Published in final edited form as:

Results Probl Cell Differ. 2011 ; 53: 135–151. doi:10.1007/978-3-642-19065-0_7.

EGG Molecules Couple the Oocyte-to-Embryo Transition with Cell Cycle Progression

Jean M. Parry and Andrew Singson*

Waksman Institute and Department of Genetics, Rutgers University, Piscataway, New Jersey 08854 USA

Abstract

The oocyte-to-embryo transition is a precisely coordinated process in which an oocyte become fertilized and transitions to an embryonic program of events. The molecules involved in this process have not been well studied. Recently a group of interacting molecules in *C. elegans* have been described as coordinating the oocyte-to-embryo transition with the advancement of the cell cycle. Genes *egg-3*, *egg-4*, and *egg-5*, represent a small class of regulatory molecules known as protein-tyrosine phosphatase-like proteins, which can bind phosphorylated substrates and act as scaffolding molecules or inhibitors. These genes are responsible for coupling the movements and activities of regulatory kinase *mbk-2* with advancement of the cell cycle during the oocyte-to-embryo transition.

1 Introduction

1.1 *C. elegans*, a model organism

During the 1960's, Dr. Sydney Brenner, Nobel Laureate, began searching for a new model organism. He put forward the free-living, non-parasitic nematode, *Caenorhabditis elegans* as an ideal model (Brenner 1974). The worms are tiny, only 1mm fully grown, reproductively fecund, producing approximately 300 progeny, and simple to culture in bulk as they feed on *E. coli* bacteria and so can be colonized on Petri dishes (Figure 1). They possess a transparent cuticle that allows live imaging of their internal processes, through both light and fluorescent microscopy. Transgenic animals are relatively easy to produce, by injection of DNA into the germline, by use of the MOS transposon recombination system, or through microparticle bombardment that can be successfully used to generate transgenic arrays (Granger et al. 2004; Kadandale et al. 2009; Praitis 2006). By this method, GFP and RFP tagged proteins can be introduced and expressed under the direction of either their own, or a tissue specific promoter. Quite uniquely, the lineage of every somatic cell in the organism, both male and hermaphrodite, can be traced from fertilization to adult (Sulston 1977). In addition, *C. elegans* represents the first multicellular organism to have a completely sequenced genome, and now the genomes of several other species of *Caenorhabditis* and other nematodes have subsequently been published, making identification and study of orthologues easy (Coghlan et al. 2006; Sulston 1977).

Much pioneering work has been completed in the humble *C. elegans*, including seminal work in apoptosis and cell death, RNAi (RNA mediated interference), and fluorescent protein tagging (GFP) (Chalfie et al. 1994; Fire 1998; Hedgecock et al. 1983). *C. elegans* also presents an excellent model for the examination of mitosis and meiosis (Greenstein 2005; Kimble 2005; L'Hernault 2006).

*singson@waksman.rutgers.edu.

1.2 *C. elegans* Reproductive Tract and Gametes

As a species, *C. elegans* exists primarily as hermaphrodites, with males arising approximately 1 in 1000 births from non-disjunction of the X chromosome. Hermaphrodites are self-fertile, while males produce only sperm and are capable of fertilizing hermaphrodites to produce outcross progeny (Figure 2A, B) (Ward and Carrel 1979). The hermaphrodite germline fills the body cavity in a bi-lobed tube. The germline begins with two DTC's (distal tip cells) one on the anterior and posterior dorsal axis (Austin and Kimble 1987). The DTC's provide a niche for the germ cells, which proliferate from dedicated germ line progenitor stem cells (Kimble 2005). Distal germ cells are maintained at mitosis by LAG-2, a protein ligand secreted by the DTC (Hubbard and Greenstein 2000; Kimble 2005). Regulation of cell cycle in the *C. elegans* germ cells is necessarily precise, and can be clearly visualized by DNA staining in fixed specimens or by live imaging of fluorescent-tagged histones. Distal germ cells progress in syncytium with one another in an organ called the rachis. As germ cell nuclei begin to differentiate, the germ cells begin to enter prophase of meiosis I in the transition zone which demarcates the change from mitosis to meiosis (Hubbard and Greenstein 2000; Kimble 2005). Well-defined regions of the rachis correspond sequentially to pachytene, diplotene, and diakinesis of prophase I (Hubbard and Greenstein 2000). *C. elegans* hermaphrodites produce first sperm during their last larval phase of development, and then switch permanently to the production of oocytes. The amoeboid spermatocytes are stored in each of two spermathecas, one corresponding to the anterior and posterior gonad arms (Figure 2) (L'Hernault 2006). As oocytes approach the bend of the gonad arm they individuate from the rachis and begin to accumulate yolk. The male of the species produces only sperm, beginning in its last larval stage. Male germ cells develop in a single gonad arm extending from a single distal tip cell to the seminal vesicle (Klass et al. 1976). The male spermatids are stored unactivated, as round non-motile spermatids, and introduced to the hermaphrodite through mating structures in the tail region. After their introduction through the hermaphrodite vulva, male-derived sperm quickly acquire motility, move to the spermatheca, and out-compete hermaphroditic self-sperm for fertilization of the oocytes (Ward and Carrel 1979).

In the *C. elegans* hermaphrodite the movement and development of sperm and oocytes are precisely coordinated. Major sperm protein (MSP) is a core component of the sperm cytoskeleton; the protein is also secreted as a signal to both oocytes and the somatic germline (Harris et al. 2006; Kuwabara 2003). As oocytes progress sequentially closer to the spermatheca, they are often referred to numerically by position with the oocyte closest to the spermatheca referred to as -1 (Figure 2 and 3). Oocytes receive the MSP signal from sperm such that the -1 oocyte is stimulated to undergo nuclear-envelope breakdown (NEBD) and cytoskeletal rearrangement in preparation for ovulation (Harris et al. 2006). The oocyte and sperm also signal to the overlying somatic sheath cells, a type of smooth muscle covering the oviduct, the spermathecal valve dilates and forces the spermatheca to engulf the -1 oocyte (Figure 2 and 3). Thus the -1 oocyte is ovulated, an event which occurs approximately every 23 minutes when sperm are abundant (McCarter et al. 1999; McNally and McNally 2005). Hermaphrodites produce a fixed number of sperm during the last larval stage, in the absence of sperm the process of ovulation drops off dramatically occurring approximately once every ten hours (McCarter et al. 1999). However, introduction of sperm through fertilization by a male or artificial insemination causes resumption of robust ovulation rates and fertilization (LaMunyon and Ward 1994). Returning to our discussion of the cell cycle, the -1 oocyte undergoes NEBD and simultaneously reenters the meiotic cell cycle at metaphase I of meiosis I approximately five minutes before ovulation will occur (Figure 3) (McCarter et al. 1999; McNally and McNally 2005).

The first ovulation events push the spermatids from the oviduct into the spermatheca (Figure 2) (L'Hernault 2006). As the subsequent oocytes enter the spermatheca they are surrounded

by the amoeboid *C. elegans* spermatozoa. Molecules including *spe-9* on the surface of the spermatozoa and *egg-1* and *egg-2* on the egg are necessary for sperm/egg interaction and sperm entry (Kadandale et al. 2005; Putiri et al. 2004; Singson et al. 1998). When sperm are abundant, fertilization occurs quickly, and the newly fertilized embryo exits the spermatheca to complete embryogenesis.

In wild-type hermaphrodites, six pairs of sister chromatids, corresponding to the six *C. elegans* chromosomes, undergo meiosis I and extrude a polar body containing the excess genetic material (Figure 3) (McNally and McNally 2005). Then six pairs of chromatids undergo the meiosis II division and extrude a second polar body. Finally the female chromosomes decondense at the anterior pole of the embryo to form the female pronucleus, while on the posterior pole the male chromosomes decondense to form the male pronucleus. Both pronuclei will eventually fuse to form a single diploid nucleus, and the mitotic divisions of embryonic development will begin.

The processes that occur just prior to fertilization and before the start of embryogenesis are referred to collectively as the oocyte-to-embryo transition (Figure 3) (Govindan and Greenstein 2007; Yamamoto et al. 2006). During these critical developmental stages the oocyte completes a maternally provided program of development and switches to an embryonic one. This process occurs in many organisms, and yet the molecules involved in this process have not been well studied. It has long been recognized that the oocyte-to-embryo transition occurs in the absence of transcription (Evsikov and Marin de Evsikova 2009). Thus cellular changes rely on translation of stored maternally provided mRNA's, modification of maternally provided proteins (adenylation, phosphorylation etc.), and the targeted destruction of maternal proteins (Evsikov and Marin de Evsikova 2009). Yet, what mechanisms drive these changes? Complex machinery must coordinate a common set of events including resumption of meiosis and advancement through the cell cycle, the block to polyspermy, cytoskeletal rearrangements, the cortical granule reaction, apposition of the male and female pronuclei followed by their fusion in many non-mammalian species and establishment of embryonic polarity (Figure 3) (Ducibella 2008). In mammals, the most studied regulatory mechanism involves the oscillation of intracellular calcium levels in the early embryo.

C. elegans presents an excellent model organism for parsing the molecular underpinnings of the oocyte-to-embryo transition. Every stage from undifferentiated stem cell, to fertilization competent egg, to developed embryo can be viewed progressively *in vivo* under light or fluorescence microscopy (Figure 2C). Many fluorescent and antigenic markers are available for cellular compartments and components, including nuclear, plasma membrane, Golgi, and endosomal (Hadwiger et al. 2010; Sato et al. 2006). Genetic deletion mutants are freely available for study, in addition to the ease of RNAi in the germline. A primary strength of RNAi in the *C. elegans* germline is the ability to perform RNAi on larval stage hermaphrodites, knocking down their maternal complement of protein, and examining their germline function at adult stages. This allows for the investigation of many genes that would otherwise be embryonic lethal.

In addition to carrying out the same basic developmental processes as mammals and other organisms during the oocyte-to-embryo transition, *C. elegans* undergo the resumption of meiosis, cytoskeletal rearrangement, ovulation, fertilization, the block to polyspermy, completion of meiosis including the extrusion of two polar bodies, extrusion of a chitinous, trilamellar eggshell, formation of a female and male pronucleus, fusion of the two pronuclei, and the initiation of the mitotic divisions (Figure 3) (McNally and McNally 2005). The genetic requirements for many of these processes have been partially explored. Much is known about how the *C. elegans* oocyte is signaled to undergo maturation and ovulation,

fertilization, how meiotic and mitotic spindles are formed, how the chitinous eggshell is formed, and how the embryonic polarity is initially formed, but these subjects are well reviewed elsewhere (Gonczy and Rose 2005; Greenstein 2005; Johnston et al. 2006; Oegema and Hyman 2006; Sundaram 2006). The subject of our research has been the coupling of the progression of the oocyte-to-embryo transition to the entry of sperm, and to the advancement of the cell cycle. Towards this end, we have elucidated several members of a gene module required for egg activation, and the oocyte-to-embryo transition.

2 The oocyte-to-embryo transition in *C. elegans*: EGG-3

The first lynchpin of the oocyte-to-embryo transition gene module is *egg-3* (F44F4.2), a gene required for maternal effect viability (Maruyama et al. 2007; Stitzel et al. 2007). This gene was identified in a screen of fertility genes performed by the Sugimoto lab (Maeda et al. 2001; Maruyama et al. 2007). A hallmark of loss of function mutant genes required for function of sperm, late oocytes, or early embryos is the production of many unshelled, non-developing oocytes (Singson et al. 2008). Mutations in genes required for sperm activation (spermiogenesis) or sperm/oocyte fusion prevents fertilization from occurring. In this situation, when observing DAPI stained embryos newly released from the spermatheca, the oocyte nuclei are visible, but the corresponding sperm nuclei are absent. However, sperm entry occurred in *egg-3* embryos, and sperm nuclei were found localized to the presumptive posterior of the embryo as in a wild type embryo. Studies of *egg-3* were carried out in tandem with a knockout supplied by National Bioresource Project for the Experimental Animal “Nematode *C. elegans*” (NBP) Japan *egg-3* (*tm1191*), and with knockdown by soaking based RNAi. Results in all cases were identical. Knockdown of *egg-3* resulted in oocyte specific lethality (Maruyama et al. 2007). Spermatogenesis and oogenesis were phenotypically normal, and no defects were visible until the one-cell stage of the embryo.

In *egg-3* mutant hermaphrodites, the first visible defects occur just after fertilization. A severe defect is observed in *egg-3* when the embryo fails to extrude a polar body at the conclusion of meiosis I (Maruyama et al. 2007). This leads to an abnormal embryo in which 12 pairs of sister chromatids undergo the division of meiosis II, before failing to extrude the second polar body. The female chromosomes eventually decondense to form a female pronucleus, which will fuse with the male pronucleus. However, as with other fertility defects, the DNA undergoes constant rounds of mitosis without cytokinesis leading to the formation of endomitotic embryos (Maruyama et al. 2007).

As previously mentioned, a highly visible marker for hermaphrodite fertility is the presence of the chitin containing eggshell. In *C. elegans* the eggshell is trilamellar with a thin vitelline layer facing outside, a chitin containing center layer, and an interior proteolipid layer, which will eventually house the first polar body (Johnston et al. 2006). In wild type, eggshell deposition begins just after fertilization, with the chitin layer being robustly detectable during meiosis I (Figure 3). Eggshell deposition fails both in sperm and oocyte mutants, including mutants causing deficiencies during the oocyte-to-embryo transition such as *egg-3*, which lacks a detectable chitin layer at the conclusion of meiosis I (Johnston et al. 2006; Maruyama et al. 2007; Singson et al. 1998).

The third notable defect in *egg-3* mutants occurs during cytoskeletal rearrangement after fertilization. In wild-type embryos, the actin cytoskeleton rearranges after fertilization such that a thick focal cap of actin forms under the plasma membrane directly over the site of sperm entry (Maruyama et al. 2007). Subsequently in wild-type embryos, the actin cap disperses throughout the posterior half of the embryo. In *egg-3* mutant embryos, an actin cap forms as normal at the site of sperm entry. However, the actin filaments disperse abnormally

throughout both posterior and anterior of the embryo (Maruyama et al. 2007). The consequences of this aberrant dispersal to the embryo are unknown.

The protein structure of EGG-3 was analyzed for further clues to its activity, and was found to contain the sequence of a protein-tyrosine phosphatase (Maruyama et al. 2007; Stitzel et al. 2007). However, protein-tyrosine phosphatases require the presence of three key amino acid residues for their catalytic activity, an aspartate, a glutamate, and a cysteine, and upon closer examination the EGG-3 protein lacked two of these residues: the aspartate and the glutamate. This sequence change indicated that the EGG-3 protein was most likely catalytically inactive, but should retain its ability to bind phosphorylated substrates. This made EGG-3 a member of a growing group of proteins referred to as protein-tyrosine phosphate-like (PTPL), or as antiphosphatases (pseudophosphatases). Previously examined molecules of this class include the Pasticcino2 gene in Arabidopsis, which is required for the regulation of cell cycle through direct interaction with cyclin dependent kinase (CDK), mouse and human genes such as *Ptpla* which is required for development, and another *C. elegans* gene, *sdf-9* which is involved in dauer formation (Bellec and Faure 2002; Da Costa et al. 2006; Jensen et al. 2007; Uwanogho et al. 1999). The structure of PTPL domains suggests two intriguing possibilities, for regulating the activity of other genes. In the first scenario, the PTPL containing protein could bind to a phosphorylated target protein, and then hold that protein as part of a complex with other proteins acting as a molecular scaffold (Govindan and Greenstein 2007). In the second scenario, the PTPL containing protein could bind to a phosphorylated target protein and then limit the target proteins access to active phosphatases acting as a competitive inhibitor. Determination of EGG-3's role as either scaffold or inhibitor would require further molecular analysis.

In order to examine the localization of the *egg-3* protein, GFP and RFP (mcherry) fusion tagged proteins were generated by microparticle bombardment. Fusions for tissue specific expression were driven by the *pie-1* promoter, which expresses in the germline of *C. elegans* hermaphrodites. It was determined that EGG-3 localizes to the cytoplasm directly below the plasma membrane, often referred to as the cortex, of developing oocytes (Figure 4) (Maruyama et al. 2007; Stitzel et al. 2007). After fertilization, and during meiosis I, the protein undergoes a dynamic shift in localization, moving to cytoplasmic punta and then being degraded (Figure 4) (Maruyama et al. 2007; Stitzel et al. 2007). The dynamic spatial localization of EGG-3 indicated that the proteins purpose might shift during meiosis I.

In order to determine the epistatic relationship between *egg-3* and the developmental pathway, we examined its interactions with other genes either involved in the oocyte-to-embryo transition, or with similar patterns of localization. The associations between EGG-3 and many other genes were analyzed, with the predominant associations occurring with CHS-1, and MBK-2.

Gene *chs-1* (T25G3.2) was selected for analysis due to its integral role in the extrusion of the chitinous eggshell after fertilization, a process that fails in *egg-3* mutants (Johnston et al. 2006; Zhang et al. 2005). The gene encodes a large multipass transmembrane protein, chitin synthase, which is responsible for catalyzing the production of chitin from UDP-N-acetylglucosamine (UDP-GlcNAc). This chitin is required as a critical component of the center layer of the trilamellate eggshell. In addition, to the requirement for CHS-1 during eggshell production, knockdown of CHS-1 through gene specific RNAi lead to defects during meiosis and polar body formation, identical to those seen in *egg-3* mutant hermaphrodites (Johnston et al. 2006).

Also chosen for close examination was gene *mbk-2*, a kinase important for degrading maternal proteins during the oocyte-to-embryo transition and known to have an identical

pattern of protein localization to EGG-3 (Raich et al., 2003). In an example of coordinate regulation, *mbk-2* is responsible for phosphorylating and marking for degradation *mei-1*, *oma-1* and *oma-2*, and *mex-5* and *mex-6* proteins important for early post-fertilization processes, which must be destroyed before the transition to later embryonic development (Nishi 2005; Pellettieri et al. 2003; Quintin et al. 2003; Stitzel et al. 2007). The *mei-1* gene encodes an orthologue of the catalytic subunit of katanin, which acts as a microtubule severing protein during meiosis but not mitosis. Thus the MEI-1 protein has a function tightly linked to the oocyte-to-embryo transition, as its function is required during the completion of meiosis, but is deleterious to the functioning of the mitotic cycles, which mark the beginning of embryonic development (Pellettieri et al. 2003; Quintin et al. 2003; Yang et al. 2003). Similarly, the *oma-1* and *oma-2* genes, which are redundant, encode zinc finger proteins, which are necessary for setting up correct cell fate specification during early embryogenesis (Detwiler et al. 2001; Lin 2003; Pellettieri et al. 2003). Persistence of the OMA-1 or OMA-2 proteins past the first mitotic division lead to adoption of the incorrect cell fates and embryonic lethality, again making the precise and timely degradation of OMA-1 and OMA-2 critical to early embryonic development (Lin 2003). Finally, the MEX-5 and MEX-6 proteins are partially redundant proteins required for polarization at the one cell stage of the embryo. The MEX-5 and MEX-6 proteins are localized to the anterior cytoplasm of the embryo where they control the localization of other maternal proteins such as PIE-1, POS-1, and MEX-1 (Nishi et al. 2008; Schubert et al. 2000). Therefore, the highly precise temporal and spatial action of *mbk-2* is necessary to progression through the oocyte-to-embryo transition. However, knockdown of *mbk-2* leads to less severe embryonic defects than depletion of *egg-3* or *chs-1*. In the absence of *mbk-2*, the embryo completes a phenotypically normal meiosis and extrudes the chitin-containing eggshell. In *mbk-2* hermaphrodites, embryos die from the combined defects caused by aberrant functioning of MEI-1, OMA-1, OMA-2, MEX-5, and MEX-6 (Stitzel et al. 2007).

The spatial and temporal localizations of the three proteins, EGG-3, CHS-1, and MBK-2 were examined using integrated GFP or RFP tagged fusion proteins, and found to be identical during late oogenesis and early embryogenesis, indicating that a direct interaction between the proteins was possible (Maruyama et al. 2007; Stitzel et al. 2007). Epistasis analysis was performed in order to elucidate whether the localizations of the three proteins were dependent on one another. Using gene specific RNAi, it was determined that the localizations of EGG-3 and CHS-1 fusion proteins were interdependent, such that knocking down EGG-3 by RNAi lead to aberrant localization of CHS-1 and vice versa (Maruyama et al. 2007). The localization of MBK-2 was dependent on the presence of both EGG-3 and CHS-1 (Maruyama et al. 2007). While neither EGG-3 nor CHS-1 localizations were affected by the absence of MBK-2 (Maruyama et al. 2007). This result was anticipated because knockdown of MBK-2 leads to less severe defects than knockdown of EGG-3 or CHS-1, as meiosis is completed as in wild type, and the chitin containing eggshell is formed. Concurrently, it was shown that EGG-3 and MBK-2 have a direct physical interaction by co-immunoprecipitation of either protein (Stitzel et al. 2007). However, the co-dependence of these proteins does not speak to their precise dynamic localization, which in the case of MBK-2 is critical for its temporal catalytic activity. Therefore, it was critical to determine the trigger for dynamic relocation of these proteins. A double tagged strain, containing RFP:EGG-3 and GFP:HIS (histone H2B) was constructed, and live imaging with this strain showed that the redistribution of EGG-3, and by extension CHS-1, and MBK-2 occurred somewhere between metaphase I and anaphase I of meiosis I (Maruyama et al. 2007). In order to verify the timing of this event, anaphase promoting complex (APC) component *mat-1* was depleted by RNAi in GFP:EGG-3, GFP:CHS-1, and GFP:MBK-2 backgrounds (Maruyama et al. 2007; Stitzel et al. 2007). Not surprisingly, abrogation of an APC subunit arrested the fertilized embryos at metaphase of meiosis I, and caused retention of all three proteins at the cortex. Therefore, the dynamic redistribution of EGG-3, CHS-1, and MBK-2

relies on the advancement of the cell cycle (Maruyama et al. 2007; Stitzel et al. 2007). Conversely, depletion of EGG-3, CHS-1, or MBK-2 did not have an effect on the depletion of GFP:CYB-1, which is an integral part of the machinery regulating entry into meiosis I. This indicated that EGG-3, CHS-1, and MBK-2 were not required for cell cycle progression during meiosis.

Both EGG-3 and CHS-1 are required for the localization of MBK-2 during development, therefore it remained to determine the precise molecular interactions between the proteins. It was determined that both the EGG-3 and MBK-2 proteins could be co-immunoprecipitated by the other protein, indicating a direct physical interaction (Stitzel et al. 2007). In addition, it was shown that while depletion of APC component MAT-1 lead to retention of MBK-2 at the cortex, simultaneous depletion of MAT-1 and EGG-3 shifted the MBK-2 protein from the cortex to the cytoplasm, allowing it to carry out its biochemical activity (Cheng et al. 2009; Stitzel et al. 2007). The implications of this experiment are two-fold. First, this indicates that the EGG-3 protein plays a direct role in holding the complex at the cortex until signaled by cell cycle progression to redistribute. Second, this indicates that EGG-3 does not directly regulate the biochemical activity of MBK-2, rather EGG-3 only regulates the spatial and temporal localization of MBK-2. Finally, simultaneous depletion of CDK-1 and EGG-3 did not alter the timing of degradation of MBK-2 target MEI-1 (Cheng et al. 2009). This indicates that CDK-1 does not regulate MBK-2 through the scaffolding molecule EGG-3.

3 A new player in the complex: EGG-4/5

It was clear that EGG-3 alone could not account for the complex regulation of MBK-2. While abrogation of EGG-3 caused premature degradation of MBK-2 targets, it did not alter MBK-2's catalytic activity (Stitzel et al. 2007). Although mislocalization of CHS-1 might be key in preventing extrusion of the chitinous eggshell, none of the proteins elucidated thus far could be pegged absolutely as the cause of failure during polar body extrusion (Johnston et al. 2006; Maruyama et al. 2007; Stitzel et al. 2007). In addition, it was as yet unknown what factors might link EGG-3, CHS-1, and MBK-2 to the advancement of the cell cycle. Therefore, further protein-tyrosine phosphatase-like proteins were examined in the hopes of finding a second such gene, which might play a role during the oocyte-to-embryo transition. This lead to the identification of proteins EGG-4 and EGG-5 (Cheng et al. 2009; Parry et al. 2009).

Genes *egg-4* (T21E3.1) and *egg-5* (R12E2.10) were identified as PTPL motif containing proteins based on their homology with *egg-3* and other anti-phosphatases (Parry et al. 2009). Outside the PTPL domains, EGG-4 and EGG-5 amino acid sequences do not contain any other obvious homologies. The genes bear a high degree of sequence similarity being 99.2% identical and containing only a single significant alteration in amino acid. The activity of the genes was examined both through deletion alleles available from NBP Japan, and through the use of soaking based RNAi. Individually, the *egg-4* or *egg-5* genes retain partial fertility, producing broods that are 76–78% of wild type sizes (Parry et al. 2009). A double mutant containing both the *egg-4* and *egg-5* deletion alleles was constructed, and found to be completely sterile (Parry et al. 2009). The partial redundancy in function of the genes coupled with their close proximity on the chromosome (0.3 cM apart), their high degree of sequence similarity, and the presence of only a single homologue in other species of *Caenorhabditis* supports the hypothesis that genes *egg-4* and *egg-5* represent a gene duplication and that the genes are predominantly redundant.

Knockdown of *egg-4/5* leads to oocyte specific lethality (Parry et al. 2009). The *egg-4/5* hermaphrodites produce phenotypically normal sperm and oocytes, which meet and fertilize in the spermathecal compartment as in wild type. However, no cell divisions occur after

fertilization and the fertilized but non-developing embryo becomes filled with endomitotic nuclei identical to those observed in *egg-3* mutants (Maruyama et al. 2007; Parry et al. 2009). Most *egg-4/5* defects phenocopy the oocyte-to-embryo transition defects seen in *egg-3* and *chs-1* mutants. Knockdown of EGG-4/5 prevents extrusion of the polar bodies, leading to an abnormal meiosis in which 12 sister chromatids undergo the second meiotic division. This in turn gives rise to a fertilized non-developing embryo, which contains multiple endomitotic nuclei in syncytium (Maruyama et al. 2007; Parry et al. 2009). In addition, a chitin staining assay shows that knockdown of EGG-4/5 also prevents formation of the tri-lamellar eggshell. Knockdown of EGG-4/5 also causes defects in the actin cytoskeleton, which are more severe than the defects present in EGG-3 mutants, in which a robust actin cap does not form at the site of sperm entry, but remains diffuse across the posterior end of the plasma membrane and eventually diffuses across both anterior and posterior poles (Parry et al. 2009).

A defect thus far unique to knockdown of EGG-4/5 is the entry of multiple sperm into a single oocyte (polyspermy). Polyspermy has never been documented before in *C. elegans* hermaphrodites, indicating that the block to polyspermy in this species is generally quite robust. However, at a rate of 15% in EGG-4/5 RNAi knockdown mutants, and 25% in EGG-4/5 double mutants, 2 or more sperm nuclei were visible in a single egg cytoplasm. Little is known about the block to polyspermy in *C. elegans*, making the *egg-4/5* genes a rare peak into the functioning of this process.

In order to determine the localization of EGG-4/5, GFP-tagged fusions of the EGG-4 and EGG-5 proteins were constructed by microparticle bombardment as with the EGG-3 fusion proteins. In addition, peptide antibodies were raised against the EGG-4 and EGG-5 proteins to confirm localization of the protein fusions (Cheng et al. 2009; Parry et al. 2009). It was anticipated that the EGG-4/5 proteins would co-localize with EGG-3, which held true for the proteins during late oogenesis when the proteins localize to the cortex of the developing oocyte (Figure 4). However, rather than forming discrete cortical puncta at the transition between metaphase I, and anaphase I of meiosis I, the EGG-4/5 proteins either become diffusely cytoplasmic, or are degraded (Figure 4) (Parry et al. 2009). The difference in EGG-4/5 localization after fertilization could reflect divergent functions after fertilization from EGG-3. Further, if EGG-4/5 forms a complex with EGG-3, CHS-1 and MBK-2 in developing oocytes, the proteins must part ways during the first meiotic division.

Once again it was determined that preventing advancement of the cell cycle past metaphase I by depleting APC component MAT-1, lead to aberrant retention of GFP:EGG-4 and GFP:EGG-5 to the oocyte cortex (Cheng et al. 2009; Parry et al. 2009). Furthermore, depletion of cyclin-dependent kinase CDK-1 also caused aberrant retention of EGG-4/5, EGG-3, CHS-1, and MBK-2 to the cortex (Cheng et al. 2009). The *cdk-1* gene is required for meiotic re-entry of the oocyte at metaphase I, and is antagonized by kinase *wee-1* (Burrows et al. 2006). Contrary to the effects of depleting MAT-1 or CDK-1, depletion of WEE-1 caused premature relocalization of EGG-4/5, EGG-3, CHS-1, and MBK-2 away from the cortex (Cheng et al. 2009; Stitzel et al. 2007). Finally, depletion of EGG-4/5 had no effect on the degradation of GFP:CYB-1, and thus no effect on advancement of the cell cycle through meiosis (Parry et al. 2009). Therefore the dynamic redistribution of EGG-4/5 is cell cycle dependent. Conversely, advancement of the cell cycle occurs in the absence of EGG-4/5.

The EGG-3, CHS-1, and MBK-2 proteins all experience a complex interdependence on one another, therefore the EGG-4/5 proteins might similarly be required for the localization of one or more of the other proteins. In order to examine this possibility, the localizations of EGG-3, CHS-1, and MBK-2 GFP or RFP tagged fusion proteins were examined in wild-

type and EGG-4/5 RNAi knockdown backgrounds (Parry et al. 2009). It was determined that the presence of EGG-4/5 is required for the dynamic re-localization of EGG-3 and CHS-1 during the first meiotic division. Similar to the requirement for the functioning of the APC during redistribution, depletion of EGG-4/5 caused aberrant retention of EGG-3 and CHS-1 to the cortex after fertilization. This differs from the interdependence between EGG-3 and CHS-1 in which each gene is required for the localization of the other at the cortex during oocyte development (Maruyama et al. 2007; Parry et al. 2009). In addition, the localization of MBK-2 during late oogenesis and early embryogenesis requires EGG-4/5 as well as EGG-3, and CHS-1, and conversely depletion of MBK-2 has no effect on localization or dynamics of EGG-4/5 (Maruyama et al. 2007; Parry et al. 2009). Again, knockdown of MBK-2 causes less severe phenotypes than knockdown of EGG-4/5, complementing the results of the epistasis analysis.

This accumulated wealth of epistatic relationships would not be complete without proof of direct physical interactions between molecules. The yeast-two-hybrid system was used to investigate interactions between EGG-4/5, EGG-3, and MBK-2 (Parry et al. 2009). All three molecules were examined in both context of bait and prey. It was determined that EGG-4 and EGG-3, EGG-4 and MBK-2, and EGG-3 and MBK-2 interacted with one another (Parry et al. 2009). The interactions between EGG-4 and MBK-2, and EGG-3 and MBK-2 were corroborated by evidence from the Seydoux lab, which showed that these molecules interact in a co-immunoprecipitation assay (Cheng et al. 2009). It had already been shown that abrogation of MAT-1 lead to retention of EGG-4/5, EGG-3, CHS-1, and MBK-2 at the plasma membrane (Cheng et al. 2009; Maruyama et al. 2007; Parry et al. 2009; Stitzel et al. 2007). While simultaneous abrogation of both MAT-1 and EGG-3 released MBK-2 into the cytoplasm (Cheng et al. 2009; Stitzel et al. 2007). Therefore, if EGG-3 were acting as a putative scaffolding molecule for EGG-4/5, CHS-1, and MBK-2, what molecular function was EGG-4/5 performing in this complex? Again the answer could be found in the link between the dynamic distributions of EGG-4/5 and the advancement of the cell cycle.

Like APC component MAT-1, knocking down activity of cyclin dependent kinase CDK-1 leads to retention of EGG-4/5, EGG-3, CHS-1, and MBK-2 at the cortex. However, as discussed above, unlike MAT-1, simultaneous depletion of EGG-3 and CDK-1 does not remove MBK-2 to the cytoplasm (Cheng et al. 2009). What then is the regulatory relationship between CDK-1 and the complex? Evidence from kinase assays and co-immunoprecipitation assays shows that cyclin dependent kinase CDK-1 is capable of phosphorylating MBK-2 at S68 (Cheng et al. 2009). Furthermore, wild-type GFP:MBK-2 but not an unphosphorylatable form GFP:MBK-2(S68E), was able to rescue the lethality of *mbk-2(pk1427)* (Cheng et al. 2009). Therefore CDK-1 dependent phosphorylation of MBK-2 is necessary for its cellular activity. However, CDK-1 becomes active during late oogenesis before the oocyte has made its resumption of meiosis I, and as previously mentioned it is critical that MBK-2 not carry out its catalytic functions in developing oocytes or the very early embryo. A mechanism must be in place to prevent premature activity of MBK-2.

The role of inhibitor for MBK-2 seems to be fulfilled by EGG-4/5. As mentioned above, EGG-4 and MBK-2 were found to interact both by yeast-two hybrid, co-immunoprecipitation assay, and GST/FLAG pull down assay (Cheng et al. 2009). The specific phosphorylation of the MBK-2 active loop also increased the efficiency of the binding reaction between EGG-4/5 and MBK-2. Additionally, evidence from *in vitro* kinase assays also indicates that MBK-2 kinase activity, phosphorylating target MEI-1, is inhibited by the presence of EGG-4/5 (Cheng et al. 2009). Therefore, rather than acting as a scaffolding molecule like fellow PTPL protein EGG-3, EGG-4/5 seems to behave as a mixed inhibitor for MBK-2 (Tonks 2009).

4 Perspectives

The process of completing the transition between fully developed, mature oocyte, and fertilized developing embryo requires precise spatial and temporal control of molecular events. In short order, the *C. elegans* oocyte is contacted by a protein signal from sperm in the nearby spermatheca to complete the process of maturation, including nuclear envelope breakdown, cytoskeletal rearrangement, and resumption of meiosis I from prophase to metaphase I. As maturation is completed the oocyte immediately enters the spermatheca where it should be fertilized by a single sperm, before passing into the uterus. What follows is a highly complex series of events that allow the newly fertilized embryo to complete its transition from maternal program to embryonic program. The complex module of proteins described in these pages, is required for coupling the precise timing of necessary transitory events to the advancement of the cell cycle. The genes *egg-3*, *chs-1*, and *egg-4/5* are minimally required for production of the chitin eggshell during meiosis, an error-free meiosis and polar body extrusion, and in the case of *egg-3* and *egg-4/5* a normal formation and/or distribution of the cytoskeletal actin cap. All of these genes, *egg-3*, *chs-1*, and *egg-4/5* are also required for the precise spatial and temporal localization of regulatory kinase *mbk-2*. Because *mbk-2* is required to mark several time sensitive maternal proteins for degradation, its localization and activity must be precisely restricted during the oocyte-to-embryo transition. To this end, several interrelated regulatory mechanisms have been elucidated. First it has been determined that the redistribution of *egg-3*, *chs-1*, *mbk-2*, and *egg-4/5* depends on advancement of the cell cycle past metaphase I, and that the redistribution is signaled in an EGG-3 dependent manner. It has also been shown that the activity of MBK-2 depends on phosphorylation by cyclin dependent kinase CDK-1, a protein that becomes active during late oogenesis. However, it is critical that MBK-2 does not have access to its targets until the completion of meiosis. Therefore a second regulatory protein, in the form of EGG-4/5, is required for holding MBK-2 and preventing its premature activity. This complex protein module, EGG-3, EGG-4/5, CHS-1, MBK-2, and CDK-1 represents a complex and novel method of regulating the transition between maternal oocyte programming and embryonic programming. Given the high degree of complexity inherent in this model, it is predicted that other molecular regulators and effectors must be active during the oocyte-to-embryo transition. Genetic and biochemical analyses are currently underway to seek out new players in this highly dynamic process.

Acknowledgments

The authors would like to thank members of the Singson Lab for input and discussion. Work in the Singson Lab has been supported by funds from the Waksman Institute and a grant from the United States National Institutes of Health (R01 HD054681).

References

- Austin J, Kimble J. *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans*. *Cell*. 1987; 51:589–599. [PubMed: 3677168]
- Bellec Y, Harrar Y, Butaeye C, Darnet S, Bellini C, Faure JD. Pasticcino2 is a protein tyrosine phosphatase-like involved in cell proliferation and differentiation in Arabidopsis. *The Plant Journal*. 2002; 32:713–722. [PubMed: 12472687]
- Brenner S. The genetics of *Caenorhabditis elegans*. *Genetics*. 1974; 77:71–94. [PubMed: 4366476]
- Burrows AE, Scurman BK, Kosinski ME, Richie CT, Sadler PL, Schumacher JM, Golden A. The *C. elegans* Myt1 ortholog is required for the proper timing of oocyte maturation. *Development*. 2006; 133:697–709. [PubMed: 16421191]
- Chalfie M, Tu Y, Euskirchen G, Ward W, Prasher D. Green fluorescent protein as a marker for gene expression. *Science*. 1994; 263:802–805. [PubMed: 8303295]

- Cheng KC, KR, Singson A, Seydoux G. Regulation of MBK-2/DYRK by CDK-1 and the pseudophosphatases EGG-4 and EGG-5 during the oocyte-to-embryo transition. *Cell*. 2009; 139:560–572. [PubMed: 19879842]
- Coghlan A, Stajich JE, Harris TW. Comparative genomics in *C. elegans*, *C. briggsae*, and other *Caenorhabditis* species. *Methods Mol Biol*. 2006; 351:13–29. [PubMed: 16988423]
- Da Costa M, BL, Landrieu I, Bellec Y, Catrice O, Brown S, De Veylder L, Lippens G, Inzé D, Faure JD. Arabidopsis PASTICCINO2 is an antiphosphatase involved in regulation of cyclin-dependent kinase A. *Plant Cell*. 2006; 18:1426–1437. [PubMed: 16698944]
- Detwiler MR, Reuben M, Li X, Rogers E, Lin R. Two zinc finger proteins, OMA-1 and OMA-2, are redundantly required for oocyte maturation in *C. elegans*. *Dev Cell*. 2001; 1:187–199. [PubMed: 11702779]
- Ducibella TFR. The roles of Ca²⁺, downstream protein kinases, and oscillatory signaling in regulating fertilization and the activation of development. *Developmental Biology*. 2008; 315:257–279. [PubMed: 18255053]
- Evsikov AV, Marin de Evsikova C. Gene expression during the oocyte-to-embryo transition in mammals. *Mol Reprod Dev*. 2009; 76:805–818. [PubMed: 19363788]
- Fire A. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998; 391:806–811. [PubMed: 9486653]
- Gonczy, P.; Rose, LS. Asymmetric cell division and axis formation in the embryo. *WormBook*; 2005. p. 1-20.
- Govindan JA, Greenstein D. Embryogenesis: anchors away! *Curr Biol*. 2007; 17:R890–892. [PubMed: 17956750]
- Granger L, Martin E, Segalat L. Mos as a tool for genome-wide insertional mutagenesis in *Caenorhabditis elegans*: results of a pilot study. *Nucleic Acids Res*. 2004; 32:e117. [PubMed: 15310838]
- Greenstein, D. Control of oocyte meiotic maturation and fertilization. *WormBook*; 2005. p. 1-12.
- Hadwiger G, Dour S, Arur S, Fox P, Nonet ML. A monoclonal antibody toolkit for *C. elegans*. *PLoS One*. 2010; 5:e10161. [PubMed: 20405020]
- Harris JE, Govindan JA, Yamamoto I, Schwartz J, Kaverina I, Greenstein D. Major sperm protein signaling promotes oocyte microtubule reorganization prior to fertilization in *Caenorhabditis elegans*. *Dev Biol*. 2006; 299:105–121. [PubMed: 16919258]
- Hedgecock E, Sulston J, Thomson J. Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. *Science*. 1983; 220:1277–1299. [PubMed: 6857247]
- Hubbard EJ, Greenstein D. The *Caenorhabditis elegans* gonad: a test tube for cell and developmental biology. *Dev Dyn*. 2000; 218:2–22. [PubMed: 10822256]
- Jensen VL, Albert PS, Riddle DL. *Caenorhabditis elegans* SDF-9 enhances insulin/insulin-like signaling through interaction with DAF-2. *Genetics*. 2007; 177:661–666. [PubMed: 17660545]
- Johnston WL, KA, Dennis JW. The eggshell is required for meiotic fidelity, polar-body extrusion and polarization of the *C. elegans* embryo. *BMC Biology*. 2006; 4. [PubMed: 16469111]
- Kadandale P, Chatterjee I, Singson A. Germline transformation of *Caenorhabditis elegans* by injection. *Methods Mol Biol*. 2009; 518:123–133. [PubMed: 19085141]
- Kadandale P, Stewart-Michaelis A, Gordon S, Rubin J, Klancer R, Schweinsberg P, Grant BD, Singson A. The egg surface LDL receptor repeat-containing proteins EGG-1 and EGG-2 are required for fertilization in *Caenorhabditis elegans*. *Curr Biol*. 2005; 15:2222–2229. [PubMed: 16360684]
- Kimble, J.; CS. Germline proliferation and its control. *WormBook*; 2005. p. 1-14.
- Klass M, Wolf N, Hirsh D. Development of the male reproductive system and sexual transformation in the nematode *Caenorhabditis elegans*. *Dev Biol*. 1976; 52:1–18. [PubMed: 986968]
- Kuwabara PE. The multifaceted *C. elegans* major sperm protein: an ephrin signaling antagonist in oocyte maturation. *Genes Dev*. 2003; 17:155–161. [PubMed: 12533505]
- L'Hernault, SW. Spermatogenesis. *WormBook*; 2006. p. 1-14.
- LaMunyon CW, Ward S. Assessing the viability of mutant and manipulated sperm by artificial insemination of *Caenorhabditis elegans*. *Genetics*. 1994; 138:689–692. [PubMed: 7851766]

- Lin R. A gain-of-function mutation in *oma-1*, a *C. elegans* gene required for oocyte maturation, results in delayed degradation of maternal proteins and embryonic lethality. *Dev Biol.* 2003; 258:226–239. [PubMed: 12781695]
- Maeda I, Kohara Y, Yamamoto M, Sugimoto A. Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. *Curr Biol.* 2001; 11:171–176. [PubMed: 11231151]
- Maruyama R, VN, Klancer R, Gordon S, Kadandale P, Parry JM, Hang JS, Rubin J, Stewart-Michaelis A, Schweinsberg P, Grant BD, Piano F, Sugimoto A, Singson A. EGG-3 regulates cell-surface and cortex rearrangements during egg activation in *Caenorhabditis elegans*. *Current Biology.* 2007; 17:1555–1560. [PubMed: 17869112]
- McCarter J, Bartlett B, Dang T, Schedl T. On the control of oocyte meiotic maturation and ovulation in *Caenorhabditis elegans*. *Dev Biol.* 1999; 205:111–128. [PubMed: 9882501]
- McNally KL, McNally FJ. Fertilization initiates the transition from anaphase I to metaphase II during female meiosis in *C. elegans*. *Dev Biol.* 2005; 282:218–230. [PubMed: 15936342]
- Nishi YLR. DYRK2 and GSK-3 phosphorylate and promote the timely degradation of OMA-1, a key regulator of the oocyte-to-embryo transition in *C. elegans*. *Developmental Biology.* 2005; 288:139–149. [PubMed: 16289132]
- Nishi Y, Rogers E, Robertson SM, Lin R. Polo kinases regulate *C. elegans* embryonic polarity via binding to DYRK2-primed MEX-5 and MEX-6. *Development.* 2008; 135:687–697. [PubMed: 18199581]
- Oegema, K.; Hyman, AA. Cell division. *WormBook*; 2006. p. 1-40.
- Parry JM, VN, Lefkovith AJ, Zegarek MH, Hang JS, Ohm J, Klancer R, Maruyama R, Druzhinina MK, Grant BD, Piano F, Singson A. EGG-4 and EGG-5 Link Events of the Oocyte-to-Embryo Transition with Meiotic Progression in *C. elegans*. *Current Biology.* 2009; 19:1752–1757. [PubMed: 19879147]
- Pellettieri J, Reinke V, Kim SK, Seydoux G. Coordinate activation of maternal protein degradation during the egg-to-embryo transition in *C. elegans*. *Dev Cell.* 2003; 5:451–462. [PubMed: 12967564]
- Praitis V. Creation of transgenic lines using microparticle bombardment methods. *Methods Mol Biol.* 2006; 351:93–107. [PubMed: 16988428]
- Putiri E, Zannoni S, Kadandale P, Singson A. Functional domains and temperature-sensitive mutations in SPE-9, an EGF repeat-containing protein required for fertility in *Caenorhabditis elegans*. *Dev Biol.* 2004; 272:448–459. [PubMed: 15282160]
- Quintin S, MP, Zinke A, Hyman AA. The *mbk-2* kinase is required for inactivation of MEI-1/katanin in the one-cell *Caenorhabditis elegans* embryo. *EMBO Reports.* 2003; 4:1175–1181. [PubMed: 14634695]
- Raich WB, Moorman C, Lacefield CO, Lehrer J, Bartsch D, Plasterk RH, Kandel ER, Hobert O. Characterization of *Caenorhabditis elegans* homologs of the Down syndrome candidate gene DYRK1A. *Genetics.* 2003; 163:571–580. [PubMed: 12618396]
- Sato K, Sato M, Audhya A, Oegema K, Schweinsberg P, Grant BD. Dynamic regulation of caveolin-1 trafficking in the germ line and embryo of *Caenorhabditis elegans*. *Mol Biol Cell.* 2006; 17:3085–3094. [PubMed: 16672374]
- Schubert CM, Lin R, de Vries CJ, Plasterk RH, Priess JR. MEX-5 and MEX-6 function to establish soma/germline asymmetry in early *C. elegans* embryos. *Mol Cell.* 2000; 5:671–682. [PubMed: 10882103]
- Singson A, Hang J, Parry JM. Genes required for the common miracle of fertilization. *International Journal of Developmental Biology.* 2008; 52:647–656. [PubMed: 18649278]
- Singson A, Mercer KB, L'Hernault SW. The *C. elegans spe-9* gene encodes a sperm transmembrane protein that contains EGF-like repeats and is required for fertilization. *Cell.* 1998; 93:71–79. [PubMed: 9546393]
- Stitzel ML, CK, Seydoux G. Regulation of MBK-2/Dyrk kinase by dynamic cortical anchoring during the oocyte-to-zygote transition. *Current Biology.* 2007; 17:1545–1554. [PubMed: 17869113]
- Sulston J, Horvitz H. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Developmental Biology.* 1977; 56:110–156. [PubMed: 838129]

- Sundaram, M. RTK/Ras/MAPK signaling. WormBook; 2006. p. 1-19.
- Tonks N. Pseudophosphatases: grab and hold on. Cell. 2009; 139:464–465. [PubMed: 19879835]
- Uwanogho DA, Hardcastle Z, Balogh P, Mirza G, Thornburg KL, Ragoussis J, Sharpe PT. Molecular cloning, chromosomal mapping, and developmental expression of a novel protein tyrosine phosphatase-like gene. Genomics. 1999; 62:406–416. [PubMed: 10644438]
- Ward S, Carrel JS. Fertilization and sperm competition in the nematode *Caenorhabditis elegans*. Dev Biol. 1979; 73:304–321. [PubMed: 499670]
- Yamamoto I, Kosinski ME, Greenstein D. Start me up: cell signaling and the journey from oocyte to embryo in *C. elegans*. Dev Dyn. 2006; 235:571–585. [PubMed: 16372336]
- Yang HY, McNally K, McNally FJ. MEI-1/katanin is required for translocation of the meiosis I spindle to the oocyte cortex in *C. elegans*. Dev Biol. 2003; 260:245–259. [PubMed: 12885567]
- Zhang Y, Foster JM, Nelson LS, Ma D, Carlow CK. The chitin synthase genes *chs-1* and *chs-2* are essential for *C. elegans* development and responsible for chitin deposition in the eggshell and pharynx, respectively. Dev Biol. 2005; 285:330–339. [PubMed: 16098962]

Figure 1.

A. N2 wild-type population with worms in various stages of development.

B. An adult sterile hermaphrodite.

Figure 2.

A. Adult male, wild-type N2 worm.

B. Adult hermaphrodite, wild-type N2 worm.

C. Close-up of adult hermaphrodite proximal germline. At left are proximal most oocytes, at center the spermatheca containing mature spermatozoa, at left fertilized and developing embryos.

D. Inactive spermatids dissected from a wild-type N2 male.

E. Activated spermatozoa dissected from a wild-type N2 male, and activated with pronase.

Figure 3.

A. The events of the oocyte-to-embryo transition

1. The proximal most (–1) oocyte.
2. As the proximal most oocyte approaches the spermatheca it undergoes NEBD and cytoskeletal rearrangement. The chromosomes condense.
3. Contractions of the somatic gonad sheath and spermatheca dilation lead to ovulation of the oocyte. After spermathecal entry, the egg is rapidly fertilized by a single sperm.
4. Metaphase I of meiosis I. The chromosomes are arranged in a characteristic pentagonal array. The sperm DNA marks the presumptive posterior of the embryo.
5. Anaphase I of meiosis I. The chromosomes separate and a single polar body is extruded.
6. Metaphase II of meiosis II.
7. Anaphase II of meiosis II. By this stage a robust chitin rich eggshell has been extruded.
8. The male and female DNA decondense and form pronuclei.
9. Cytoskeletal rearrangements bring the pronuclei together, they fuse.
10. The one cell embryo contains a single nucleus.
11. The beginning of mitotic divisions.

Figure 4.

A. The EGG-4 and EGG-5 proteins rings the oocyte cortex. Again, the heaviest confluorescence occurs as the oocyte undergoes nuclear envelope breakdown. The protein remains at the cortex until the transition between metaphase I and anaphase I of meiosis I. At that point the protein washes across the cell in a distinct wave, becoming diffusely cytoplasmic.

B. The EGG-3, CHS-1, and MBK-2 proteins rings the oocyte cortex. The heaviest confluorescence occurs as the oocyte undergoes nuclear envelope breakdown. The protein remains at the cortex until the transition between metaphase I and anaphase I of meiosis I. At that point the protein forms discrete cortical puncta.

C. The hermaphrodite reproductive tract centered on the proximal most oocytes, spermatheca, and newest embryos. The animal contains a RFP:EGG-3 fusion protein.