Oogenesis: The Universal Process

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Foreword

August Weissman dedicated his book, 'The Germ-Plasm' (1892) to the memory of Charles Darwin. Weissman understood the urgent need for a proper theory of heredity, knew that Darwin's ideas on the subject were inadequate, and equally clearly recognized that, unlike "the perishable body of the individual" something —the "hereditary substance"—had to be passed from generation to generation in eggs and sperm and hence, "the continuity of the germ-plasm". It took another 10–15 years before Thomas Hunt Morgan accepted that the behaviour of chromosomes explained Mendel's laws (of which Weissman was unaware; indeed, neither 'chromosomes' nor 'nucleus' feature in the index of his book), and one might say that it took the structure of DNA, and the idea that "DNA makes RNA makes protein" to bring biology into the modern era. We don't think twice, these days, about the continuity of life on earth, and accept without question that cells only arise from pre-existing cells; this is all so integral to the biologist's world view that a number of great mysteries hardly ever come to light. Broadly speaking, these underlie the topic of this collection of essays about oogenesis. How does the germ-plasm manage to avoid the body's mortality?

Quite apart from deep questions of this kind, the details of how eggs come to be eggs are fascinating and instructive well beyond the relatively narrow field of reproductive biology. Likewise the events just before and after fertilization, when the egg meets the sperm and starts to become a new body. This book contains a series of essays, authoritative and fascinating reviews of all aspects of oogenesis.

The reviews follow a kind of chronological or developmental order from questions about sex determination in worms to assisted reproduction in humans. The simple-sounding decision of what sex to become is anything but, and we are reminded that it is quite possible to be a hermaphrodite and survive perfectly successfully. We discuss the setting-aside of germ cells from the soma early in development as well as the surprisingly complicated decision-making processes that lead to the differentation of eggs or sperm. Meiosis is a necessary common process for both kinds of gamete, and we have reviews of what is known about meiotic chromosome pairing and homologous recombination. In oocytes, the meiotic divisions often take place shortly before the cell becomes a fully-fledged, fertilizable egg, and is subject to some elaborate controls that are still far from completely understood.

The choice between becoming an egg or a sperm is one of the most complex of development, and it is made long before changes in cell morphology take place. This fate decision depends on sex chromosomes and depends on interactions between gonadal somatic cell lineages and the germ cells themselves. Indeed, metazoans have evolved a complex array of interactions between the soma and germ line that regulate reproductive success. During the growth period of oogenesis, meiotically-arrested

oocytes accumulate large quantities of dormant maternal mRNAs. Meiotic resumption requires cascades of successive unmasking, translation, and discarding of these maternal mRNAs. Not only is the the timing of specific translation finely regulated during this period, but the embryonic axis and even the establishment of the next generation of germ cells are also defined through the localization of such dormant mRNAs within the oocyte. And of course, meiosis is an integral component of the oogenesis program, accomplishing the essential reduction of diploid chromosome number to a haploid complement in preparation for zygotic development. Crossovers between homologous chromosomes not only generate genetic diversity, but are actually required for the accurate segregation of homologous chromosomes in most organisms. At a fundamental level, the ability to reduce chromosome number two-fold requires the formation of correct pairwise associations between homologous chromosomes and further recombination. Chromosomes in the germ line exhibit unique structural and functional properties that are essential to coordinate the complex events of meiosis with subsequent changes leading towards nuclear and epigenetic maturation during gametogenesis.

Once meiosis is (almost) complete and sufficient growth has been achieved, the oocyte is ready to exit the prophase I arrest of meiosis and undergo the two meiotic divisions. Once again, communication between somatic cells and the oocyte are required to control this unique prophase-to-metaphase transition. The oocyte normally undergoes a highly asymmetric division that is critical to ensure the formation of a competent resource-rich egg, capable of generating a living euploid descendent after fertilization. In the last few years, our understanding of the principles of meiotic spindle assembly has significantly improved, due to the elucidation of common mitotic and meiotic principles as well as special features that apply to female meiosis and the generation of extreme asymmetry in the formation of polar bodies. There is great interest in the business of chromosome segregation from a medical standpoint, since chromosome non-disjunction produces all kinds of problems including developmental arrest, miscarriages, or severe birth defects such as Down's syndrome. The basis for these errors are still a matter of intense investigation, with a long-term view to prevention as well as diagnosis.

The regulation of the cell cycle during the life of an oocyte is extremely interesting, with multiple arrest points. Here, there is tremendous specificity and variability from organism to organism, bewildering to the unwary. In some species, it is the arrival of the sperm that reinitiates meiosis. In others, hormonal signals prepare the oocyte for fertilization, and elaborate mechanisms exist to ensure that the sperm hits the egg at the right phase of the cell cycle. So clams release oocytes into the sea and the arrival of the sperm initiates completion of meiosis; frogs and women lay eggs that are arrested in second meiotic metaphase waiting for the sperm to arrive, but sea urchins complete both meiotic divisions and arrest in a dormant G-zero state to await fertilization. Limpets and starfish eggs like to be fertilized while meiotic divisions are in progress; sometimes one marvels that there are any successful matings at all! Extensive studies have gradually revealed the core signalling components required for oocytes to wait for the sperm, and show how common components can be used and reused in different ways to achieve the same end by a variety of routes.

Fertilization marks the completion and culmination of oogenesis. It is a multi-step event that leads to the fusion of two complementary gametes. Compatibility of the particular egg with the correct sperm is determined before the gametes fuse in a variety of ways including the complex behaviour of courtship as well as gamete attraction and gamete molecular recognition and adhesion. The extracellular molecules on each gamete that participate in this species-selective process are thought to co-evolve within a species while diversifying from sister organisms so as to minimize cross-species interactions. But fertilization also initiates early development, and, germane to the oocyte to embryo transition, is the need to dispose of some maternal products. This is achieved via their specific and timely degradation, triggered by the arrival of the sperm.

The mammalian ovary is endowed with a fixed number of follicles because in the female, germline stem cells have been exhausted around the time of birth. The reserve population of potential oocytes, represented by primordial follicles, is gradually depleted by recruitment to the growing stages of oogenesis, but most of these would-be eggs undergo atresia by apoptosis. Over the course of the reproductive lifespan in human females, the total number of follicles declines from about a million to a threshold of around one thousand, below which ovulatory cycles are unsustainable and the menopause intervenes. Thus, ageing of the follicle population commences from the moment it has been established, and is irreversible, but the initial reserve is normally sufficient for fecundity until mid-life. Such basic knowledge of the journey of an oocyte has major implications for our understanding of the molecular mechanisms of aneuploidy as well as the design of clinical procedures to address infertility. Understanding ovarian follicle development is crucial for physicians interested to determine the best assisted reproductive technologies to use for women with fertility-threatening diseases and for scientists to develop experimental foeto-protective strategies.

The study of oocytes has made enormous contributions to the understanding of the molecular composition of the factors promoting M-phase entry. The power and complementarity of investigations into the mechanisms of maturing oocytes on the one hand and yeast genetic studies on the other, coupled with the revolution in molecular cloning allowed us to unravel the basis of cell cycle regulation. But although the heroic phase of the story of maturation promoting factor and points of no return may be over, the study of oocyte and oogenesis is still producing new seeds and comes up with interesting new model organisms that give evolutionary perspective to sexual reproduction. For example, the jellyfish *Clytia* offers a fresh perspective on regulation of oogenesis and its evolutionary history because of the phylogenetic position of the organism and by the simplicity, transparency and experimental accessibility of the female gonad. The development of diverse model systems will surely bring answers to this fascinating question of the evolutionary origins and advantages of sex.

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Section I

Oocyte determination

1

The sperm/oocyte decision, a *C. elegans* perspective

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> No trumpets sound when the important decisions of our life are made. Destiny is made known silently.
>
> *Agnes de Mille*

1.1 Introduction

The decision of germ cells to differentiate as spermatocytes or oocytes is dramatically different from other decisions made during development. First, the magnitude of the response is far greater than in most cell-fate decisions. For example, microarray analyses identified at least 250 oocyte-enriched genes and 650 spermatocyte-enriched genes in *Caenorhabditis elegans* (Reinke *et al.*, 2000). By contrast, touch-receptor cells are defined by only a few dozen genes (reviewed by Goodman, 2006; Bounoutas and Chalfie, 2007). Second, most cell-fate decisions occur in individual cells, or pairs of daughter cells that are being formed by division. However, germ cells retain cytoplasmic contacts with their neighbours during much of development. In *C. elegans,* for example, primordial germ cells begin spermatogenesis or oogenesis as part of a syncytium. Indeed, some cells connected to the syncytium undergo spermatogenesis while others are initiating oogenesis. Third, developing oocytes contain a variety of messenger RNAs and proteins that are needed for embryonic development, and some of these molecules must be prevented from influencing the sperm/oocyte decision itself. Thus, this regulatory decision is unique. Since sperm and oocytes are the most ancient sexually dimorphic cells (reviewed by White-Cooper,

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Doggett and Ellis, 2009), evolution has had a long time to shape solutions to these problems.

In most animals, primordial germ cells differentiate into spermatocytes in males or oocytes in females. However, hermaphrodites like *C. elegans* make both types of gametes in the same gonad, which simplifies the study of how these fates are controlled. In particular, hermaphrodite genetics makes it easy to identify and maintain sterile mutants. Furthermore, these animals are transparent, so developing germ cells can be observed in living worms. Finally, mutant hermaphrodites that make only sperm or only oocytes are easy to identify. Thus, research has been able to create a detailed picture of how the sperm/oocyte decision is regulated in *C. elegans.*

1.2 *C. elegans* hermaphrodites are modified females

Although most species of nematodes produce males and females, hermaphroditism has arisen independently on many occasions (Kiontke and Fitch, 2005). Even in the genus *Caenorhabditis,* two species appear to have acquired this trait independently (Cho *et al.*, 2004; Kiontke *et al.*, 2004). In these species, the *XX* hermaphrodites develop female bodies, but some of their germ cells undergo spermatogenesis late in larval development, producing a small supply of sperm that are stored in the spermatheca. Early in adulthood, hermaphrodites switch to the production of oocytes, which can be fertilized by their own sperm. This pattern of development shows that primordial germ cells have the ability to form either spermatocytes or oocytes, and analysis of *C. remanei* confirms that this capacity is found in related male/female species (Haag, Wang and Kimble, 2002).

Two traits make self-fertile hermaphrodites like *C. elegans* different from cross-fertile hermaphrodites, which are able to mate with each other. First, these nematodes produce sperm by altering germ cell fates in *XX* animals for a short period of time, prior to the onset of oogenesis. Thus, the number of self-sperm is limited by the duration of production. Second, self-fertile hermaphrodites have female gonads, so they provide an excellent model for oogenesis. By contrast, most cross-fertile hermaphrodites have male and female gonads.

1.3 The hermaphrodite gonad provides the normal environment for oogenesis

In many species, the female gonad is essential for germ cells to initiate and carry out oogenesis. This is not true for nematodes, since some mutations that alter the sperm/oocyte decision cause males to make oocytes (for examples, see Barton and Kimble, 1990; Ellis and Kimble, 1995). However, the hermaphrodite gonad does provide the normal setting for oogenesis in nematodes, and oocytes in males do not progress to fertilization. Furthermore, some experiments imply that cells in the somatic gonad directly influence the sperm/oocyte decision (McCarter *et al.*, 1997).

1.3.1 Structure of the hermaphrodite gonad

In *C. elegans,* the hermaphrodite gonad is composed of two symmetrical tubes that meet at a central uterus (Figure 1.1). Each tube contains a large ovotestis and a spermatheca, which adjoins the uterus. The entire process of germ cell differentiation takes place in the two ovotestes, which are each composed of a distal tip cell and five pairs of sheath cells (Figure 1.1; McCarter *et al.*, 1997; Hall *et al.*, 1999, and see www.wormatlas.org for a concise review). Each stage of oogenesis occurs in a separate region of the ovotestis.

The distal tip cells create a stem cell niche, where mitosis continues throughout the animal's life. In the area just beyond the distal tip cells (known as the transition zone), germ cells begin meiosis. This region is not ensheathed by cells of the somatic gonad, although it is covered by a basement membrane. Next, most developing oocytes arrest in the pachytene phase of prophase I while in contact with the large sheath cell 1 pair. Near the bend in the ovotestis, under the sheath cell 2 pair, most oocytes resume progression through meiosis, and some undergo apoptosis (Gumienny *et al.*, 1999). Finally, sheath

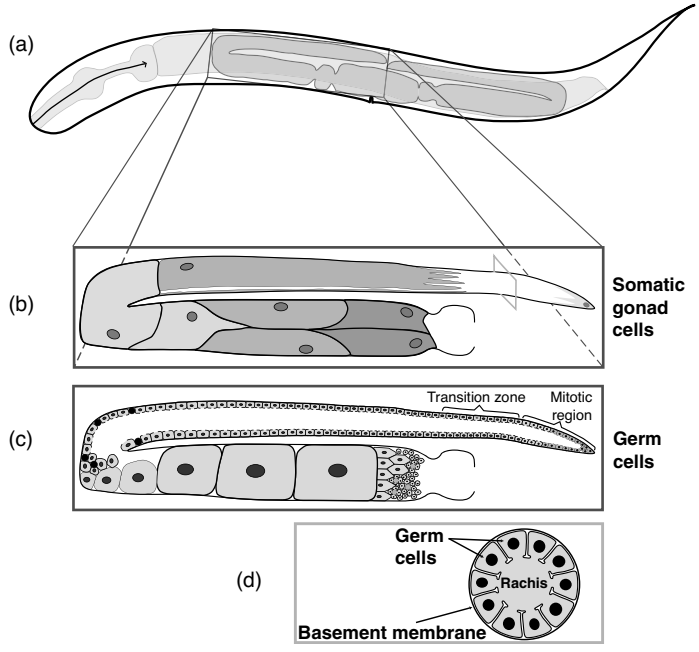

Figure 1.1 Structure of the hermaphrodite gonad. (a) Diagram of a young adult hermaphrodite, showing the digestive system in light green, and the gonad in grey. Anterior is to the left, and ventral is down. (b) Inset diagram of the anterior ovotestis, showing cells of the somatic gonad. The distal tip cell is yellow. Sheath cell 1 is dark blue, sheath cell 2 is light blue, and sheath cell 3 is tan. The second member of each pair is on the opposite side of the gonad, with only the edge of sheath cell 1 visible. Sheath cell pair 4 is peach, and sheath cell pair 5 is orange. (c) Inset diagram of the anterior ovotestis, showing the germ cells. Cells expressing female transcripts and proteins are pink, and those expressing male transcripts are blue. Cell corpses are black circles, and residual bodies are blue circles. (d) Cross-section of the gonad. A full colour version of this figure appears in the colour plate section.

cells 3, 4 and 5 contain extensive actin/myosin networks that support rapidly growing oocytes and control ovulation.

1.3.2 Interactions between gonad and germline

The somatic gonad is descended from two founder cells present in newly hatched larvae (Kimble and Hirsh, 1979). The simplicity of this lineage allows the elimination of groups of gonadal cells by killing their ancestors with a laser microbeam (Kimble and White, 1981; McCarter *et al.*, 1997). When a sheath/spermatheca (SS) precursor cell is killed, the ovotestis contains only a single member of each sheath cell pair, and often produces oocytes instead of sperm (McCarter *et al.*, 1997). Thus, the somatic gonad appears to influence the sperm/oocyte decision. However, killing germ cells sometimes causes animals to make oocytes instead of sperm, so it remains possible that the somatic gonad influences the sperm/oocyte decision indirectly, by promoting robust growth of the germline.

1.4 The core sex-determination pathway regulates somatic and germ cell fates

In *C. elegans,* the same genes regulate sexual fates in both the soma and germline. They act through a signal transduction pathway to control the master transcription factor TRA-1 (Figure 1.2).

1.4.1 The *X*: A ratio determines sex

In nematodes, sexual identity is specified by the ratio of *X* chromosomes to sets of autosomes (Madl and Herman, 1979). Signalling elements on these chromosomes regulate the activity of *xol-1,* a gene that promotes male development (reviewed by Wolff and Zarkower, 2008). In males, XOL-1 represses three *sdc* genes, allowing the expression of HER-1. In hermaphrodites, the absence of XOL-1 allows the SDC

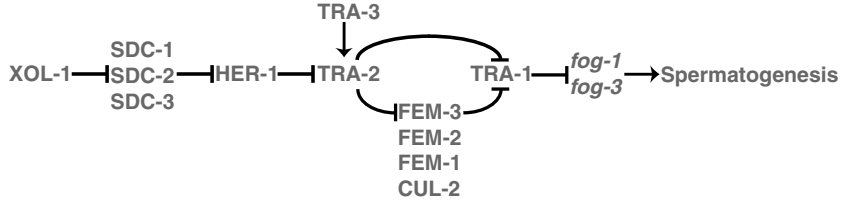

Figure 1.2 The core sex-determination pathway. Genes promoting male fates are blue, and those promoting female fates are pink. Arrows indicate positive interactions, and '⊣' indicates negative interactions. Proteins are indicated by capital letters, and genes by lowercase italics. A full colour version of this figure appears in the colour plate section.

proteins to block the transcription of *her-1*. The SDC proteins also promote dosage compensation (reviewed by Wolff and Zarkower, 2008).

1.4.2 Sexual fates are coordinated by the secreted protein HER-1

HER-1 is a small, secreted protein that causes somatic cells to adopt male fates and germ cells to become sperm. Thus, it acts like a male sex hormone. In *XX* animals, ectopic expression of HER-1 is sufficient to cause spermatogenesis (Perry *et al.*, 1993). In *XO* animals, *her-1* mutations result in hermaphroditic development and the production of oocytes, so *her-1* is required to maintain spermatogenesis (Hodgkin, 1980). However, it is not needed for spermatogenesis *per se,* since null mutants make sperm before switching to oogenesis (Hodgkin, 1980). Although most cells secrete HER-1, mosaic analyses indicate that the germline is most strongly influenced by production from the intestine, which is the major site for protein production and secretion in the worm, and possibly by the somatic gonad as well (Hunter and Wood, 1992).

1.4.3 HER-1 inactivates the TRA-2 receptor

The only target of HER-1 is TRA-2. It produces a large transcript that encodes the transmembrane protein TRA-2A, and two small transcripts that encode the intracellular fragment TRA-2B (Okkema and Kimble, 1991). HER-1 binds the TRA-2A receptor (Okkema and Kimble, 1991; Kuwabara, Okkema and Kimble, 1992; Kuwabara and Kimble, 1995) at an interaction site defined by a dominant mutation in *tra-2* that transforms *XO* animals into hermaphrodites (Hodgkin and Albertson, 1995; Kuwabara, 1996). The complementary site on HER-1 was identified by mutations that block binding in HEK 293 cells (Hamaoka *et al.*, 2004). Although genetic analyses imply that HER-1 inactivates TRA-2A, how it works is unknown. However, *tra-3* behaves like a positive regulator of *tra-2* (Hodgkin, 1980). Since TRA-3 is a calpain protease (Barnes and Hodgkin, 1996) that cleaves TRA-2A *in vitro* (Sokol and Kuwabara, 2000), it might cleave TRA-2A *in vivo* to release an active, intracellular fragment. If so, perhaps the interaction between HER-1 and TRA-2A prevents cleavage.

1.4.4 TRA-2 prevents the FEM proteins from causing TRA-1 degradation

The pathway branches at TRA-2. First, TRA-2 negatively regulates three *fem* genes, which are needed for spermatogenesis and male development (Doniach and Hodgkin, 1984; Kimble, Edgar and Hirsh, 1984; Hodgkin, 1986). FEM-1 has ankyrin repeats (Spence, Coulson and Hodgkin, 1990), FEM-2 is a type 2C protein phosphatase (Pilgrim *et al.*, 1995), and FEM-3 is novel (Ahringer *et al.*, 1992). These proteins cooperate to lower the activity of TRA-1, a transcription factor that controls all sexual fates in the nematode (Hodgkin and Brenner, 1977; Zarkower and Hodgkin, 1992). To do this, FEM-1 binds to CUL-2, a member of the E3 ubiquitin ligase complex that promotes male fates (Starostina *et al.*, 2007), and these four

proteins act together to target TRA-1 for ubiquitinylation and degradation. The net effect is that TRA-1 protein levels are low in males and high in hermaphrodites (Figure 1.3; Schvarzstein and Spence, 2006). Since TRA-2 binds to FEM-3 (Mehra *et al.*, 1999), it might work by inhibiting this FEM/CUL-2 complex and protecting TRA-1.

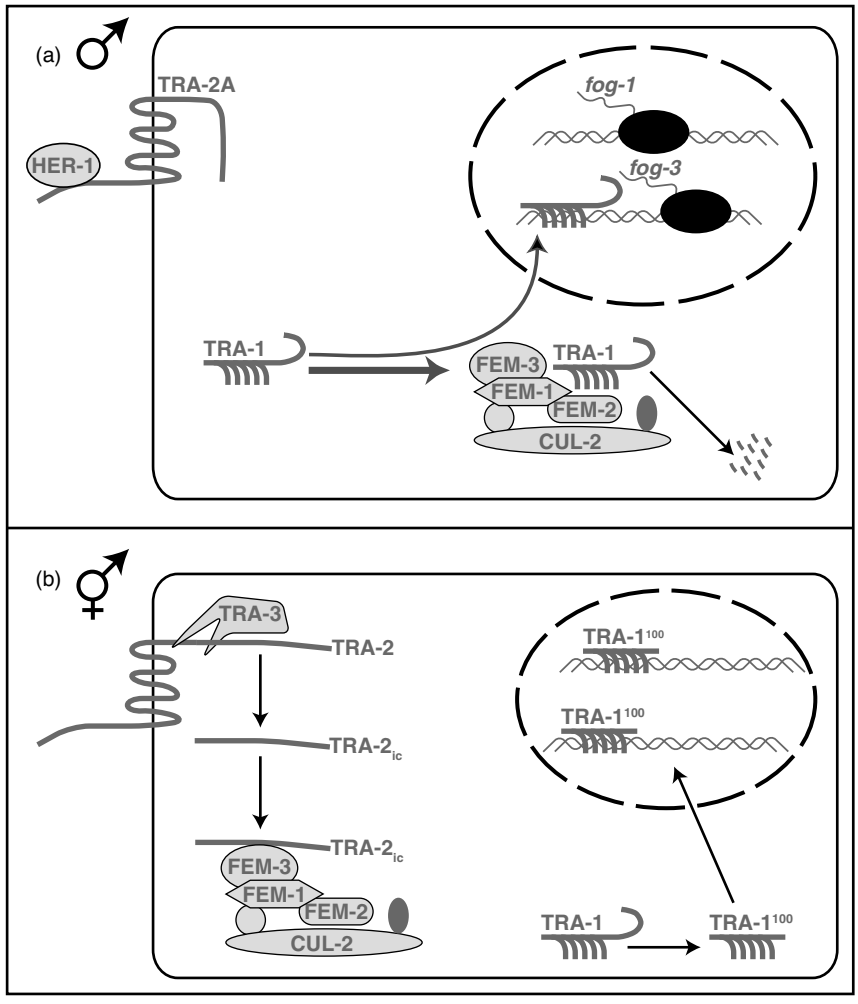

Figure 1.3 Model for the sperm/oocyte decision in adults. (a) In males, HER-1 binds to and represses the TRA-2A receptor; in this diagram, we do not depict cleavage of TRA-2A, but it has not yet been proven that HER-1 prevents this cleavage. The FEM/CUL-2 complex degrades full length TRA-1, which is needed to maintain spermatogenesis in older animals; thus, some TRA-1A is shown being degraded, and some entering the nucleus and regulating targets. The *fog-1* and *fog-3* genes are transcribed and promote spermatogenesis. In the figure, the black ellipses represent RNA polymerase, and the dark blue ellipsis represents ubiquitin. (b) In adult hermaphrodites, TRA-2 and TRA-3 are active, and prevent the FEM/CUL-2 complex from degrading TRA-1A. One possibility is that cleavage of TRA-2A by TRA-3 releases an intracellular fragment that inhibits the FEM complex by binding FEM-3. TRA-1 is cleaved to produce an aminoterminal fragment that represses transcription. A full colour version of this figure appears in the colour plate section.

1.4.5 TRA-2 also regulates TRA-1 directly

TRA-2 also regulates sexual fates through a second branch in the pathway, which involves direct contact with TRA-1 (Lum *et al.*, 2000; Wang and Kimble, 2001). The sites required for this interaction were identified by deletion studies in the yeast two-hybrid system, and are located on the intracellular portion of TRA-2A, a region also found in the smaller protein TRA-2B. Furthermore, several unusual *tra-2* mutations, often called mixomorphic alleles, disrupt TRA-2/TRA-1 binding. These alleles slightly decrease *tra-2* activity in somatic tissues, causing some cells to adopt male fates (Doniach, 1986; Schedl and Kimble, 1988). However, in the germline they are dominant and cause hermaphrodites to produce only oocytes, just like females. Thus, the interaction between TRA-2 and TRA-1 is necessary for hermaphrodites to make sperm, though it is not clear if this interaction regulates sexual fates in other tissues. An intracellular fragment of TRA-2 can be imported into the nucleus (Lum *et al.*, 2000), so it might interact with TRA-1 there *in vivo*. This fragment could be produced by cleavage of TRA-2A, or by translation of the smaller *tra-2* transcripts.

1.4.6 TRA-2, FEM-1 and FEM-3 stability is also regulated

Mutations in RPN-10, a component of the 26S proteasome, prevent hermaphrodite spermatogenesis and cause males to make yolk (Shimada *et al.*, 2006). In the intestine, these mutations increase the amount of TRA-2 protein in nuclei, so wild-type RPN-10 probably helps degrade TRA-2. Perhaps *rpn-10* mutations affect only the sperm/oocyte decision and yolk production, because these processes are more sensitive to changes in TRA-2 activity than other aspects of sex determination.

A similar but opposite effect involves *sel-10,* an F-box protein that regulates the levels of FEM-1 and FEM-3 (Jager *et al.*, 2004). Co-immunoprecipitation experiments show that SEL-10 binds both FEM-1 and FEM-3 and targets them for ubiquitinylation and degradation (Jager *et al.*, 2004), and yeast two-hybrid experiments indicate that SEL-10 also binds SKR-1, a component of the E3 ubiquitin ligase complex (Killian *et al.*, 2008). Mutations in *sel-10* alter some somatic fates and can suppress *tra-2(mixomorphic)* alleles in the germline.

1.5 Transcriptional control of germ cell fates

The two branches of the sex-determination pathway converge on TRA-1, a member of the Ci and Gli family of transcription factors (Zarkower and Hodgkin, 1992). Although *tra-1* produces two transcripts, only *tra-1A* has a known function, so its product is called TRA-1 below.

1.5.1 TRA-1 represses male genes in the germline and soma

Mutations that inactivate *tra-1* cause *XX* animals to develop male bodies (Hodgkin, 1987). Several somatic targets of TRA-1 have been identified, including: *egl-1,* a gene

that regulates apoptosis (Conradt and Horvitz, 1998; Conradt and Horvitz, 1999); *mab-3,* a homologue of *Drosophila doublesex* that specifies many male cell fates (Shen and Hodgkin, 1988; Raymond *et al.*, 1998; Yi, Ross and Zarkower, 2000); *ceh-30,* a gene that prevents specific cell deaths in males (Peden *et al.*, 2007; Schwartz and Horvitz, 2007); and *dmd-3,* another *doublesex* homologue (Mason, Rabinowitz and Portman, 2008). So far, all of these somatic targets are male genes that are repressed by TRA-1 in *XX* animals.

Somatic targets of TRA-1 usually have a single binding site, either in the promoter, an intron, or an enhancer. By contrast, the major targets of TRA-1 in germ cells have multiple binding sites in their promoters, near the start of transcription (Chen and Ellis, 2000; Jin, Kimble and Ellis, 2001b). Both of these targets, *fog-1* and *fog-3,* are essential for spermatogenesis (Barton and Kimble, 1990; Ellis and Kimble, 1995). Mutations in either gene are epistatic to mutations in *tra-1,* and cause males to make oocytes. Furthermore, inactivation of *tra-1* increases *fog-3* expression (Chen and Ellis, 2000). Thus, TRA-1 controls germ cell fates by repressing transcription.

1.5.2 TRA-1 might also activate targets in the germline

If TRA-1 only worked by repressing *fog-1* and *fog-3*, then null alleles of *tra-1* should cause spermatogenesis. Instead, these mutations cause both *XX* and *XO* animals to produce sperm early in life, and then switch to oogenesis (Hodgkin, 1987; Schedl *et al.*, 1989). This result leads to two major conclusions. First, *tra-1* is not essential for either germ cell fate, since null mutants make both sperm and oocytes. And second, *tra-1* normally represses spermatogenesis in young animals, but promotes spermatogenesis in older males. One set of transgenic experiments is consistent with these observations: mutations in some of the *tra-1* binding sites of *fog-3* inactivate the transgene, implying that those sites mediate activation by TRA-1 (Chen and Ellis, 2000).

1.5.3 TRA-1 cleavage might be critical for oogenesis and female development

If TRA-1 indeed acts both as a repressor and an activator in the germline, how does it work? The Ci and Gli proteins also act as repressors in some contexts, and activators in others (Alexandre, Jacinto and Ingham, 1996; Ruiz i Altaba, 1999). The N-termini of these proteins contain five zinc fingers that are essential for repression, and the C-termini contain sequences required for activation. The full-length protein activates transcription of some targets, but cleavage releases an N-terminal fragment that represses transcription (reviewed by Jiang, 2002).

In *C. elegans,* TRA-1 is cleaved to produce a shorter product, called TRA-1^{100} (Schvarzstein and Spence, 2006). This product is abundant in adult hermaphrodites, which are producing oocytes. Furthermore, some *tra-1* nonsense mutations are dominant and cause oogenesis if the system for nonsense-mediated decay has also been disrupted. Since these mutants encode only the N-terminal half of TRA-1, the TRA-1^{100}

fragment must specify oogenesis. Although animals that lack a germline do not accumulate full-length TRA-1, they do make TRA-1^{100} in the soma, where it promotes female cell fates. By contrast, animals that are producing only sperm accumulate significant amounts of full-length TRA-1 (Schvarzstein and Spence, 2006). Thus, one simple model is that TRA-1^{100} promotes female development and oogenesis, whereas full-length TRA-1 promotes spermatogenesis (Figure 1.3).

1.5.4 Do other transcription factors cooperate with TRA-1 in germ cells?

In the soma, *tra-4* works with *tra-1* to repress transcription of male genes (Grote and Conradt, 2006). TRA-4 is a homologue of the transcriptional repressor PLZF, and appears to act in a complex with NASP-1, a histone chaperone, and HDA-1, a histone deacetylase. Thus, these proteins are likely to repress male genes by altering chromatin structure. So far, there is no evidence that members of this complex regulate the sperm/oocyte decision. However, the transcript levels of many genes that act during spermatogenesis are high in males and low in adult hermaphrodites (reviewed by L'Hernault, 2006), and transgenic experiments confirm that several genes active during spermatogenesis are regulated transcriptionally (Merritt *et al.*, 2008). Thus, it is likely that transcriptional control of germ cell fates occurs downstream of *tra-1*. Perhaps either TRA-4 or a group of germline genes regulates chromatin structure as part of the sperm/oocyte switch.

1.6 Translational regulation of the sperm/oocyte decision

Both *fog-1* and *fog-3* act at the end of the sex-determination pathway to control germ cell fates. If either gene is inactive, all germ cells differentiate as oocytes, so *fog-1* and *fog-3* are needed to specify spermatogenesis (Barton and Kimble, 1990; Ellis and Kimble, 1995).

1.6.1 FOG-1 is a cytoplasmic polyadenylation element binding protein

The *fog-1* gene makes two transcripts, but only the larger one has a known function. It encodes a CPEB protein with two RNA recognition motifs and a zinc finger (Luitjens *et al.*, 2000; Jin, Kimble and Ellis, 2001b). All of these RNA-binding domains are essential for activity, and FOG-1 interacts with its own 3′UTR (Jin *et al.*, 2001a), so it probably regulates translation like other CPEB proteins (reviewed by Richter, 2007). Antibody staining revealed that FOG-1 is expressed in germ cells long before a sperm-specific marker, which is consistent with models in which FOG-1 controls the sperm/oocyte decision (Figure 1.4c; Lamont and Kimble, 2007). Although *fog-1* itself, *fog-3,* and other genes have potential FOG-1 binding sites in their 3′UTRs, the steps that occur between FOG-1 activation and the expression of genes involved in spermatogenesis are not known.

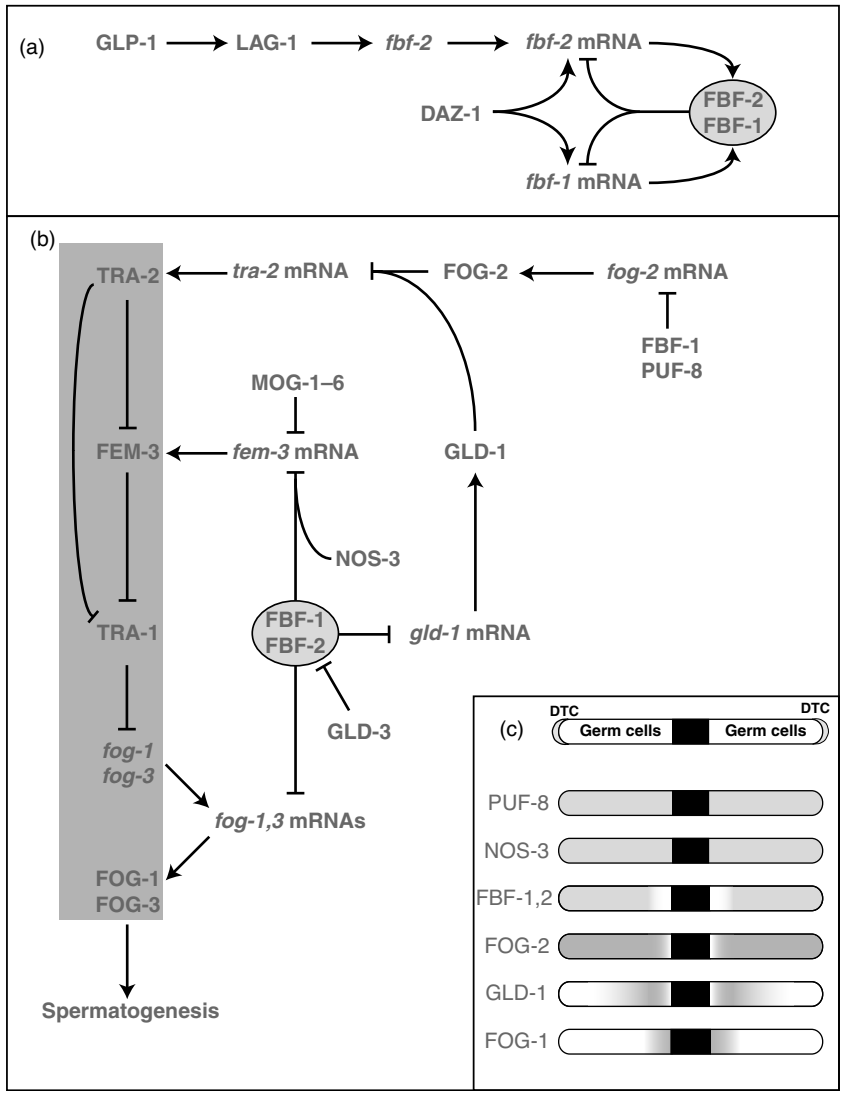

Figure 1.4 Translational regulation of germ cell fates. (a) The distal tip cell promotes FBF activity. In germ cells, the GLP-1 (Notch) receptor is activated by a signal from the distal tip cells (reviewed by Kimble and Crittenden, 2007). Working through the transcription factor LAG-1, it promotes transcription of *fbf-2*. The FBF proteins in turn promote mitotic proliferation or female germ cell fates. Through a feedback loop, they also inhibit their own translation; repression of *fbf-1* by FBF-2 and repression of *fbf-2* by FBF-1 have been demonstrated, and auto-repression is inferred. Proteins are shown in uppercase, and genes in lower case. Arrows indicate positive interactions, and '⊣' indicates negative interactions. (b) Modulation of the core sex-determination pathway by translational regulators (highlighted in grey; see text). The FBF proteins act at several points in the sex-determination pathway to prevent the translation of messenger RNAs that promote spermatogenesis. Similarly, GLD-1 acts with FOG-2 to prevent translation of *tra-2* messages, which normally promote oogenesis. GLD-1 also binds *tra-1* messages. All molecules that promote male fates are blue, and those that promote female fates are pink. (c) Expression of translational regulators in L3 hermaphrodites. A schematic of the L3 gonad is shown at top, with the distal tip cells (DTC, yellow) at either end, and

1.6.2 FOG-3 is a tob protein that might function with FOG-1

FOG-3 acts at the same step in the pathway as FOG-1, and both genes are essential for spermatogenesis. In fact, the only genetic distinction between them is that *fog-1* is very sensitive to changes in gene dose, whereas *fog-3* is not (Barton and Kimble, 1990; Ellis and Kimble, 1995). For example, *fog-1/+* males cannot sustain spermatogenesis, and eventually begin producing oocytes.

FOG-3 is the only nematode member of the large Tob and BTG family of proteins (Chen *et al.*, 2000). Other family members bind a diverse set of regulatory proteins, but in most cases their biochemical functions are not clear (reviewed by Jia and Meng, 2007). However, recent studies show that human Tob protein can promote the deadenylation of target messenger RNAs (Ezzeddine *et al.*, 2007). It does this by binding both the CCR4–CAF1 deadenylation complex and poly(A)-binding protein. If FOG-3 acts similarly, then both FOG proteins might control the translation of mRNAs by regulating their poly(A) tails. However, it remains possible that FOG-3 cooperates with unknown genes to do something else, like regulate transcription.

1.6.3 The three FEM proteins directly promote spermatogenesis

The primary function of the FEM proteins is to eliminate TRA-1. However, they have a second function in *C. elegans*, revealed by the fact that *tra-1; fem* double mutants make oocytes, even though they have male bodies (Hodgkin, 1986) and express high levels of *fog-3* (Chen and Ellis, 2000). How the FEM proteins promote spermatogenesis is not known. However, this activity seems to be a recent innovation, since it is not found in the related species *C. briggsae* (Hill *et al.*, 2006).

1.7 Other translational regulators specify hermaphrodite development

Male nematodes make sperm because HER-1 inactivates the TRA-2 receptor, allowing the FEM proteins to eliminate TRA-1 (Figures 1.2 and 1.3). Since hermaphrodites don't express HER-1, how do they produce sperm? Researchers have identified several translational regulators that modulate the activity of the sex-determination pathway to allow hermaphroditic development (Figure 1.4).

Figure 1.4 (*Continued*) other somatic cells (black) in the centre. Rough sketches of the protein levels of key translational regulators are shown below; since none of these studies compared different proteins in the same animals, the regions shown are only approximate. The PUF-8 expression pattern is based on a PUF-8::GFP transgene (Ariz, Mainpal and Subramaniam, 2009). NOS-3 is based on antibody staining (Kraemer *et al.*, 1999), as are FBF (Zhang *et al.*, 1997), FOG-2 (Clifford *et al.*, 2000), GLD-1 (Jones, Francis and Schedl, 1996) and FOG-1 (Lamont and Kimble, 2007). A full colour version of this figure appears in the colour plate section.

1.7.1 FOG-2 and GLD-1 repress translation of *tra-2* to allow spermatogenesis

Mutations in *fog-2* transform *XX* animals into true females, but do not affect males (Schedl and Kimble, 1988). Thus, *fog-2* alters the sperm/oocyte decision to allow hermaphroditic development. Mutations in *gld-1* affect many aspect of oogenesis, so *XX* animals are sterile rather than female (Francis *et al.*, 1995a). However, one of the phenotypes controlled by *gld-1* is hermaphrodite spermatogenesis; in null mutants all germ cells begin oogenesis instead of spermatogenesis, although they fail to complete it (Francis *et al.*, 1995a; Jones, Francis and Schedl, 1996). Genetic tests imply that both *fog-2* and *gld-1* act upstream of *tra-2* (Schedl and Kimble, 1988; Francis, Maine and Schedl, 1995b).

Cloning revealed that FOG-2 was created by a gene duplication event and co-opted into the sex-determination pathway to allow hermaphrodite development, and that it contains an F-box (Clifford *et al.*, 2000). Although many F-box proteins work as part of the E3 ubiquitin ligase complex to mark targets for degradation (reviewed by Kipreos and Pagano, 2000; Kipreos, 2005), FOG-2 associates with GLD-1 but does not destabilize it (Clifford *et al.*, 2000). This interaction with GLD-1 is mediated by the carboxyl terminus of FOG-2, which has been under positive selection during recent evolution (Nayak, Goree and Schedl, 2005).

GLD-1 is a translational regulator that contains a KH domain (Jones and Schedl, 1995) and appears to act as a dimer (Ryder *et al.*, 2004). It binds the 3′UTR of *tra-2* messenger RNAs, and can form a ternary complex that includes FOG-2 (Clifford *et al.*, 2000) and blocks translation (Jan *et al.*, 1999). The target site is defined by dominant mutations in two Direct Repeat Elements of the *tra-2* 3′UTR, which cause hermaphrodites to make oocytes rather than sperm (Doniach, 1986; Goodwin *et al.*, 1993); deletion of these repeats prevents GLD-1 binding (Jan *et al.*, 1999). Thus, FOG-2 and GLD-1 lower TRA-2 levels in young hermaphrodites to allow spermatogenesis. GLD-1 also regulates many other messages in the developing germline (Lee and Schedl, 2001; Marin and Evans, 2003; Mootz, Ho and Hunter, 2004; Schumacher *et al.*, 2005), including *tra-1* (Lakiza *et al.*, 2005), but none of these interactions appears to require FOG-2.

1.7.2 The FBF proteins repress translation of *fem-3* to allow oogenesis

Although FOG-2 and GLD-1 allow spermatogenesis to begin, hermaphrodites need to ensure that some germ cells eventually differentiate as oocytes. Mutations in several genes show that the level of FEM-3 is restricted so that this change can happen at the appropriate time.

As with *tra-2,* dominant mutations have been identified in the 3′UTR of *fem-3*, but they have the opposite effect, causing all germ cells to differentiate as sperm (Barton, Schedl and Kimble, 1987; Ahringer and Kimble, 1991; Ahringer *et al.*, 1992). These mutations disrupt a point mutation element (PME) that binds to and is regulated by FBF-1 and FBF-2 (Zhang *et al.*, 1997), two nematode members of the PUF family of translational regulatory proteins (reviewed by Wickens *et al.*, 2002). Since inactivation of both proteins causes constitutive spermatogenesis, just like the dominant mutations