-embryo transition

Gene	Encoded protein	Function
Fertilization		
spe-9	EGF repeat (10)-containing protein with a short intracellular domain	Fertilization (in sperm)
spe-13	Unknown	Fertilization (in sperm)
spe-36	Unknown	Fertilization (in sperm)
spe-38	Novel four-pass transmembrane protein	Fertilization (in sperm)
spe-41/trp-3	Calcium-permeable cation channels that are members of the TRPC (transient receptor potential canonical) subfamily of TRP channels	Fertilization; calcium influx (in sperm)
spe-42	Novel sperm-specific seven-pass transmembrane protein; two functional domains: DC-STAMP (dendritic cell-specific transmembrane protein) and C4C4-type RING finger	Fertilization (in sperm)
fer-14	Unknown	Fertilization (in sperm)
egg-1/2	LDL-receptor repeat-containing protein	Fertilization (in oocytes)
Oocyte-to-embryo transition		
mbk-2	DYRK (dual-specificity Yak1-related) kinase	Early embryo development; Substrates include MEI-1, OMA-1, OMA-2, MEX-5, and MEX-6
egg-3	Member of the protein tyrosine phosphatase-like (PTPL) family	Polar body emission; eggshell extrusion; MBK-2 regulation
egg-4/5	Member of the protein tyrosine phosphatase-like (PTPL) family	Polar body emission; eggshell extrusion; MBK-2 regulation; block to polyspermy
chs-1	Chitin synthase	Chitin polymerization; MBK-2, EGG-3, and EGG-4/5 localization
spe-11	Novel protein	Polar body emission; eggshell extrusion
cyk-4	Rho GAP (Rho guanosine triphosphatase (GTPase) activating protein)	Establishes anteroposterior axis along with small GTPase RhoA (Ras homolog gene family, member A) and ECT-2 (a RhoA guanine nucleotide-exchange factor)
sep-1	Separase cysteine protease	CG exocytosis; eggshell formation
rab-11.1	Small GTPase homologous to the Rab GTPases	CG exocytosis; eggshell formation
syn-4	Syntaxin-related t-SNARE	CG exocytosis; eggshell formation
gna-2	Glucosamine 6-phosphate *N*-acetyltransferase	Synthesis of UDP-*N*-acetylglucosamine (UDP-GlcNAc), *N*-acetylgalactosamine (UDP-GalNAc), and chitin; eggshell formation

could be used for membrane fusion events, including sperm–egg fusion; however, the mechanism by which DC-STAMP domains mediate fusion is unclear, and currently there is no evidence to support this hypothesis (Wilson et al. 2011; Kroft et al. 2005). The C-terminal tail of SPE-42 forms a RING finger domain that has eight conserved cysteine residues that are thought to coordinate Zn^{2+} ions; a mutation in any of these cysteine residues abolishes SPE-42 function (Wilson et al. 2011).

At present, not much is known about how the *spe-9* class of genes interact on the protein level. Recent work has shown that SPE-38 and SPE-41/TRP-3 interact both genetically and physically (Singaravelu et al. 2012). In *spe-38* mutant spermatozoa, SPE-41/TRP-3 is trapped in the MO, suggesting that SPE-38 is required for the trafficking of SPE-41/TRP-3 following sperm activation. In contrast, the localization of SPE-38 is unperturbed in *spe-41/trp-3* mutant sperm, suggesting that SPE-38 regulates SPE-41/TRP-3 trafficking and not vice versa. Split ubiquitin yeast-two hybrid results show that SPE-38 and SPE-41/TRP-3 can interact with each other. Further studies could elucidate whether SPE-38 modulates channel activity of SPE-41/TRP-3.

The SPE-9 class proteins may potentially function in sperm to mediate either recognizing, adhering, or fusing with the oocyte. All SPE-9 class proteins are membrane proteins and thus have the potential to be a sperm ligand. SPE-9 is the most likely candidate to serve as a sperm ligand because of the presence of multiple EGF repeats and the selective localization of SPE-9 to the pseudopod (Singson et al. 1998). Proteins that localize to the pseudopod are the best candidates to mediate fusion with the oocyte, as the pseudopod is thought to make initial contact with the oocyte (Zannoni et al. 2003). Delineating whether these SPE-9 class proteins function to organize the sperm membrane appropriately or as an adhesion molecule or fusogen will provide insight into the molecular basis of fertilization.

11.4.3 Oocyte Genes Necessary for Fertilization

Currently, only two genes on the oocyte are known to be necessary for fertilization: *egg-1* and *egg-2* (*egg-1/2*) (Kadandale et al. 2005). EGG-1 and EGG-2 are single-pass transmembrane proteins that contain low-density lipoprotein (LDL) receptor repeats on the plasma membrane of the oocyte (Kadandale et al. 2005). EGG-1 and EGG-2 are 67% identical and are likely to be functionally redundant (Kadandale et al. 2005). In *C. elegans*, the hermaphrodites lacking function *egg-1* and *egg-2*, either by mutation or RNAi, lay unfertilized oocytes and have severe reductions in progeny production (Kadandale et al. 2005; Lee and Schedl 2001; Maeda et al. 2001; Johnston et al. 2010). Analysis of *egg-1*(*tm1071*) indicates that oocytes lacking *egg-1* are ovulated and make contact with sperm, but a majority of the oocytes are not fertilized (Kadandale et al. 2005; Johnston et al. 2010). Proteins related to LDL receptors bind with diverse ligands such as lipoproteins, viruses, and signaling molecules, and thus could mediate fertilization (Nykjaer and Willnow 2002). The identification of additional transmembrane proteins in the oocyte is paramount to

Table 11.2 Species-specific fertilization of the *elegans* group

	Fem./herm.			
Male	*C. elegans*	*C. briggsae*	*C. remanei*	*C. brenneri*
C. elegans	X	–	+	+
C. briggsae	–	X	+	+
C. remanei	–	–	X	+
C. brenneri	Not tested	Not tested	–	X

our understanding of *C. elegans* fertilization and could provide insight into how fertilization is mediated in all species.

11.4.4 Species-Specific Fertilization in Caenorhabditis

Fertilization in the *Caenorhabditis* species is specific; however, a selected number *Caenorhabditis* species are able to fertilize the oocytes of other species (Table 11.2) (Baird et al. 1992; Sudhaus and Kiontke 2007; Baird and Yen 2000; Hill and L'Hernault 2001; Kiontke and Fitch 2005). The *elegans* group of *Caenorhabditis* contains four species: *C. elegans, C. briggsae, C. remanei*, and *C. brenneri* (Kiontke and Fitch 2005). *C. elegans* is actually a sister species of a clade (a species and all of its descendents) made up of *C. briggsae, C. remanei*, and *C. brenneri* (Kiontke and Fitch 2005). Like *C. elegans*, *C. briggsae* is a hermaphrodite–male species while *C. remanei* and *C. brenneri* are female–male species (Kiontke and Fitch 2005). Interestingly, *C. brenneri* oocytes can be fertilized by sperm from any of the three other members of the *elegans* group, indicating that *C. brenneri* oocytes possess receptor(s) that can recognize ligand(s) on *C. elegans*, *C. briggsae*, and *C. remanei* sperm and mediate fusion (Baird and Yen 2000). On the other hand, *C. elegans* and *C. briggsae* oocytes can only be fertilized by sperm from their own species (Baird et al. 1992). Despite the fact that fertilization can occur between species of the *elegans* group, these fertilization events do not result in fertile offspring, indicating that there is post-zygotic incompatibility (Baird et al. 1992; Sudhaus and Kiontke 2007; Baird and Yen 2000; Sudhaus et al. 2007). Studying the species specificity of fertilization in the *elegans* group may provide clues as to which proteins mediate sperm–egg fusion as well as insight into the evolutionary origins of the species.

11.5 Egg Activation

The transition from an oocyte to an embryo is comprised of two steps: meiotic maturation and egg activation (Stitzel and Seydoux 2007). During meiotic maturation, the oocyte exits meiotic prophase, initiates the meiotic division phase, and becomes fertilization competent (see Chap. 10, Kim et al. 2012; Stitzel and Seydoux 2007;

Horner and Wolfner 2008; Yamamoto et al. 2006). Egg activation is the transition of a fertilized egg to a developing embryo. Egg activation entails a number of dynamic cellular processes: completion of meiosis, maternal protein degradation, cytoskeletal rearrangements, activation of embryogenesis, and eggshell formation (Stitzel and Seydoux 2007; Horner and Wolfner 2008).

11.5.1 Regulation of Meiotic Resumption

In *C. elegans* it is critical that meiotic resumption and cell cycle progression are tightly regulated because of the continual production of oocytes and rapid fertilization (McCarter et al. 1999). In many animals, oocytes arrest in late meiotic prophase to temporally control utilization and often have a second arrest during the meiotic divisions; in most vertebrates, oocytes arrest in prophase I until they are cyclically recruited and then arrest again in metaphase II as they wait for a fertilization-competent sperm (Masui and Clarke 1979; Eppig 1996; McCarter et al. 1999). However, in *C. elegans*, oocytes are produced continually in the presence of sperm, so there is no need for oocytes to arrest in meiotic prophase (although they do arrest in late meiotic prophase in the absence of sperm) (McCarter et al. 1999). Additionally, since sperm signal both maturation and ovulation, there is no need to arrest the oocyte cell cycle in order to wait for the arrival of competent sperm (McCarter et al. 1999; Miller et al. 2001). In fact, in the absence of sperm, oocytes progress to anaphase I (McNally and McNally 2005). As a result, meiotic resumption and completion as well as egg activation events associated with fertilization occur concurrently, making tight regulatory control critical for proper embryogenesis.

In *C. elegans*, fertilization occurs as the oocyte enters the spermatheca, after germinal vesicle breakdown and while the meiosis I spindle is assembling (McNally and McNally 2005) (Fig. 11.3a). Fertilization is accompanied by an increase in free cytosolic calcium levels (Samuel et al. 2001). The increase in cytoplasmic calcium levels at fertilization occurs in other animals; however, the functional consequence of this change in calcium dynamics in *C. elegans* needs to be more fully explored (Samuel et al. 2001; Horner and Wolfner 2008). In *Xenopus*, a calcium signal triggers the exit from meiosis II by activating the anaphase-promoting complex APC/C (Tunquist et al. 2002; Liu and Maller 2005; Rauh et al. 2005). Like *Xenopus*, the

Fig. 11.3 (continued) The MBK2-, EGG-3, EGG-4/5, CHS-1 complex is still in the cortex in metaphase I but disassembles as the embryo transitions to meiotic metaphase II (**c**). During the transition to metaphase II, EGG-3, EGG-4/5, and SPE-11 are all necessary for polar body extrusion and eggshell production. Eggshell production is also dependent upon CHS-1 to polymerize chitin and upon the release of chondroitin proteoglycans to the lipid layer via CG exocytosis during this transition. At meiotic metaphase II, MBK-2, EGG-3, and CHS-1 are localized to the cytoplasmic puncta. As the embryo enters mitosis (**d**), the complex dissociates and MBK-2 is free to phosphorylate its substrates and promote embryo development. The eggshell is impermeable by mitotic entry and consists of three layers: the outer vitelline layer, the middle chitin layer, and the inner lipid-rich layer

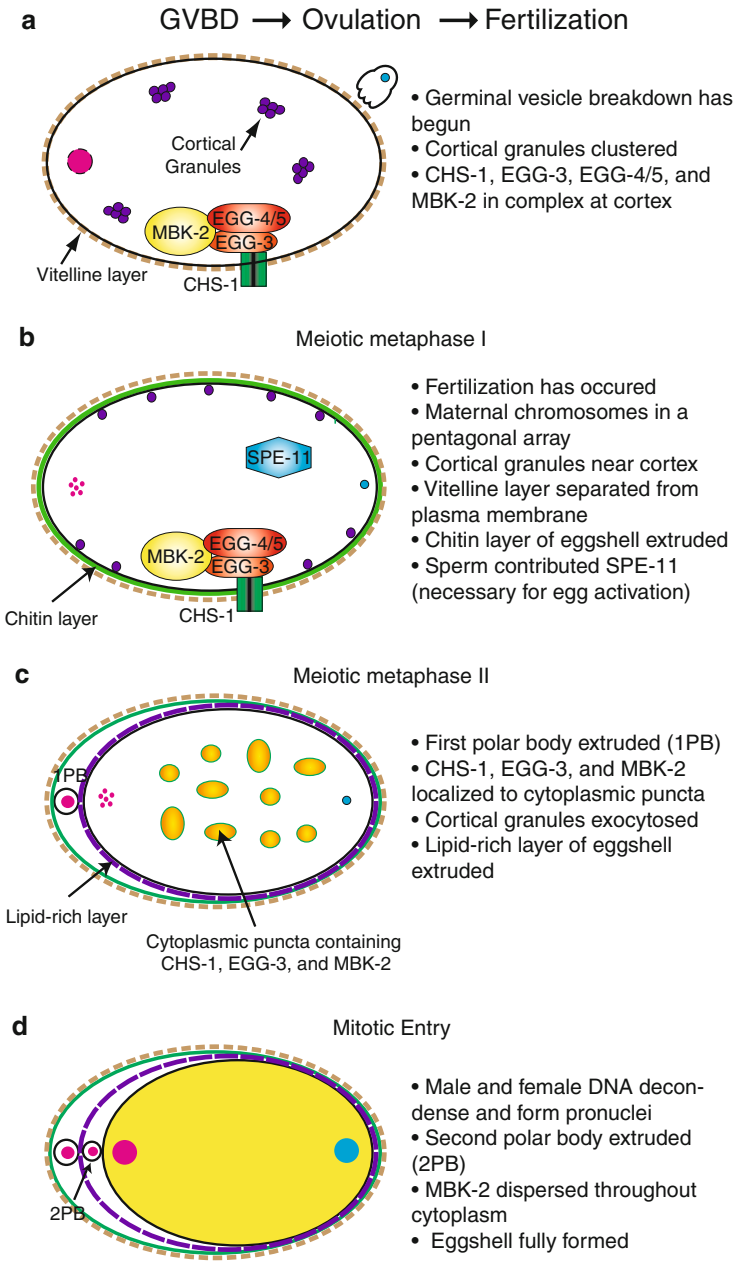

Fig. 11.3 Oocyte-to-embryo transition in *C. elegans*. During germinal vesicle breakdown (GVBD) and ovulation as well as immediately before fertilization (**a**), MBK-2, EGG-3, and EGG-4/5 are localized at the cortex through interactions with CHS-1 and cortical granules (CGs) are clustered. SPE-11 is supplied by the sperm during fertilization and is necessary for polar body extrusion and eggshell production. In meiotic metaphase I (**b**), CGs redistribute to the cortex to prepare for exo cytosis.

completion of meiosis in *C. elegans* requires APC/C; however, the role of calcium is unknown (Furuta et al. 2000; Golden et al. 2000; Siomos et al. 2001; Davis et al. 2002; Shakes et al. 2011). In *C. elegans*, activation of APC/C is fertilization independent (McNally and McNally 2005). Oocytes that are ovulated but not fertilized complete the metaphase-to-anaphase transition in meiosis I (McNally and McNally 2005). In the absence of fertilization, oocytes will progress through anaphase I but cannot transition to metaphase II, as they fail to form polar bodies or assemble meiosis II spindles (McNally and McNally 2005). Unfertilized oocytes, which lack centrioles/MTOC from sperm, undergo continuous cycles of DNA replication without cytokinesis, termed endoreplication or endo-mitotic reduplication (McNally and McNally 2005; Ward and Carrel 1979). Unfertilized oocytes are able to progress through anaphase I because cyclin B is partially degraded by APC/C, but full cyclin B degradation will not occur without fertilization (McNally and McNally 2005). Fertilization acts as the trigger to degrade cyclin B further (possibly through activation of the ZYG-11/CUL-2 E3 ubiqutin ligase) and allows for the progression into meiosis II (McNally and McNally 2005).

The rapid succession of the meiotic divisions and the requirement of fertilization for completion of the second meiotic division represent unique challenges for regulation, especially since both divisions rely on many common factors (i.e., cyclin B and APC/C). Identifying the factors that account for and regulate the two meiotic divisions is important as will be understanding how the increase in cytoplasmic calcium is originated, transmitted, and interpreted.

11.5.2 Coordination of Egg Activation Events

As noted above, unfertilized oocytes can progress through anaphase I without fertilization (McNally and McNally 2005). In addition, egg activation events such as maternal protein degradation, cytoskeletal rearrangements, and the activation of early embryogenesis cues can also occur without fertilization (McNally and McNally 2005; Pellettieri et al. 2003; Stitzel et al. 2006). However, fertilization is necessary for the proper spatial and temporal control of these events as well as the completion of meiosis, secretion of the eggshell, and proper timing of embryogenesis (McNally and McNally 2005; Parry et al. 2009; Maruyama et al. 2007; Cheng et al. 2009; Stitzel et al. 2007).

Recent studies have identified three pseudo-phosphatases, EGG-3, EGG-4, and EGG-5, that are required to coordinate egg activation events (Maruyama et al. 2007; Stitzel et al. 2007; Parry et al. 2009; Cheng et al. 2009) (Fig. 11.3). As pseudo-phosphatases, EGG-3, EGG-4, and EGG-5 contain inactive protein tyrosine phosphatase (PTP) domains that allow them to bind to phosphotyrosine residues without promoting their hydrolysis (Maruyama et al. 2007; Stitzel et al. 2007; Parry et al. 2009; Cheng et al. 2009). *egg-4* and *egg-5* are functionally redundant genes with 99.2% amino acid identity; for this reason, this pair of genes and their products is referred to as *egg-4/5* and EGG-4/5 (Parry et al. 2009). In the absence of EGG-3 and EGG-4/5, fertilized oocytes fail to extrude polar bodies, form a

proper eggshell, or yield embryos that develop properly (Maruyama et al. 2007; Parry et al. 2009).

Exactly how EGG-3 and EGG-4/5 coordinate meiotic completion, fertilization, and embryogenesis is not well understood. However, it is clear that one of the main functions of EGG-3 and EGG-4/5 is to regulate MBK-2 (minibrain kinase-2), a kinase responsible for embryonic development (Maruyama et al. 2007; Stitzel et al. 2007; Parry et al. 2009; Cheng et al. 2009; Pellettieri et al. 2003). MBK-2 is a kinase that modifies many oocyte proteins, which are essential for multiple steps of embryonic development (Pellettieri et al. 2003). Known MBK-2 substrates include MEI-1, OMA-1, OMA-2, MEX-5, and MEX-6 (see Chap. 12, Robertson and Lin 2012; Guven-Ozkan et al. 2008; Nishi et al. 2008; Pang et al. 2004; Pellettieri et al. 2003; Quintin et al. 2003). MEI-1, a homolog of the microtubule severing protein katanin p60, is necessary for meiotic spindle function, and it is signaled for degradation through phosphorylation by MBK-2 (Bowerman and Kurz 2006; Lu and Mains 2007; Stitzel et al. 2006). OMA-1 and OMA-2 prevent precocious embryogenesis by sequestering the general transcription factor component TAF-4 (TATA-binding protein associated factor 4) in the cytoplasm, thus repressing transcription in early embryonic blastomeres (Detwiler et al. 2001; Guven-Ozkan et al. 2008). Phosphorylation by MBK-2 activates OMA-1 and OMA-2 and facilitates OMA-1/2 interaction with TAF-4 (Detwiler et al. 2001; Guven-Ozkan et al. 2008). MEX-5 and MEX-6 regulate embryonic polarity (Schubert et al. 2000). Phosophorylation by MBK-2 is a prerequisite for MEX-5 and MEX-6 activation by the polo kinases PLK-1 and PLK-2 (Schubert et al. 2000; Nishi et al. 2008). The variety of MBK-2 substrates necessitates tight control of MBK-2 activity by EGG-3 and EGG-4/5 in order to prevent aberrant spatial and temporal activation of many critical factors in embryogenesis.

MBK-2 activity is controlled by EGG-3 and EGG-4/5 in two ways. First, MBK-2 is physically sequestered to the oocyte cortex in a complex with EGG-3 and EGG-4/5 that is localized to the plasma membrane through an interaction between the integral membrane protein CHS-1 and EGG-3 (Harris et al. 2000; Maruyama et al. 2007; Veronico et al. 2001; Zhang et al. 2005; Singson et al. 2008) (Fig. 11.3b). Secondly, 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 (Cheng et al. 2009).

The full release of MBK-2 inhibition is also accomplished sequentially. First, MBK-2 is phosphorylated by the MPF component CDK-1 (Cheng et al. 2009). Second, at meiotic anaphase I, EGG-3 is targeted for and degraded by the ubiquitin ligase containing APC/C. These events lead to the dissociation of the MBK-2–EGG-3–EGG-4/5 complex, thereby allowing MBK-2 to be released and to act on its substrates (Stitzel et al. 2007) (Fig. 11.3d). The regulation of MBK-2 by the EGG complex is dependent upon cell cycle progression but not fertilization. However, embryogenesis will ultimately fail because, without fertilization, the oocyte DNA will undergo endoreplication because of a number of reasons, including the inability to form polar bodies, assemble meiosis II spindles, or degrade cyclin B further (Maruyama et al. 2007; Stitzel et al. 2007; Cheng et al. 2009; Parry et al. 2009).

Unlike other factors in this cascade, EGG-3 and EGG-4/5 regulate fertilization-independent events, including MBK-2 activity, as well as fertilization-dependent

events, including actin cap formation, polar body extrusion, and secretion of the eggshell (Maruyama et al. 2007; Stitzel et al. 2007; Cheng et al. 2009; Parry et al. 2009; McNally and McNally 2005). Future directions include the identification of additional regulatory elements necessary for the coordination of egg activation events to fertilization, as well as signal transduction pathways that promote meiotic completion and eggshell exocytosis.

11.5.3 The Role of Sperm in Egg Activation and Embryogenesis

Fertilization provides multiple sperm components that are necessary to coordinate meiotic progression and egg activation and to ensure proper embryogenesis. Each haploid sperm has a single pair of centrioles and, after fertilization, these centrioles duplicate and ultimately generate the two active centrosomes that establish the two poles of the embryo's first mitotic spindle (Albertson 1984; Dammermann et al. 2008; Pelletier et al. 2006; Albertson and Thomson 1993). *C. elegans* sperm also supply the centrosome that specifies the anterior–posterior axis (O'Connell et al. 2000; Cowan and Hyman 2004; Hamill et al. 2002; Wallenfang and Seydoux 2000). In addition to providing organelles, sperm provide proteins necessary for egg activation (SPE-11) and embryogenesis (CYK-4) (Browning and Strome 1996; Jenkins et al. 2006).

spe-11 is predicted to encode a sperm-specific novel cytoplasmic protein that exhibits a perinuclear localization pattern (Browning and Strome 1996). Sperm produced by *spe-11* mutants are capable of fertilizing oocytes but are unable to activate them, and oocytes fertilized by *spe-11* mutants have defects in meiosis (spindle orientation and cytokinesis) and embryogenesis, do not secrete an eggshell, and do not produce polar bodies (Hill et al. 1989; McNally and McNally 2005). *spe-11* paternal-effect lethal embryos can assemble the meiosis I spindle; however, the anaphase I chromosomes do not segregate and instead collapse back together and individualize (McNally and McNally 2005). Despite the defect in chromosome segregation at anaphase I, *spe-11* embryos are able to form a meiosis II spindle and undergo normal anaphase II separation, ultimately producing two female pronuclei (McNally and McNally 2005). The assembly of a meiosis II spindle in *spe-11* embryos indicates a role for sperm contributions to meiosis spindle assembly and cytokinesis, as unfertilized oocytes do not assemble a meiosis II spindle (McNally and McNally 2005). Using transgenes to ectopically supply *spe-11* gene function through maternal expression in oocytes, rescues *spe-11* function suggesting it acts when it is incorporated into the embryo after fertilization (Browning and Strome 1996).

In *C. elegans*, the sperm-enriched *cyk-4* gene product, a Rho guanosine triposphatase (GTPase)-activating protein (GAP), is necessary for establishing anterior–posterior polarity (Portereiko et al. 2004; Portereiko and Mango 2001; Jenkins et al. 2006). CYK-4 is enriched in the MO of sperm and is detected in the posterior cortex of the one-cell embryo after fertilization (Jenkins et al. 2006). CYK-4, along

with the small GTPase RhoA (Ras homolog gene family, member A) and ECT-2, a RhoA guanine nucleotide-exchange factor, modulate myosin light-chain activity to create an actomyosin gradient (Jenkins et al. 2006). The actomyosin gradient is necessary to establish the anterior domain of the embryo and properly localizes the polarity proteins PAR-3 and PAR-6 (Jenkins et al. 2006). Additional sperm proteins that regulate and coordinate egg activation are yet to be identified, but it is clear that sperm entry plays an important role in these processes (McNally and McNally 2005).

11.5.4 Additional Sperm Contributions

Paternal mitochondria: When sperm–egg fusion occurs, paternal mitochondria in the sperm are incorporated into the oocyte cytoplasm. To ensure that the maternal mitochondrial DNA (mtDNA) is inherited in *C. elegans*, paternal mitochondria are eliminated by autophagy in the oocyte (Sato and Sato 2011; Al Rawi et al. 2011). Autophagy allows for the degradation of cytoplasmic proteins and organelles by sequestering them to autophagosomes and eventually lysosomes where they are broken down (Nakatogawa et al. 2009; Sato and Sato 2011; Mizushima 2007; Xie and Klionsky 2007; Al Rawi et al. 2011). Two recent reports found that autophagosomes engulf paternal mitochondria and that the mitochondria are degraded during early embryogenesis (Sato and Sato 2011; Al Rawi et al. 2011). Both reports also found that *lgg-1*, a gene necessary for autophagosome formation, was necessary for clearance of paternal mitochondria (Sato and Sato 2011; Al Rawi et al. 2011). LGG-1 and LGG-2 are orthologs of *S. cerevisae* Atg8 and mammalian LC3 (Nakatogawa et al. 2009; Mizushima 2007; Xie and Klionsky 2007; Alberti et al. 2010). *lgg-1* null mutant homozygotes were able to produce fertilized eggs; however, 36% of the eggs were unable to hatch and 59% of the embryos that did hatch died at the L1 larval stage (Sato and Sato 2011). These findings provide evidence that paternal mitochondria are actively degraded and that degradation, not dilution of paternal mtDNA by an excess of maternal mtDNA, is the primary mode of ensuring maternal mtDNA inheritance (Sato and Sato 2011; Al Rawi et al. 2011). This paradigm may also hold true in mammals, as mouse autophagy has been shown to be necessary for preimplantation development (Tsukamoto et al. 2008; Sato and Sato 2011). Moreover, the paternal mitochondria in mouse sperm, which are located in the midpiece of the flagellum, stain positively with an anti-LC3 antibody after fertilization indicating that autophagy plays a role in the degradation of paternal mitochondria (Al Rawi et al. 2011).

peel-1/zeel-1 element: Sperm also deliver another other component that is actually toxic if the proper antidote is not present in the embryo (Seidel et al. 2011; Seidel et al. 2008). The *C. elegans* gene *peel-1* (*paternal effect epistatic embryonic lethal-1*) encodes a four-pass transmembrane protein that localizes to MOs in spermatids and is delivered to the embryo during fertilization (Seidel et al. 2011). PEEL-1 is a potent toxin that disrupts the development of muscle and epidermal tissue during late embryogenesis (Seidel et al. 2011). To neutralize the toxicity of

PEEL-1 the embryo transiently expresses *zeel-1* (*zygotic epistatic embryonic lethal-1*) (Seidel et al. 2011). ZEEL-1 is six-pass transmembrane protein, the transmembrane domain of which is necessary for its function as an antidote to PEEL-1 (Seidel et al. 2011). The cause of PEEL-1 toxicity and the mechanism by which ZEEL-1 mitigates PEEL-1 toxicity are unknown (Seidel et al. 2011). Seidel et al. hypothesize that ZEEL-1 could promote the degradation of PEEL-1 or prevent PEEL-1 from binding to its target (Seidel et al. 2011).

Despite its deleterious effects on zygotic development, *peel-1* maintains its place in the genome through an inseparable association with its antidote *zeel-1* (Seidel et al. 2008, 2011). *peel-1* and *zeel-1* are adjacent genes and cannot be separated by homologous recombination; they are referred to as the *peel-1/zeel-1* element (Seidel et al. 2008). The *peel-1/zeel-1* element persists because, in *C. elegans*, homozygosity, not heterozygosity, is most common as a result of a low number of males in the population (Seidel et al. 2011). The evolutionary origins of the *peel-1/zeel-1* element are unknown (Seidel et al. 2008, 2011). The *peel-1/zeel-1* element is intriguing from the perspective of fertilization and embryogenesis because it may provide insight into how sperm-supplied factors are interpreted by and influence zygotic transcription and embryonic development.

11.6 The Eggshell

11.6.1 Eggshell Structure

The chitin eggshell is deposited and assembled after fertilization and is essential for *C. elegans* embryogenesis (Rappleye et al. 1999; Bembenek et al. 2007; McNally and McNally 2005). Structural support provided by the eggshell allows for the completion of meiosis, polar body extrusion, and establishment of embryo polarity, and it also forms an osmotic barrier that protects the developing embryo from osmotic changes (Rappleye et al. 1999; Siomos et al. 2001; Kaitna et al. 2000; McNally and McNally 2005). The *C. elegans* eggshell is made of three layers: the outer vitelline layer, the middle chitin layer, and the inner lipid-rich layer (Rappleye et al. 1999). At the time of fertilization, the outer vitelline layer that surrounds the developing oocyte begins to separate from the plasma membrane as the chitin layer is formed (Rappleye et al. 1999; Bembenek et al. 2007). The middle chitin layer is formed during metaphase I and provides the eggshell with its mechanical strength (Rappleye et al. 1999; Maruyama et al. 2007). Chitin is formed through the polymerization of UDP-*N*-acteylglucosamine (UDP-GlcNAc), which is catalyzed by the oocyte membrane protein CHS-1, and the chitin-binding protein CBD-1 is necessary for the formation of a continuous chitin layer (Rappleye et al. 1999; Zhang et al. 2005; Maruyama et al. 2007; Johnston et al. 2010). The lipid-rich inner layer of the eggshell begins to form during anaphase I and provides osmotic and mechanical strength (Rappleye et al. 1999; Bembenek et al. 2007; Sato et al. 2008). The eggshell performs diverse roles for the *C. elegans* embryo and, without proper eggshell deposition,

the embryo is not able to divide properly and is susceptible to many mechanical and osmotic stresses (Rappleye et al. 1999; McNally and McNally 2005).

11.6.2 Cortical Granule Exocytosis

Cortical granules (CGs) are Golgi-derived secretory vesicles that are stored at the cortex of oocytes (Horner and Wolfner 2008). In *C. elegans*, CGs are necessary for proper eggshell formation (Bembenek et al. 2007; Sato et al. 2008). CG exocytosis occurs during anaphase I in response to APC/C activation—not fertilization—and requires a number of cell cycle components, including the *C. elegans* ortholog separase-1 SEP-1 as well as the small GTPase RAB-11.1 and the target-SNARE SYN-4 (Bembenek et al. 2007; Sato et al. 2008). CGs contain chondroitin proteoglycans and, upon exocytosis, these chondroitin proteoglycans are released to the extracellular space surrounding the embryo (Hwang and Horvitz 2002; Bembenek et al. 2007; Sato et al. 2008).

The role of chondroitin proteoglycans in eggshell formation is unclear. Two chondroitin proteoglycans, CPG-1 and CPG-2, are known to bind chitin, thus suggesting a role for CPG-1 and CPG-2 in the formation or maintenance of the chitin layer (Bembenek et al. 2007; Olson et al. 2006). However, chondroitin proteoglycans are present in CGs and are not exocytosed until after the chitin layer has formed during anaphase I, implicating a role for chondroitin proteoglycans in the formation of the lipid-rich layer (Bembenek et al. 2007; Sato et al. 2008). Determining the precise localization and role for chondroitin proteoglycans in eggshell formation will aid in understanding how the eggshell provides osmotic and mechanical strength.

11.6.3 Membrane Block to Polyspermy

C. elegans have a robust block to polyspermy (Ward and Carrel 1979). In addition to the ovulated oocyte, the spermatheca also contains multiple sperm in an extremely tight space (Ward and Carrel 1979). Despite contact with multiple sperm, *C. elegans* oocytes are only fertilized by a single sperm (Ward and Carrel 1979). Once fertilization occurs, the oocyte prevents additional sperm from entering (Ward and Carrel 1979). Recently, embryos with multiple fused sperm have been described; however, these polyspermic events occur in a very small percentage of the embryos examined (Parry et al. 2009; Johnston et al. 2010). In *C. elegans*, polyspermy has been observed after the depletion of *chs-1, gna-2*, or *egg-4/5* (Parry et al. 2009; Johnston et al. 2010). As discussed earlier, CHS-1 is necessary for the polymerization of the chitin component UDP-*N*-acteylglucosamine (Zhang et al. 2005). *gna-2* encodes a GLD-regulated glucosamine-6-P N acetyltransferase that supplies UDP-*N*-acetyl glucosamine for chitin biosynthesis (Johnston et al. 2006). The role of *chs-1* and *gna-2* in chitin synthesis indicates a potential role for chitin in the block to polyspermy.

In other organisms, cortical granule exocytosis is known to contribute to polyspermy blocks (Wessel et al. 2001). The connection between CG exocytosis and the block to polyspermy in *C. elegans* is unclear, but evidence from *egg-4/5* embryos indicates that CG exocytosis does not act in the block to polyspermy (Parry et al. 2009). *egg-4/5* embryos seem to have normal CG exocytosis but polyspermic embryos can still be detected (Parry et al. 2009). However, since the majority of *egg-4/5* embryos are not polyspermic, there may be other contributions to the block to polyspermy or more subtle details about CG exocytosis that are not yet understood (Parry et al. 2009).

11.7 Conclusion

Fertilization in *C. elegans* involves a vast number of cell processes that must be executed with high fidelity so as to ensure the successful propagation of the species. First, *C. elegans* must produce haploid gametes with extraordinary differences in morphology and function to ensure they are able to locate one another. When the gametes finally do meet, they will only fuse if they are from the correct species despite the fact sperm from other species look similar and can enter the reproductive tract and migrate to the site of fertilization. Finally, the oocyte must only allow one sperm to enter, sort out which components of the sperm are necessary for embryogenesis, and then initiate embryonic development. Many questions remain about how fertilization is regulated in *C. elegans*. What are the signal transduction pathways that allow sperm to respond to prostaglandin signals from the oocyte? How is the developmental program regulated during the oocyte-to-embryo transition? How does the eggshell form? How is the rapid block to polyspermy triggered? Finally, the central question remains—how is sperm–egg fusion mediated?

The genetic and molecular basis of fertilization is not well understood. The study of fertilization in *C. elegans* provides an excellent opportunity to understand the molecular functions of the genes that are necessary for fertilization and to discover previously unpredicted genes necessary for this fundamental process of sexual reproduction. The study of mammalian fertilization has been difficult, partially because the ability to obtain and successfully manipulate gametes is challenging. *C. elegans* is an emerging model system to study fertilization. Many parallels have already been identified between cellular processes in *C. elegans* and other species. The molecular and genetic techniques available for use in *C. elegans* allow for relatively easy gene discovery. It is possible that many of the genes that are necessary for fertilization in *C. elegans* will also be important in mammalian systems because of the conserved features of sexual reproduction.

Acknowledgments We thank the anonymous reviewers and Drs. Tim Schedl, Diane Shakes, and David Greenstein for their helpful comments and suggestions. We also thank the members of the Singson laboratory, Roopa Kalyanaraman Marcello, and Dr. Sara K. Olson for critical comments and editorial assistance. This work was supported by a grant from the National Institutes of Health to AS [R01HD054681-06]. MRM is a UMDNJ-RWJMS IRACDA Postdoctoral Fellow supported by NIH Grant GM093854.

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