REVIEW

Every Sperm Is Sacred: Fertilization in *Caenorhabditis elegans*

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The nematode *Caenorhabditis elegans* is an attractive model system for the study of fertilization. *C. elegans* exists as a self-fertilizing hermaphrodite or as a male. This unusual situation provides an excellent opportunity to identify and maintain sterile mutants that affect sperm and no other cells. Analysis of these mutants can identify genes that encode proteins required for gamete recognition, adhesion, signaling, fusion, and/or activation at fertilization. These genes can also provide a starting point for the identification of additional molecules required for fertility. This review describes progress in the genetic and molecular dissection of fertilization in *C. elegans* and related studies on sperm competition.
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INTRODUCTION

For sexually reproducing species, events leading to the fusion of gametes are prerequisites for the development of a new individual with a unique combination of genes. Gametes are highly specialized in order to assure conception. Eggs are large and essentially stationary cells that must contain most of the raw materials required to sustain zygotes through embryogenesis. In contrast, sperm are small cells that often contain little more than a haploid nucleus, mitochondria for energy generation, and cellular structures devoted to motility. Sperm must also be adapted to the particular environment in which they function (i.e., sea water or reproductive tract). Consequently, sperm morphology varies considerably between species. Despite this variability, all sperm must accomplish similar tasks. First, they must locate and move toward an egg. Second, they must bind to the egg, often in a species-specific manner. Third, they must fuse with or enter the egg and trigger the developmental program that results in a new individual. For general reviews on fertilization see Yanagimachi (1994), Vacquier (1998), and Wassarman (1999). The fundamental mecha-

nisms that underlie events of fertilization are conserved in many other important cell–cell interactions during the life and development of multicellular organisms.

The nematode *Caenorhabditis elegans* is a well-established model system for the study of many biological processes (Riddle *et al.*, 1997). *C. elegans* is also emerging as an attractive system for studying the complexities of fertilization. Many of the genetic and molecular tools developed for *C. elegans* are not available or very difficult to utilize in the other organisms traditionally used for studying fertilization. The amoeboid sperm of *C. elegans*, despite lacking an acrosome and flagellum (Fig. 1), carry out the same basic functions common to all sperm. The most significant advantage of *C. elegans* is the ability to isolate and maintain mutants that affect sperm and no other cells. This is accomplished by selecting sterile hermaphrodites that cannot produce self-progeny, but whose oocytes can be fertilized by wild-type male sperm. Another advantage of the worm is its transparent cuticle. This allows for direct observation of gametogenesis, gamete behavior, and fertilization in wild-type or mutant animals. Additionally, various molecular probes, together with modern microscopy, can be used to detect cellular behaviors and physiological changes as they occur *in vivo*. Gene expression data, made possible by the sequencing of the worm genome and DNA microarrays, can simplify the identification and analysis of genes required for fertility (Reinke *et al.*, 2000). Finally,

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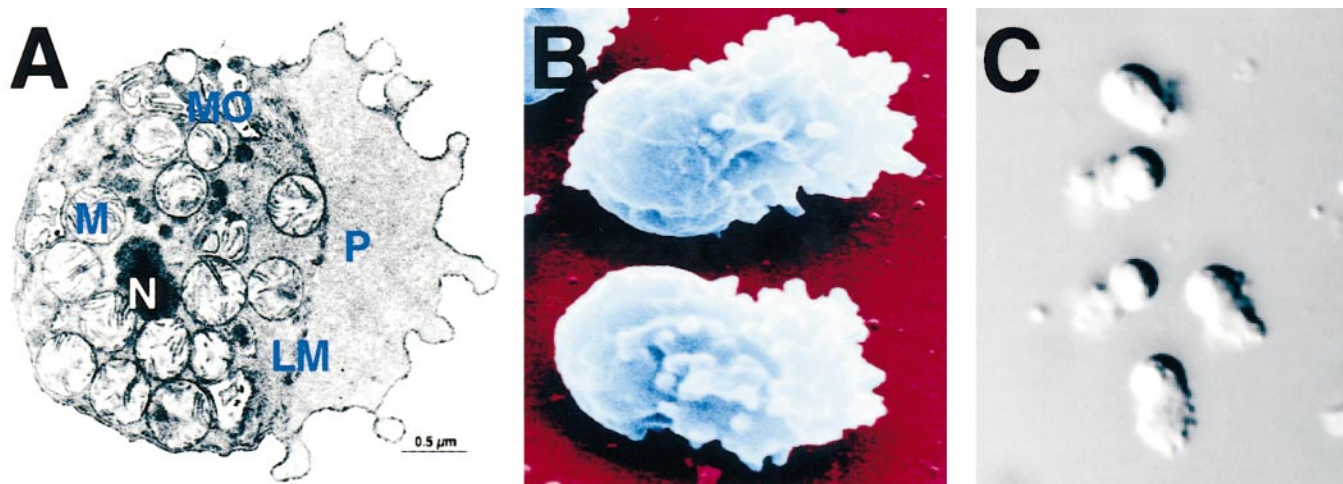
FIG. 1. *C. elegans* spermatozoa. (A) Transmission electron micrograph. P = pseudopod, N = nucleus, M = mitochondria, MO = membranous organelles, LM = laminar membranes. (B) Scanning electron micrograph. (C) Light micrograph.

both sperm (L'Hernault and Roberts, 1995) and oocytes (Aroian *et al.*, 1997) can be isolated in relatively large quantities. Therefore, biochemical approaches can also be applied to the study of *C. elegans* reproductive biology.

Fertilization in *C. elegans* has been previously described in a number of excellent papers and reviews (Kemphues and Strome, 1997; Kimble and Ward, 1988; Ward and Carrel, 1979; Hirsh *et al.*, 1976; Honda, 1925). Here, I review the events of fertilization in *C. elegans* with an emphasis on new insights and important unresolved questions. I will also consider important work with *C. elegans* concerning issues of sperm competition, an important form of sexual selection.

## GENERAL FEATURES OF *C. elegans* REPRODUCTIVE BIOLOGY

As noted above, *C. elegans* exists primarily as self-fertile hermaphrodites (karyotype 5AA, XX) that make both sperm and eggs or as males that make only sperm (karyotype 5AA, XØ) (reviewed by Meyer, 1997). Hermaphrodites, during their last larval stage, produce about 300 sperm and then switch to producing oocytes. Therefore, adult hermaphrodites are essentially females with stored sperm. Adult males continually produce sperm and can mate with hermaphrodites to produce cross-progeny. The internal fertilization of *C. elegans* is extremely efficient. An unmated hermaphrodite will use all of its sperm and produce about 300 progeny. When a hermaphrodite is mated to males, they can produce as many as 1400 progeny (Kimble and Ward, 1988). A male worm has the potential to sire more than 2800 progeny (Hodgkin, 1983).

HERMAPHRODITE AND MALE REPRODUCTIVE TRACTS

For an in-depth description of *C. elegans* reproductive tract development and structure, see Hubbard and Greenstein (2000), Schedl (1997), Hirsh *et al.* (1976), and Klass *et al.* (1976). The adult reproductive tract, much like the worm body, is a tubelike structure with a distal (tip of the gonad) -to-proximal (opening to the exterior) axis (Fig. 2A). Hermaphrodites have two U-shaped gonad arms that each terminate proximally at a spermatheca. The spermatheca is a convoluted tube that serves as the site of sperm storage and fertilization (Fig. 2B). A distal constriction separates the spermatheca from the gonad arm while a proximal constriction (spermathecal valve) separates the spermatheca from the uterus (Hirsh *et al.*, 1976; Ward and Carrel, 1979). Both spermathecae are connected to a common uterus with a central vulval opening. Males have a single J-shaped testis connected to a cloaca via a seminal vesicle, a valve region, and vas deferens (Fig. 2A) (Klass *et al.*, 1976). Males use mating structures on their tail to locate the hermaphrodite vulva and deposit sperm into the uterus. For a full description of male mating behavior see Liu and Sternberg (1995) and Emmons and Sternberg (1997). Both hermaphrodites and males have a basement membrane that surrounds the gonad. The proximal end of the hermaphrodite gonad is also surrounded by a contractile myoepithelial sheath (McCarter *et al.*, 1997; Hall *et al.*, 1999). In both sexes, gametes differentiate as they move proximally.

GAMETE MATURATION

There are a number of key events just prior to the meeting of sperm and oocytes that prepare them for fertilization.
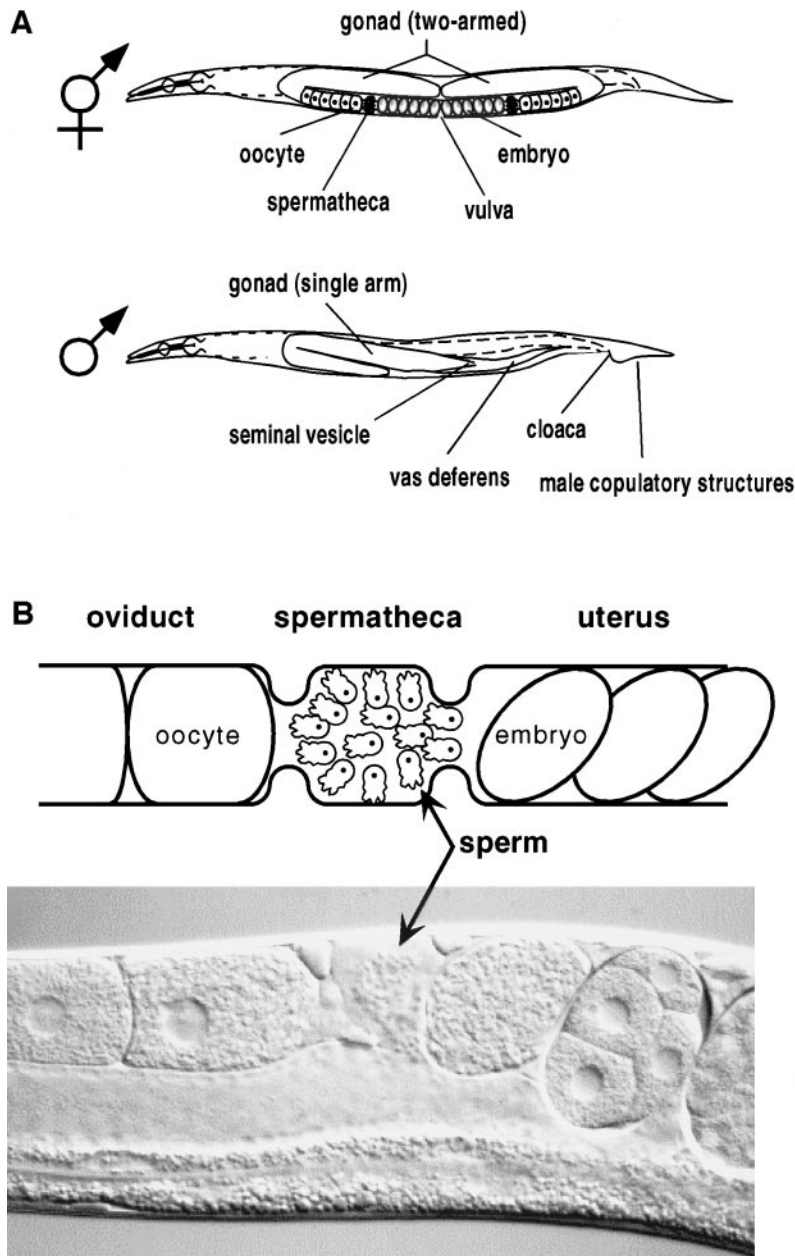
FIG. 2. (A) The hermaphrodite and male reproductive tracts. (B) A schematic and light micrograph of the *C. elegans* reproductive tract in the region of the spermatheca.

Oocytes must undergo oocyte maturation and ovulation, while spermatids must undergo spermiogenesis to become fully functional and motile spermatozoa. For a description of gametogenesis prior to the events described here see Hubbard and Greenstein (2000), Schedl (1997) and L'Hernault (1997).

Oocyte Maturation and Ovulation

Late-stage oocytes in diakinesis of prophase I enlarge, mature, and are ovulated from the oviduct in an assembly line-like fashion roughly every 20 min (McCarter *et al.*, 1999; Ward and Carrel, 1979). During late oogenesis, the oocyte nucleus migrates distally. The significance of this apparent polarization, although important in other nematode species, is unclear since the sperm entry point specifies the future anterior–posterior axis of the embryo in *C. elegans* (Goldstein *et al.*, 1998; Goldstein and Hird, 1996; Golden, 2000). Oocyte maturation refers to the transition from diakinesis to metaphase I (McCarter *et al.*, 1999). Nuclear envelope breakdown occurs followed by a cortical

rearrangement that transforms the oocyte to a rounder shape. Just prior to ovulation, the gonadal sheath cells significantly increase their contractile activity. Finally, the distal spermatheca dilates and appears to be pulled over the oocyte at ovulation (McCarter *et al.*, 1999; Ward and Carrel, 1979).

Several gamete signaling events are required for high levels of oocyte maturation and ovulation (McCarter *et al.*, 1999). First, sperm are necessary to trigger oocyte maturation and basal gonadal sheath activity. Second, the maturing oocyte triggers the intense sheath activity and spermathecal dilation at ovulation. The nature of these signals is largely unknown. However, spermathecal dilation seems to require LET-23 (EGF-receptor) function (J. McCarter and T. Schedl, unpublished observation) in conjunction with an IP_3-mediated pathway (Clandinin *et al.*, 1998). Additionally, major sperm protein (MSP, see below), a molecule that plays a central role in pseudopod motility, can promote oocyte maturation and sheath contraction (M. Miller and D. Greenstein, unpublished observation). Analysis of mutants that affect ovulation should shed more light on the nature of these signaling pathways. When events surrounding ovulation are not coordinated, oocytes are often damaged (McCarter *et al.*, 1999, 1997; Clandinin *et al.*, 1998; Greenstein *et al.*, 1994). Furthermore, since sperm are a limited resource in an unmated hermaphrodite, these signaling systems may help prevent the worm from wasting metabolically costly oocytes when sperm are not present in the reproductive tract.

Spermiogenesis and Motility

Spermiogenesis, or sperm activation, refers to the process where round, sessile spermatids are converted to bipolar, crawling spermatozoa capable of fertilizing an egg. The cellular events of spermiogenesis include (1) protrusion of spikelike structures that eventually coalesce into a single pseudopod; (2) fusion of sperm-specific membranous organelles (MO, Fig. 1A) with the plasma membrane; and (3) initiation of motility (Shakes and Ward, 1989). The precise function of the MO is not known. However, mutations that block MO fusion produce sperm with motility defects and the worms are sterile (Achanzar and Ward, 1997). Upon fusion with the sperm plasma membrane, the primarily glycoprotein contents of the MOs are exocytosed (Ward *et al.*, 1981). Additionally, the MOs contribute membrane glycoproteins to the cell surface (Roberts *et al.*, 1986).

The amoeboid sperm of *C. elegans* crawl with a single pseudopod that projects from and drags the cell body (Fig. 1). Interestingly, nematode sperm motility is not actin based. Rather it depends on the dynamic polymerization of a 14-kDa major sperm protein (Italiano *et al.*, 1996). For reviews on nematode sperm motility, see Ward *et al.* (1982), Theriot (1996), or Roberts and Stewart (2000).

Spermiogenesis occurs under different conditions for hermaphrodites and males. In hermaphrodites, the first ovulated oocyte pushes spermatids from the proximal gonad arm into the spermatheca and spermiogenesis occurs during this passage (Ward and Carrel, 1979). In males, spermiogenesis occurs upon mating. Mutations in a number of genes (*fer-15, spe-8, spe-12, spe-27,* and *spe-29*) cause worms to accumulate spermatids that do not undergo spermiogenesis (Nance *et al.*, 1999; Minniti *et al.*, 1996; Shakes and Ward, 1989; L'Hernault, 1997). There must be distinct mechanisms for male and hermaphrodite activation because the *spe-8, spe-12, spe-27,* and *spe-29* mutants block spermiogenesis only in hermaphrodites. Hermaphrodite and male activators are not known. However, seminal fluid can activate spermatids from both sexes (Shakes and Ward, 1989; Ward *et al.*, 1983). Because the *spe-12* and *spe-27* genes encode novel proteins, the overall nature of the spermiogenesis signaling pathway is still unclear (Nance *et al.*, 1999; Minniti *et al.*, 1996).

After spermiogenesis, hermaphrodite-derived sperm remain in the spermatheca awaiting passage of the next oocyte. The amoeboid nature of *C. elegans* sperm seems exquisitely adapted to the cramped environment in which they function. The lumen of the spermatheca presents a highly involuted surface where sperm tightly imbed their pseudopods (Ward and Carrel, 1979). Male-derived sperm, following deposition into the uterus just under the vulva, must crawl the length of the uterus past a "boulder field" of developing embryos to a spermatheca (Fig. 2). Additionally, since the volume of the spermatheca is not much larger than passing oocytes, sperm are often "swept" into the uterus. In an older hermaphrodite, remaining sperm may have been migrating against a flow of oocytes for five or more days. The movement of sperm toward the spermatheca appears directed and nonrandom (Ward and Carrel, 1979). It is unknown what attracts sperm to the spermatheca.

FERTILIZATION

In *C. elegans*, fertilization occurs in the spermatheca. If an oocyte passes through a spermatheca without being fertilized, it does not complete meiosis or secrete an eggshell. Unfertilized oocytes undergo rounds of endomitotic DNA replication without cytokinesis (Ward and Carrel, 1979). These polyploid and unhealthy cells are eventually laid by hermaphrodites and can be easily seen as large mushy dark brown cells. Additionally, fertilization will not occur in the proximal oviduct. In mutants that do not ovulate, fertilization is not observed (Clandinin *et al.*, 1998; Iwasaki *et al.*, 1996).

When an oocyte is ovulated, it can come in contact with hundreds of sperm in the spermatheca. There is a very potent but unknown block to polyspermy since each oocyte is fertilized by only a single sperm (Ward and Carrel, 1979). Fertilization occurs very rapidly and often before the oocyte has fully entered the spermatheca (Ward and Carrel, 1979; A. Samuel, V. Murthy, and M. Hengartner, unpublished observations). The final interactions of sperm and oocyte in *C. elegans* have not yet been captured by electron microscopy. Therefore, there are still a number of mysteries

concerning sperm–oocyte interactions. For example, the cellular region of sperm that contacts the oocyte at fertilization is not known. Additionally, it is unclear as to whether sperm fuse with oocytes at the plasma membrane or are engulfed. In other nematode species, the pseudopod interacts with the oocyte surface and gamete membranes fuse almost immediately (Foor, 1970, 1968).

C. elegans oocytes are somewhat unusual in that they apparently lack an egg coat. It is uncertain whether "wispy" material seen in some micrographs of *C. elegans* oocytes represents an egg coat (D. Hall, personal communication). Some nematode species clearly have extracellular material surrounding the oocyte (Foor, 1970, 1968). However, these "extraneous egg coats" do not pose a significant barrier to sperm penetration and could explain why nematode sperm lack an acrosome (Foor, 1970).

The sperm entry point in *C. elegans* fertilization usually occurs at the leading edge of the oocyte just as it enters the spermatheca (Goldstein and Hird, 1996; A. Samuel, V. Murthy, and M. Hengartner, unpublished observations). There is not, however, a single sperm entry point since sperm have the ability to enter anywhere on the oocyte surface (Goldstein and Hird, 1996).

EGG ACTIVATION AND THE SPERM'S CONTRIBUTION TO THE ZYGOTE

After being fertilized in the spermatheca, the zygote completes meiosis, begins embryogenesis, secretes a tough chitinous eggshell, passes through the uterus, and is laid prior to hatching. Sperm entry specifies the embryonic axis, activates the embryonic program, and triggers a constellation of events that together are generally referred to as egg activation. The exact mechanism of egg activation and its relationship to the embryonic program is not well understood. In *C. elegans*, the events of egg activation include (1) a single transient rise in intracellular $[Ca^{2+}]$ originating from the sperm entry point (A. Samuel, V. Murthy, and M. Hengartner, unpublished observations); (2) completion of the meiotic cell cycle and entry into the mitotic cell cycle; and (3) cytoplasmic rearrangements associated with the specification of the embryonic axis (reviewed by Kemphues and Strome, 1997; Golden, 2000).

Sperm also make a number of important contributions to the *C. elegans* zygote. The paternal haploid nucleus is required for a diploid embryo to develop. In a beautiful set of experiments, Sadler and Shakes (2000) demonstrated that anucleate sperm can fertilize oocytes, complete meiosis, secrete an eggshell, direct the embryonic axis, and develop quite normally before arresting. Centrosomes are also inherited paternally and they nucleate microtubules that mediate pronuclear migration and compose the mitotic spindle (Albertson, 1984; Albertson and Thomson, 1993). Additionally, components of the sperm pronucleus/centrosome complex are thought to signal to the egg cell cortex to promote changes that drive cell polarity (O'Connell *et al.*, 2000; Kemphues, 2000). The *spe-11* pro-
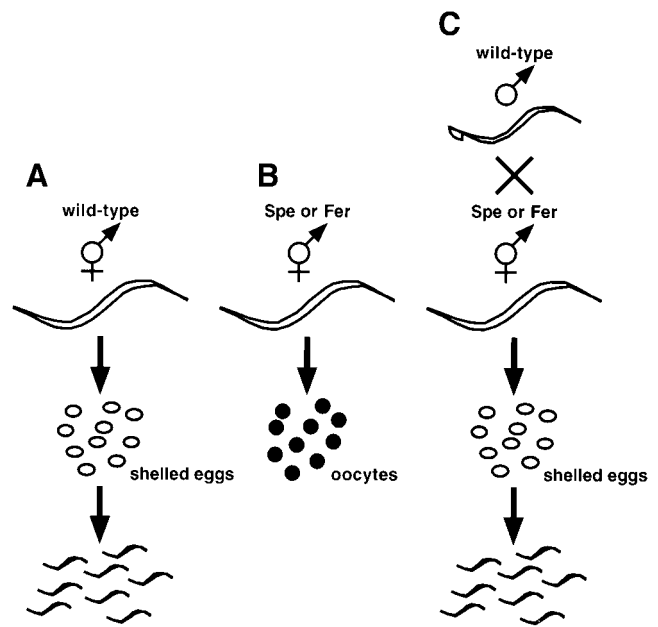
FIG. 3. The spermatogenesis (Spe) defective or fertilization (Fer) defective phenotype. (A) Wild-type worms are self-fertile. (B) Spe and Fer mutant worms are self-sterile and lay oocytes. (C) When Spe or Fer mutants are crossed to wild-type males (a source of sperm), they can produce outcross-progeny.

tein has also been implicated in some aspects of egg activation. Loss of function mutations in *spe-11* lead to a paternal-effect lethal phenotype where embryos fail to complete meiosis, form only a thin eggshell, show defects in spindle orientation, and fail to undergo cytokinesis (Hill *et al.*, 1989). Sperm normally supply the novel *spe-11* protein to the zygote. However, genetically engineered worms that express *spe-11* in oocytes can fully rescue embryo viability (Browning and Strome, 1996). Finally, since unfertilized embryos undergo repeated DNA replication, sperm are involved in the choice between endomitosis and meiotic completion (Sadler and Shakes, 2000). The exact relationship between the sperm supplied factors discussed here and egg activation need to be more fully determined.

## GENETIC AND MOLECULAR ANALYSIS OF FERTILIZATION

The hermaphrodite/male nature of *C. elegans* facilitates the identification of mutants that affect sperm and no other cells. Mutant hermaphrodites that are spermatogenesis-defective (*spe*) or fertilization-defective (*fer*) are self-sterile and lay unfertilized oocytes (Fig. 3). However, when these otherwise healthy worms are crossed to wild-type males as a source of sperm, they can produce progeny. Genetic screens for this phenotype have identified more than 40

genes that affect sperm development or function (for reviews see L'Hernault and Singson, 2000; L'Hernault, 1997). The *fer* gene designation has been discontinued and all new mutants that display the phenotype depicted in Fig. 3 are now given the *spe* designation. Based on the number of *spe* and *fer* genes with multiple alleles and the large number of known sperm-specific transcripts, the worm genome is still far from saturation for mutants with this phenotype (S. L'Hernault, personal communication; S. Ward, personal communication). Not surprisingly, the majority of *spe* or *fer* mutations identified thus far alter or arrest sperm development. The study of *C. elegans spe* and *fer* genes has helped provide important insights into the nature of cellular morphogenesis, asymmetric cell divisions, cellular motility, signal transduction, the regulation of lifespan, and cell-cycle regulation in sperm development (L'Hernault and Roberts, 1995; L'Hernault, 1997). Only more recently have worms been used as a model system for fertilization. A subset of the *spe* and *fer* mutations are particularly relevant to fertilization (*spe-9, spe-13, spe-19, spe-36, spe-38,* and *fer-14*). Mutations in any of these genes cause worms to produce sperm with normal morphology and motility that cannot fertilize oocytes even after gamete contact (Singson *et al.*, 1998; L'Hernault *et al.*, 1988; A. Singson and S. L'Hernault, unpublished observations). Therefore, these genes are specifically required for sperm function at fertilization.

spe-9

As introduced above, *spe-9* mutants were originally selected as hermaphrodites that cannot produce self-progeny but do produce oocytes that can be fertilized by wild-type male-derived sperm (Singson *et al.*, 1998; L'Hernault *et al.*, 1988). Male worms that are homozygous for mutations in *spe-9* are also sterile. The morphology of *spe-9* mutant sperm isolated from both male and hermaphrodite worms is indistinguishable from wild-type sperm when examined by light and electron microscopy. Sperm from *spe-9* mutants undergo normal spermiogenesis both *in vivo* and *in vitro*. Furthermore, *spe-9* mutant sperm can be seen in contact with oocytes passing through the spermathecae without fertilization taking place. Finally, *spe-9* mutant sperm can induce wild-type levels of ovulation, are motile, can locate the spermathecae, and can participate in sperm competition (see below; Singson *et al.*, 1999, 1998).

The *spe-9* gene encodes a sperm-specific transmembrane protein that consists primarily of 10 epidermal growth factor (EGF)-like repeats in its extracellular domain (Singson *et al.*, 1998). A common feature of proteins that include EGF-like motifs is their involvement in extracellular functions such as adhesive and ligand-receptor interactions (Davis, 1990). Moreover, the SPE-9 EGF-like repeats are most similar to those seen in the extracellular domains of the Notch/LIN-12/GLP-1 family of transmembrane receptors and their ligands (Singson *et al.*, 1998). This class of EGF-like repeat is a defining feature of the Notch family of molecules (Artavanis-Tsakonas *et al.*, 1999). Furthermore, the overall structure of SPE-9 suggests
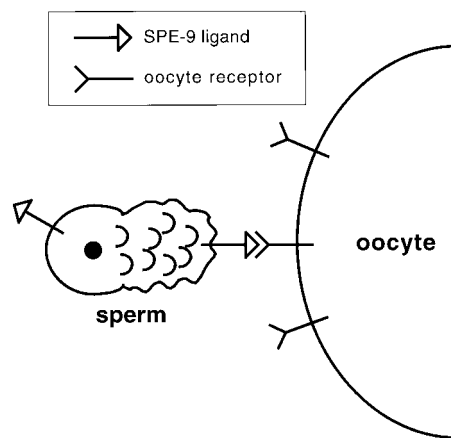
FIG. 4. A model for SPE-9 function during *C. elegans* fertilization. SPE-9 on the surface of sperm interacts with a receptor on the oocyte and mediates gamete recognition, adhesion, and/or signaling during fertilization. It is not yet known if SPE-9 is localized to a particular region of the mature sperm plasma membrane.

that it is more similar to the ligand class of these molecules (Delta and Serrate from *Drosophila*, APX-1 and LAG-2 from *C. elegans,* and the Jagged proteins from mammals). The Notch pathway is known to play a role primarily in juxtacrine cell–cell signaling that results in specific cell fate decisions during the differentiation of many tissues in a variety of organisms (Artavanis-Tsakonas *et al.*, 1999). Point mutations and an ongoing structural analysis of SPE-9 indicate that its EGF-like motifs are critical for its biological activity (Singson *et al.*, 1998; S. Zannoni and A. Singson, unpublished observations). Additionally, secreted forms of the SPE-9 extracellular domain are nonfunctional in transgenic worms. This result supports the observation that *spe-9* mutants act cell autonomously. In other words, *spe-9* mutant sperm will never be able to fertilize an oocyte even if they are mixed with wild-type sperm. Preliminary immunolocalization experiments indicate that SPE-9 localizes to the sperm plasma membrane (A. Singson, unpublished observations). Taken together, these data suggest that SPE-9 is involved in the specialized cell–cell interactions required for fertilization. A simple model for SPE-9 function is presented in Fig. 4. In this model, SPE-9 functions as a sperm ligand for an as yet unknown receptor on the surface of the oocyte. The interaction of these two molecules would mediate gamete recognition, adhesion, and/or signaling events required for fertilization. The confirmation of this model will depend on the continued analysis of the *spe-9* pathway and identification of the hypothesized receptor.

Other Genes With spe-9-like Mutant Phenotypes

There are a number of other genes that when mutated can apparently lead to a *spe-9*-like phenotype. Since *spe-9* was the first gene of its type to be studied in depth, I will refer to this group of genes as the *spe-9* class. Although a detailed phenotypic and molecular analysis of many of the *spe-9*

class genes is still incomplete, their analysis should prove very informative. The mutant phenotypes associated with the *spe-9* class genes suggest that their gene products could possibly mediate several different sperm functions during fertilization. Like *spe-9*, these genes could function in gamete recognition, adhesion, and/or signaling events that are required for fertilization. Additionally, some of the *spe-9* class genes could encode components of the cellular machinery required for membrane fusion or egg activation. It is also possible that some *spe-9* class genes could have a more indirect role in fertilization. For instance, these genes could encode proteins required for the subcellular localization of other sperm surface proteins. The sperm of many species are known to have distinct membrane domains associated with different sperm functions (Primakoff and Myles, 1983). Other *spe-9* class genes could encode proteins that are required for the proper maturation or processing of molecules more directly involved in fertilization. Several molecules required for fertility in other systems are thought to be cleaved or modified before they are functional (Primakoff and Myles, 2000; Ikawa *et al.*, 1997). If any of the *spe-9* class genes do function in the localization or maturation of other molecules, their role should be fairly specific since these mutant sperm accomplish most functions normally. Finally, it should be noted that many of the functions mentioned above might not be mutually exclusive. For example, some cell surface molecules are known to have both adhesive and signaling functions (Giancotti and Ruoslahti, 1999; Greenspan, 1990).

SPERM COMPETITION

The study of *C. elegans* reproductive biology has been useful for related studies on the mechanism of sexual selection. These processes are important forces that influence species survival, reproductive behavior, morphology, and physiology (Karr and Pitnick, 1999). Worms have proven to be particularly useful in addressing the mechanisms of sperm competition, a major type of sexual selection.

Sperm competition in *C. elegans* refers to the process whereby male-derived sperm are utilized preferentially to fertilize oocytes over hermaphrodite-derived sperm (Ward and Carrel, 1979). This bias in sperm utilization ensures that outcrossing will occur in this predominately self-fertilizing species. A landmark series of experiments by LaMunyon and Ward have made it clear that a major mechanism in determining paternity in *C. elegans*, and probably in other nematode species, is sperm size (LaMunyon and Ward, 1994, 1995, 1998, 1999). Male worms seem to invest more in their sperm in that they make larger sperm with larger pseudopods. These larger sperm can move more efficiently than smaller sperm and this increased motility allows male-derived sperm to displace smaller hermaphrodite-derived sperm from the spermathecae. Large sperm size is particularly evident in gonochoristic nematode species (males and females, a state thought to be ancestral to hermaphroditism) where sperm competition

between the sperm of different males may occur frequently (LaMunyon and Ward, 1999). In *C. elegans*, sperm competition leads to the functional suppression of hermaphrodite self-fertility. Sperm produced by males with any of several different *spe-9* class mutations, although unable to fertilize oocytes, can also suppress hermaphrodite self-fertility (Singson *et al.*, 1999). This result leads to the somewhat counterintuitive conclusion that sperm competition mechanisms are independent of fertilization in *C. elegans*.

There are several emerging lines of evidence that suggest that factors other than just sperm size may influence paternity in nematodes. Recent experiments examining cross-species inseminations have been done in which large sperm from one species occupy the spermathecae of another species with smaller sperm. Although species barriers may be a factor, the large sperm did not have any impact on the utilization of the smaller sperm (K. Hill and S. L'Hernault, unpublished observations). It has also been observed that in some reduced fertility mutant worms, hermaphrodites have a sperm selection mechanism that can influence sperm utilization (P. Kadandale and A. Singson, unpublished observations). Finally, in *C. briggsae*, X-bearing sperm that give rise to hermaphrodite progeny are more competitive than nullo-X sperm that give rise to males (LaMunyon and Ward, 1997). The nature of these phenomena and their relationship to sperm size need to be more fully investigated.

PERSPECTIVES

Fertilization is a process of fundamental importance in developmental biology. Despite intense study, sperm–egg interactions are still poorly understood at the molecular level. *C. elegans* is proving to be an excellent model system for the study of fertilization and related questions on sperm competition. A primary advantage of studying fertilization in worms is the ability to make mutants that affect fertility. A group of genes has been identified that are required for sperm function during fertilization. Characterization of these genes should identify new components of the fertilization pathway and it is likely that more genes with functions in fertilization remain to be identified. Furthermore, not every gene required for fertilization will be sperm specific. To date, no specific search has been conducted for mutants that affect egg function at fertilization. It is possible that this type of mutant was isolated and discarded in screens for maternal effect lethals (D. Shakes, personal communication). Promising new screening strategies and technologies such as RNA interference (RNAi) may make this type of screen feasible. Additionally, modifier screens starting with mutant sperm genes may lead to the identification of important egg proteins. For instance, new mutations that suppress the sterility of *spe-9* mutants may help to identify an egg receptor. Finally, with the worm genome sequenced, candidate gene and reverse genetic approaches can be applied to questions of fertility.

There are a number of fundamental questions concerning worm reproductive biology that still remain unresolved. For

example, the signal(s) that attracts sperm to the spermathecae, the precise way that sperm and egg first meet, and the nature of the block to polyspermy are unknown. The development of new experimental approaches will help to answer these questions. For instance, *in vitro* fertilization has not yet been achieved in *C. elegans* (Goldstein and Hird, 1996; S. L'Hernault, personal communication). *In vitro* fertilization assays could help assign function to newly discovered molecules. The development of new techniques that complement existing approaches for the study of worm fertilization is one of the major challenges of the future. As the molecular nature of fertilization in *C. elegans* is revealed, it should have implications for parasitic nematode control (Scott, 1996), complement studies in other systems, and provide new insights into how sperm and egg come together to initiate the life of a new individual.

ACKNOWLEDGMENTS

REFERENCES

Achanzar, W. E., and Ward, S. (1997). A nematode gene required for sperm vesicle fusion. *J. Cell Sci.* **110,** 1073–1081.

Albertson, D. G. (1984). Formation of the first cleavage spindle in nematode embryos. *Dev. Biol.* **101,** 61–72.

Albertson, D. G., and Thomson, J. N. (1993). Segregation of holocentric chromosomes at meiosis in the nematode, *Caenorhabditis elegans. Chromosome Res.* **1,** 15–26.

Aroian, R. V., Field, C., Pruliere, G., Kenyon, C., and Alberts, B. M. (1997). Isolation of actin-associated proteins from *Caenorhabditis elegans* oocytes and their localization in the early embryo. *EMBO J.* **16,** 1541–15549.

Artavanis-Tsakonas, S., Rand, M. D., and Lake, R. J. (1999). Notch signaling: Cell fate control and signal integration in development. *Science* **284,** 770–776.

Browning, H., and Strome, S. (1996). A sperm-supplied factor required for embryogenesis in *C. elegans. Development* **122,** 391–404.

Clandinin, T. R., DeModena, J. A., and Sternberg, P. W. (1998). Inositol trisphosphate mediates a RAS-independent response to LET-23 receptor tyrosine kinase activation in *C. elegans. Cell* **92,** 523–533.

Davis, C. G. (1990). The many faces of epidermal growth factor repeats. *New Biol.* **2,** 410–419.

Emmons, S. W., and Sternberg, P. W. (1997). Male development and mating behavior. *In* "*C. Elegans* II" (D. L. Riddle., T. Blumenthal,

B. J. Meyer, and J. R. Priess, Eds.), pp. 295–334. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Foor, W. E. (1968). Zygote formation in *Ascaris lumbricoides* (Nematoda). *J. Cell Biol.* **39,** 119–134.

Foor, W. E. (1970). Spermatozoan morphology and zygote formation in nematodes. *Biol. Reprod.* **2** (Suppl 2), 177–120.

Giancotti, F. G., and Ruoslahti, E. (1999). Integrin signaling. *Science* **285,** 1028–1032.

Golden, A. (2000). Cytoplasmic flow and the establishment of polarity in *C. elegans* 1-cell embryos. *Curr. Opin. Genet. Dev.* **10,** 414–420.

Goldstein, B., Frisse, L. M., and Thomas, W. K. (1998). Embryonic axis specification in nematodes: Evolution of the first step in development [published erratum appears in *Curr. Biol.* (1998), **8** (5), 303]. *Curr. Biol.* **8,** 157–160.

Goldstein, B., and Hird, S. N. (1996). Specification of the anteroposterior axis in *Caenorhabditis elegans. Development* **122,** 1467–1474.

Greenspan, R. J. (1990). The *Notch* gene, adhesion, and developmental fate in the *Drosophila* embryo. *New Biol.* **2,** 595–600.

Greenstein, D., Hird, S., Plasterk, R. H., Andachi, Y., Kohara, Y., Wang, B., Finney, M., and Ruvkun, G. (1994). Targeted mutations in the *Caenorhabditis elegans* POU homeo box gene ceh-18 cause defects in oocyte cell cycle arrest, gonad migration, and epidermal differentiation. *Genes Dev.* **8,** 1935–1948.

Hall, D. H., Winfrey, V. P., Blaeuer, G., Hoffman, L. H., Furuta, T., Rose, K. L., Hobert, O., and Greenstein, D. (1999). Ultrastructural features of the adult hermaphrodite gonad of *Caenorhabditis elegans*: Relations between the germ line and soma. *Dev. Biol.* **212,** 101–123.

Hill, D. P., Shakes, D. C., Ward, S., and Strome, S. (1989). A sperm-supplied product essential for initiation of normal embryogenesis in *Caenorhabditis elegans* is encoded by the paternal-effect embryonic-lethal gene, *spe-11* [published erratum appears in *Dev. Biol.* (1990), **139** (1), 230]. *Dev. Biol.* **136,** 154–166.

Hirsh, D., Oppenheim, D., and Klass, M. (1976). Development of the reproductive system of *Caenorhabditis elegans. Dev. Biol.* **49,** 200–219.

Hodgkin, J. (1983). Male phenotypes and mating efficiency in *Caenorhabditis elegans. Genetics* **103,** 43–64.

Honda, H. (1925). Experimental and cytological studies on bisexual and hermaphroditic free-living nematodes with special reference to the problems of sex. *J. Morphol.* **40,** 191–233.

Hubbard, E. J., and Greenstein, D. (2000). The *Caenorhabditis elegans* gonad: A test tube for cell and developmental biology. *Dev. Dyn.* **218,** 2–22.

Ikawa, M., Wada, I., Kominami, K., Watanabe, D., Toshimori, K., Nishimune, Y., and Okabe, M. (1997). The putative chaperone calmegin is required for sperm fertility. *Nature* **387,** 607–611.

Italiano, J. E., Jr., Roberts, T. M., Stewart, M., and Fontana, C. A. (1996). Reconstitution in vitro of the motile apparatus from the amoeboid sperm of *Ascaris* shows that filament assembly and bundling move membranes. *Cell* **84,** 105–114.

Iwasaki, K., McCarter, J., Francis, R., and Schedl, T. (1996). emo-1, a *Caenorhabditis elegans* Sec61p gamma homologue, is required for oocyte development and ovulation. *J. Cell Biol.* **134,** 699–714.

Karr, T. L., and Pitnick, S. (1999). Sperm competition: Defining the rules of engagement. *Curr. Biol.* **9,** R787–R790.

Kemphues, K. (2000). PARsing embryonic polarity. *Cell* **101,** 345–348.

Kemphues, K. J., and Strome, S. (1997). Fertilization and establishment of polarity in the embryo. *In* "*C. Elegans* II" (D. L. Riddle.,

T. Blumenthal, B. J. Meyer, and J. R. Priess, Eds.), pp. 241–269. Cold Spring Harbor. Laboratory Press, Cold Spring Harbor, NY.

Kimble, J., and Ward, S. (1988). Germ-line development and fertilization. *In* "The Nematode *Caenorhabditis elegans* " (W. B. Wood, Ed.), pp. 191–213. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Klass, M., Wolf, N., and Hirsh, D. (1976). Development of the male reproductive system and sexual transformation in the nematode *Caenorhabditis elegans. Dev. Biol.* **52**, 1–18.

L'Hernault, S. W. (1997). Spermatogenesis. *In* "*C. Elegans* II" (D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Priess, Eds.), pp. 271–294. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

L'Hernault, S. W., and Roberts, T. M. (1995). Cell biology of nematode sperm. *Methods Cell Biol.* **48**, 273–301.

L'Hernault, S. W., Shakes, D. C., and Ward, S. (1988). Developmental genetics of chromosome *I* spermatogenesis-defective mutants in the nematode *Caenorhabditis elegans. Genetics* **120**, 435–452.

L'Hernault, S. W., and Singson, A. (2000). Developmental genetics of spermatogenesis in the nematode *Caenorhabditis elegans. In* "The Testis: From Stem Cell to Sperm Function, Serono Symposium USA" (E. Goldberg, Ed.), pp. 109–119. Springer-Verlag Inc., New York.

LaMunyon, C. W., and Ward, S. (1994). Assessing the viability of mutant and manipulated sperm by artificial insemination of *Caenorhabditis elegans. Genetics* **138**, 689–692.

LaMunyon, C. W., and Ward, S. (1995). Sperm precedence in a hermaphroditic nematode *Caenorhabditis elegans* is due to competitive superiority of male sperm. *Experientia* **51**, 817–823.

LaMunyon, C. W., and Ward, S. (1997). Increased competitiveness of nematode sperm bearing the male X chromosome. *Proc. Natl. Acad. Sci. USA* **94**, 185–189.

LaMunyon, C. W., and Ward, S. (1998). Larger sperm outcompete smaller sperm in the nematode *Caenorhabditis elegans. Proc. R. Soc. Lond. Ser. B Biol. Sci.* **265**, 1997–2002.

LaMunyon, C. W., and Ward, S. (1999). Evolution of sperm size in nematodes: Sperm competition favours larger sperm. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **266**, 263–267.

Liu, K. S., and Sternberg, P. W. (1995). Sensory regulation of male mating behavior in *Caenorhabditis elegans. Neuron* **14**, 79–89.

McCarter, J., Bartlett, B., Dang, T., and Schedl, T. (1997). Soma-germ cell interactions in *Caenorhabditis elegans*: Multiple events of hermaphrodite germline development require the somatic sheath and spermathecal lineages. *Dev. Biol.* **181**, 121–143.

McCarter, J., Bartlett, B., Dang, T., and Schedl, T. (1999). On the control of oocyte meiotic maturation and ovulation in *C. elegans. Dev. Biol.* **205**, 111–128.

Meyer, B. J. (1997). Sex determination and X chromosome dosage compensation. *In* "*C. Elegans* II" (D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Priess, Eds.), pp. 209–240. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Minniti, A. N., Sadler, C., and Ward, S. (1996). Genetic and molecular analysis of *spe-27*, a gene required for spermiogenesis in *Caenorhabditis elegans* hermaphrodites. *Genetics* **143**, 213–223.

Nance, J., Minniti, A. N., Sadler, C., and Ward, S. (1999). *spe-12* encodes a sperm cell surface protein that promotes spermiogenesis in *Caenorhabditis elegans. Genetics* **152**, 209–220.

O'Connell, K. F., Maxwell, K. N., and White, J. G. (2000). The *spd-2* gene is required for polarization of the anteroposterior axis and formation of the sperm asters in the *Caenorhabditis elegans* zygote. *Dev. Biol.* **222**, 55–70.

Primakoff, P., and Myles, D. G. (1983). A map of the guinea pig sperm surface constructed with monoclonal antibodies. *Dev. Biol.* **98**, 417–428.

Primakoff, P., and Myles, D. G. (2000). The ADAM gene family: Surface proteins with adhesion and protease activity. *Trends Genet.* **16**, 83–87.

Reinke, V., Smith, H. E., Nance, J., Wang, J., Van. Doren., C., Begley, R., Jones, S. J., Davis, E. B., Scherer, S., Ward, S., and Kim, S. K. (2000). A global profile of germline gene expression in *C. elegans. Mol. Cell.* **6**, 605–616.

Riddle, D. L., Blumenthal, T., Meyer, B. J., and Priess, J. R. (1997). "*C. elegans* II," pp. 1222. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Roberts, T. M., Pavalko, F. M., and Ward, S. (1986). Membrane and cytoplasmic proteins are transported in the same organell complex during nematode spermatogenesis. *J. Cell Biol.* **102**, 1787–1796.

Roberts, T. M., and Stewart, M. (2000). Acting like actin. The dynamics of the nematode major sperm protein (msp) cytoskeleton indicate a push-pull mechanism for amoeboid cell motility. *J. Cell Biol.* **149**, 7–12.

Sadler, P. L., and Shakes, D. C. (2000). Anucleate *Caenorhabditis elegans* sperm can crawl, fertilize oocytes and direct anterior-posterior polarization of the 1-cell embryo. *Development* **127**, 355–366.

Schedl, T. (1997). Developmental genetics of the germ line. *In* "*C. Elegans* II" (D. L. Riddle, T. Blumenthal, B. J. Meyer, and J. R. Priess, Eds.), pp. 241–269. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Scott, A. L. (1996). Nematode sperm. *Parasitol. Today* **12**, 425–430.

Shakes, D. C., and Ward, S. (1989). Initiation of spermiogenesis in *C. elegans*: A pharmacological and genetic analysis. *Dev. Biol.* **134**, 189–200.

Singson, A., Hill, K. L., and L'Hernault, S. W. (1999). Sperm competition in the absence of fertilization in *Caenorhabditis elegans. Genetics* **152**, 201–208.

Singson, A., Mercer, K. B., and L'Hernault, S. W. (1998). The *C. elegans spe-9* gene encodes a sperm transmembrane protein that contains EGF-like repeats and is required for fertilization. *Cell* **93**, 71–79.

Theriot, J. A. (1996). Worm sperm and advances in cell locomotion. *Cell* **84**, 1–4.

Vacquier, V. D. (1998). Evolution of gamete recognition proteins. *Science* **281**, 1995–1998.

Ward, S., Argon, Y., and Nelson, G. A. (1981). Sperm morphogenesis in wild-type and fertilization-defective mutants of *Caenorhabditis elegans. J. Cell Biol.* **91**, 26–44.

Ward, S., and Carrel, J. S. (1979). Fertilization and sperm competition in the nematode *Caenorhabditis elegans. Dev. Biol.* **73**, 304–321.

Ward, S., Hogan, E., and Nelson, G. A. (1983). The initiation of spermiogenesis in the nematode *Caenorhabditis elegans. Dev. Biol.* **98**, 70–79.

Ward, S., Roberts, T. M., Nelson, G. A., and Argon, Y. (1982). The development and motility of *Caenorhabditis elegans* spermatozoa. *J. Nematol.* **14**, 259–266.

Wassarman, P. M. (1999). Mammalian fertilization: Molecular aspects of gamete adhesion, exocytosis, and fusion. *Cell* **96**, 175–183.

Yanagimachi, R. (1994). Mammalian fertilization. *In* "The Physiology of Reproduction" (E. Knobil and J. D. Neill, Eds.), pp. 189–317. Raven Press, New York.