Fertilization: what we can learn from worms

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Infertility and development of contraceptive methods have profound societal affects; however, the genetic mechanisms underlying this are still largely unknown. Here, we describe how using the small worm *Caenorhabditis elegans* has helped us to discover the genes involved in these processes. Nobel Laureate Sydney Brenner established the nematode worm *C. elegans* as a genetic model system with a powerful ability to discover genes in many biological pathways through mutagenesis. In this tradition, many labs have been using the substantial genetic tools established by Brenner and the 'worm' research community to discover genes required for uniting sperm and egg. Our understanding of the molecular underpinnings of the fertilization synapse between sperm and egg rivals that of any organism. Genes have been discovered in worms that share homology and mutant phenotypes with mammals. We provide an overview of the state of our understanding of fertilization in worms as well as exciting future directions and challenges.

Infertility treatment and development of novel contraceptive targets have acute societal importance

Approximately one in 10 couples worldwide face difficulties in conceiving a baby according to the Centers for Disease Control Division of Reproductive Health. For most couples facing infertility there are no visible defects in the sperm or eggs, which often mean that treatment of infertility is imprecise and may not address the underlying issues. One of the reasons for this disconnection in treatment options is that many of the genes and molecules that underlie the process of fertilization are still not identified or have incomplete molecular characterization. This is a barrier to being able to develop treatments for infertility. Furthermore, the incomplete understanding of the molecular mechanisms of fertility also makes it difficult to identify novel targets for contraception. Therefore, there is a pressing need to understand the genetics and cell biology of fertilization.

Fertility is a complex process with many steps

Fertilization requires coordinated cellular events (Figure 1a). First the sperm cell must activate to be able to move towards the egg; then once the sperm and egg come in contact with one another, the sperm cell and egg cell must recognize that they're from the same species; then binding and fusion of the sperm and egg occurs. After the fusion of the sperm and egg, the fertilized egg will activate to start the early stages of embryonic development. Each of these steps in fertilization requires both signalling between the sperm and egg cell as well as within the individual sperm and egg cells. If any steps in fertilization aren't successful, this can lead to infertility. These conserved steps occur in many different species of animals from worms to humans to flies and mice. There is some diversity in the morphology of the sperm and egg in different species dependent on the environment where fertilization happens (Figure 1a). For example, fertilization in a wet environment for some species of frogs will require specialized sperm movement structures. *Caenorhabditis elegans* sperm are amoeboid and crawl. Apparently, this is the most effective mode of motility in a crowded tunnel like reproductive tract. Although the sperm and egg cells might look slightly different in different organisms, all reproductive cells have to complete the same steps. The ability to study fertilization in different organisms is highly advantageous. Due to ethical considerations, we do not do many invasive fertility experiments on humans. Other animals such as rats and mice have contributed greatly to the body of knowledge surrounding fertilization; however, generating mutants in these animals can take months and are quite expensive to maintain in a laboratory setting. In our work, we use the worm *C. elegans*, which has a quick generation time, the ability to do unbiased gene discovery and a plethora of available genetic tools and is relatively inexpensive to maintain.

C. elegans: a model for fertility research

The nematode *C. elegans* has been a pioneer in facilitating the study of cell biology and genetics.

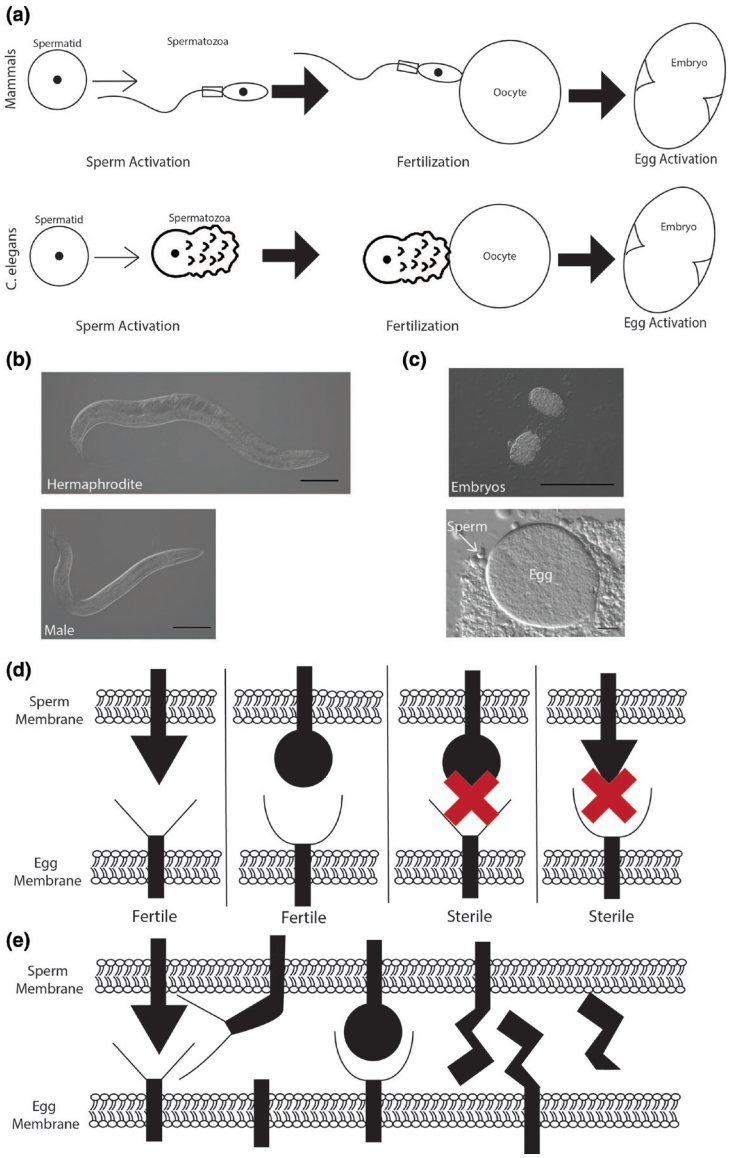

Figure 1. Fertilization is a largely conserved process with complex molecular interactions. (A) Steps of fertilization: the *C. elegans* sperm turns from a round immotile spermatid into a motile ameoboid spermatozoon after sperm activation, just as mammalian sperm form a flagellar 'tail'. Following sperm activation, fertilization and egg activation occurs. (B) Representative images of a *C. elegans* hermaphrodite and male. A full-grown adult hermaphrodite is about the size of a period on a printed page. Scale bar 100 μM. (C) Developing embryos, scale bar 100 μM, and gametes (sperm and egg, scale bar 10 μM). (D) Historical receptor–ligand model of fertilization: one protein on the surface of the sperm would bind with one protein on the surface of the egg and fertilization would occur. Mismatch in these proteins on the surface of the cells would block fertilization. There must be species specificity for binding to occur between the sperm and egg cells. (E) Current model of the fertilization synapse containing multiple receptor–ligand pairs as well as secreted proteins and other protein interactions.

This has led to three Nobel Prizes for discoveries in *C. elegans*. First, in 2002, a Nobel Prize for the genetic regulation of organ development and programmed cell death, next in 2006, for the discovery of gene silencing by double-stranded RNA and, most recently in 2008, for the development of green fluorescent protein as a tool for cell biology. Each of these amazing advances in science has contributed to establishing the worm as a model for reproductive biology.

C. elegans are small 1-mm roundworms (Figure 1b), which have approximately 80% of gene homology with humans. They exist as one of two sexes, either hermaphrodite animals that produce sperm during a late larval stage and then eggs as

adults, or males which only produce sperm and allow mating and increased genetic diversity (Figure 1c). The hermaphrodite sex is an advantage for studying fertility as we're able to examine one animal and see if they have defects in fertility without mating to the opposite sex due to the presence of sperm and eggs in that animal. *C. elegans* develop from embryo to adult in just 3 days and an unmated hermaphrodite can produce up to 300 progeny in their lifetime. The worms are also transparent, which allows for us to examine fertilization as it is occurring in the animal without dissection. One of the most beneficial aspects of *C. elegans* is the plethora of powerful genetic tools that allow us to do unbiased genetic approaches, keep infertile animals alive to study and identify the genes. In order to understand what genetic components may relate to fertilization, we need to be able to identify mutations that prevent reproduction. *C. elegans* as a model species has temperature-sensitive conditional mutants, where at low temperatures the animal is fertile and at high temperatures the animal is sterile. This enables scientists to search for mutations that do not allow reproduction to take place. Another tool is balancer chromosomes, which are specially engineered chromosomes that select and maintain animals with fertility defects. These available tools allow us to search for sterile mutations to discover the genes involved in this process. Moreover, there is a robust research community of researchers studying genetics and cell biology in *C. elegans*.

Our work hinges on gene discovery. We isolate sterile animals from large mutagenesis screens, which randomly mutate genes, and then determine what change in the DNA occurred and how it impacts fertility. Similar to a human fertility clinic, we run a battery of tests to determine what event of fertilization is affected. Can the sperm move to the site of fertilization and make contact with the egg? Is the sperm able to bind with the egg? Can the egg activate after the sperm enters for embryonic development? Understanding the specific genes that are required for each event in fertilization helps us to understand what is occurring at the molecular and cellular level of fertilization.

The fertilization synapse: the emerging picture of molecular complexity

Research done in *C. elegans* as well as other organisms has shown that many genes and molecules are required for fertilization. Early models of fertilization envisioned a simple receptor–ligand interaction where one protein on the surface of the sperm and one protein on the surface of the egg mediated fertilization (Figure 1d). Species-specific fertilization hinged on precise molecular complementation with a ligand and a receptor. As the field of fertilization has continued to develop, it has been discovered that many proteins on the surface of both the sperm and egg must interact at the correct expression levels and places for fertilization to successfully occur (Figure 1e). The multiple interactions of cell adhesion, signalling, secretion and molecular organization that are required both between and within the sperm and egg are analogous to synapses identified in other cell types. The fertilization synapse is similar to synapses such as the immunological synapse in immune responses or neural synapses between neurons. All cellular synapses also require coordinated adhesion, signalling, secretion and specialized cellular structures just as fertilization does. The intricacies of the process of fertilization are one reason that it has been so challenging to identify the genes and molecules involved. Work being completed in *C. elegans* has helped to dissect out the emerging complexity of the fertilization synapse.

Fertility genes found in *C. elegans* have homologues in mammals

As we discover more about the genes involved in *C. elegans* fertilization, we see parallels emerging between genes involved in *C. elegans* and those found in mammals. The research done in *C. elegans* helps to set the stage to look for gene homologues in other organisms that might be functioning in similar processes. Some notable gene structure homologs are the immunoglobulin domains found in the *C. elegans* genes *spe-45, spe-51* and in the *Izumo* and *Spaca6* genes in mice. These proteins on the cell surface are important for the sperm and egg to bind to one another. Additionally, a specific type of protein, first identified as having a role in fertilization in *C. elegans,* was published in 2005 as SPE-42 and SPE-49. Recently, in 2021 homologues in mice, DCST1 and DCST2 were also found to be required for sperm–egg interactions, thus showing the value of gene discovery in *C. elegans*. Furthermore, our lab has provided the first experimental evidence for sperm-secreted proteins. Secreted proteins can stay associated with the plasma membrane or surface of the cell and can interact with other cells to signal, bind or fuse with the egg. The recent work has also shown that a recently identified sperm mammalian gene Sof1 is secreted. Currently, *C. elegans* has 12 genetically validated genes identified which are involved in fertilization, more than any other organism. The number of gene homologues that have been found in both *C. elegans* and other animals underlies how valuable *C. elegans* is as a frontline model for discovering the genes necessary for fertilization.

Further genetic and biochemical analysis is needed to continue to understand fertilization

Even as we continue to learn more about how the sperm and eggs interact with one another, there are still many areas that require further investigation. One important gap in knowledge is what genes are found on the surface of the egg that are required for fertilization. Far more sperm surface genes have been identified compared to just a few genes on the egg's surface. This is true across all organisms, highlighting the difficulty in identifying these genes. More egg genes will help to understand how egg-mediated events such as fusion of the cells are regulated. Identification of fewer egg genes can be due to biological reasons such as dosage of molecules on the surface, gene redundancy and pleiotropy (when one gene regulates multiple biological processes) as well as possible experimental bias. Additionally, as we continue to identify more of the genes required, we continue to elucidate which components interact with one another to recapitulate the fertilization synapse using genetic, biochemical and cell biological experimental approaches. Future research will also focus on better understanding genes that have redundancy or multiple roles during development addressing the pleiotropy issue. The contributions from our research into *C. elegans* fertilization will help us to understand how speciation emerges and evolution at the molecular and genetic level. This will provide us better knowledge of how species are isolated from one another as well as how species in diverse environmental conditions from land to water are able to reproduce. *C. elegans* have been and will continue to be instrumental to our growing understanding of fertilization. ■

Further Reading

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