Section I
Oocyte determination
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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,
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](image-url) 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](image.png)  
*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-1100 (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-1100...
fragment must specify oogenesis. Although animals that lack a germline do not accumulate full-length TRA-1, they do make TRA-1\textsuperscript{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\textsuperscript{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, \textit{tra-4} works with \textit{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 \textit{et al.}, 2008). Thus, it is likely that transcriptional control of germ cell fates occurs downstream of \textit{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 \textit{fog-1} and \textit{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 \textit{fog-1} and \textit{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 \textit{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 \textit{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 \textit{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 \textit{fog-1} itself, \textit{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 \textit{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 \textit{fbf-1} by FBF-2 and repression of \textit{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 \textit{tra-2} messages, which normally promote oogenesis. GLD-1 also binds \textit{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 \textit{fog-1} is very sensitive to changes in gene dose, whereas \textit{fog-3} is not (Barton and Kimble, 1990; Ellis and Kimble, 1995). For example, \textit{fog-1/þ} 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 \textit{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 \textit{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 \textit{C. elegans}, revealed by the fact that \textit{tra-1; fem} double mutants make oocytes, even though they have male bodies (Hodgkin, 1986) and express high levels of \textit{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 \textit{C. briggsae} (Hill \textit{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).
1.7.1 FOG-2 and GLD-1 repress translation of \textit{tra-2} to allow spermatogenesis

Mutations in \textit{fog-2} transform \textit{XX} animals into true females, but do not affect males (Schedl and Kimble, 1988). Thus, \textit{fog-2} alters the sperm/oocyte decision to allow hermaphroditic development. Mutations in \textit{gld-1} affect many aspect of oogenesis, so \textit{XX} animals are sterile rather than female (Francis \textit{et al.}, 1995a). However, one of the phenotypes controlled by \textit{gld-1} is hermaphrodite spermatogenesis; in null mutants all germ cells begin oogenesis instead of spermatogenesis, although they fail to complete it (Francis \textit{et al.}, 1995a; Jones, Francis and Schedl, 1996). Genetic tests imply that both \textit{fog-2} and \textit{gld-1} act upstream of \textit{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 \textit{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 \textit{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 \textit{et al.}, 2004). It binds the 3’UTR of \textit{tra-2} messenger RNAs, and can form a ternary complex that includes FOG-2 (Clifford \textit{et al.}, 2000) and blocks translation (Jan \textit{et al.}, 1999). The target site is defined by dominant mutations in two Direct Repeat Elements of the \textit{tra-2} 3’UTR, which cause hermaphrodites to make oocytes rather than sperm (Doniach, 1986; Goodwin \textit{et al.}, 1993); deletion of these repeats prevents GLD-1 binding (Jan \textit{et al.}, 1999). Thus, FOG-2 and GLD-1 lower \textit{TRA-2} levels in young hermaphrodites to allow spermatogenesis. GLD-1 also regulates many other messages in the developing germ-line (Lee and Schedl, 2001; Marin and Evans, 2003; Mootz, Ho and Hunter, 2004; Schumacher \textit{et al.}, 2005), including \textit{tra-1} (Lakiza \textit{et al.}, 2005), but none of these interactions appears to require FOG-2.

1.7.2 The FBF proteins repress translation of \textit{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 \textit{tra-2}, dominant mutations have been identified in the 3’UTR of \textit{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 \textit{et al.}, 1992). These mutations disrupt a point mutation element (PME) that binds to and is regulated by FBF-1 and FBF-2 (Zhang \textit{et al.}, 1997), two nematode members of the PUF family of translational regulatory proteins (reviewed by Wickens \textit{et al.}, 2002). Since inactivation of both proteins causes constitutive spermatogenesis, just like the dominant mutations
in the *fem-3* 3′UTR, FBF-1 and FBF-2 normally repress translation of *fem-3* messenger RNAs. Mutations in either *fbf-1* or *fbf-2* alone have more subtle but complex effects, which suggest that they also inhibit each other (Lamont et al., 2004). Finally, the FBF proteins can also bind *fog-1* messages and repress their translation, and seem likely to act on *fog-3* transcripts as well, since they contain putative binding sites (Thompson et al., 2005).

FBF-1 and FBF-2 are assisted by NOS-3, a homologue of the translational regulatory protein Nanos from *Drosophila* (Kraemer et al., 1999). Furthermore, RNA interference shows that NOS-1 and NOS-2 act redundantly with NOS-3 to prevent spermatogenesis. Since only NOS-3 binds the FBF proteins in the yeast two-hybrid system, perhaps the other NOS proteins only form a complex with FBF-1 or FBF-2 when *fem-3* messages are present. The co-regulation of *fem-3* by the FBF proteins and NOS-3 parallels the regulation of hunchback by Pumilio and Nanos in *Drosophila*, suggesting that these translational regulatory networks are ancient.

### 1.7.3 Other translational regulators reinforce these decisions

The activities of the *fbf* genes are themselves tightly regulated (Figure 1.4a). First, the translational regulator DAZ-1 can bind *fbf* messenger RNAs and promote translation, thus favouring oogenesis (Karashima, Sugimoto and Yamamoto, 2000; Otori, Karashima and Yamamoto, 2006). Second, GLD-3, a homologue of bicaudal-C, can bind the FBF proteins and inhibit their interaction with the *fem-3* 3′UTR (Eckmann et al., 2002). This inhibitory interaction is mutual, since the FBF proteins repress the expression of GLD-3 (Eckmann et al., 2004). Third, the distal tip cell acts through the Notch pathway to promote the expression of *fbf-2* (Lamont et al., 2004). Thus, FBF activity is controlled in part by translational regulation.

The activities of *fog-2* and *gld-1* are also under translational control. FBF-1 and PUF-8, a related protein, act redundantly to regulate FOG-2 proteins levels (Bachorik and Kimble, 2005). And the two FBF proteins regulate the translation of *gld-1* (Crittenden et al., 2002).

### 1.7.4 Essential RNA-binding proteins also influence the sperm/oocyte switch

Several essential genes also regulate the expression of *fem-3*. Most of these genes were identified in general screens for mutations that caused hermaphrodites to produce sperm throughout their lives, and are named *mog-1* through *mog-6* (Graham and Kimble, 1993; Graham, Schedl and Kimble, 1993). Although mutations in these genes cause constitutive spermatogenesis, the mutants do not make as many sperm as *fem-3*(gf) mutants. Since the *mog* mutations are suppressed by mutations in the *fem* genes, but not by mutations in *fog-2*, they could act upstream of *fem-3*. Furthermore, mutations in the *mog* genes activate reporter constructs that have been fused to the *fem-3* 3′UTR, which implies that the MOG proteins regulate translation of *fem-3* (Gallegos et al., 1998). For technical reasons, these experiments used transgenes that were only active in the soma,
so it is unclear if the reporters were co-regulated by the FBF proteins, which are largely
restricted to the germline (Zhang et al., 1997). However, mutations in the fem-3 PME
did increase translation of the reporter constructs, so perhaps somatic members of the
PUF family (Walser et al., 2006) can work in concert with the MOG proteins to control
their translation.

Molecular cloning revealed that MOG-1, MOG-4 and MOG-5 are DEAH helicases, a
family that includes proteins that bind RNA (Puoti and Kimble, 1999; Puoti and
Kimble, 2000). Since mog; fem-3 double mutants make oocytes that give rise to
dead eggs, these genes are also essential for embryonic development (Graham and
Kimble, 1993; Graham, Schedl and Kimble, 1993). These three helicases interact with
MEP-1, a zinc finger protein that regulates the expression of fem-3 in germ cells
(Belfiore et al., 2002), and works with PIE-1 to block the expression of germline
messages in the soma (Unhavaithaya et al., 2002). Furthermore, MOG-6 is an unusual
cyclophilin that also interacts with MEP-1 (Belfiore et al., 2004). Thus, this large group
of proteins appears essential for initiating oogenesis in hermaphrodites, and regulating
gene expression in the early embryo.

Another essential gene influences the switch from spermatogenesis to oogenesis –
mag-1 (Li, Boswell and Wood, 2000). The mag-1(RNAi) animals resemble mog
mutants in two ways: they make sperm constitutively, and mag-1(RNAi); fem-3 double
mutants make oocytes that give rise to dead eggs. But unlike mog-1, the mag-1(RNAi);
fog-2 double mutants make only oocytes, just like fog-2 animals; so mag-1 might act
upstream of fog-2. Although epistasis experiments that involve RNA interference are
not conclusive, this difference raises the possibility that MAG-1 is a positive regulator of
tra-2, rather than a negative regulator of fem-3.

MAG-1 is likely to work with RNP-4, the homologue of yeast Y14, since rnp-4
(RNAi) animals have similar phenotypes and the two proteins co-immunoprecipitate
(Kawano et al., 2004). Both MAG-1 and RNP-4 are components of the exon-junction
complex, which is formed during splicing. Since the mammalian homologues Magoh
and Y14 remain associated with mRNAs following splicing and promote translation
(Nott, Le Hir and Moore, 2004), perhaps MAG-1 and RNP-4 promote translation of a
message needed for oogenesis, like tra-2.

ATX-2 also regulates sex determination in the germline (Ciosk, DePalma and
Priess, 2004; Maine et al., 2004), since RNA interference causes many hermaphrodites
to produce sperm constitutively. Surprisingly, this phenotype is not completely sup-
pressed by fog-2(q71null) mutations, but is suppressed by tra-2(q122gf) mutations.
Thus, these mutations have distinct effects, even though both disrupt translational
regulation of tra-2. How ATX-2 promotes oogenesis is not known.

Finally, the essential gene laf-1 has the opposite effect; laf-1/+ animals make
oocytes instead of sperm, just like the fog mutants. Analysis of double mutants indicates
that laf-1 might regulate tra-2 translation (Goodwin et al., 1997; Jan et al., 1997).

1.7.5 The relative activities of TRA-2 and FEM-3 determine germ cell fates

The existence of elaborate regulatory networks focused on tra-2 and fem-3 highlights
the importance of these genes in the developing germline. In fact, several observations
support the idea that the relative levels of TRA-2 and FEM-3 are the critical factor in the sperm/oocyte decision. First, the \textit{tra-2(gf)} mutations increase the production of wild-type TRA-2 protein, causing oogenesis. Second, the \textit{fem-3(gf)} mutations, which should increase the production of wild-type FEM-3, cause constitutive spermatogenesis. And third, these mutations compensate for each other, since \textit{tra-2(gf); fem-3(gf)} double mutants are self-fertile hermaphrodites (Barton, Schedl and Kimble, 1987).

1.8 The sperm/oocyte decision is intimately linked to the initiation of meiosis

Many of the genes that modulate the sex-determination pathway in hermaphrodites also regulate the decision of germ cells to remain in mitosis or enter meiosis (reviewed by Kimble and Crittenden, 2007). For example, GLD-1 and NOS-3 work together to stop mitosis and promote meiosis, as do GLD-2 and GLD-3 and some of the MOG proteins (Belfiore \textit{et al.}, 2004; Eckmann \textit{et al.}, 2004; Hansen \textit{et al.}, 2004). By contrast, FBF-1, FBF-2, FOG-1 and FOG-3 play redundant roles promoting germ cells to remain in mitosis (Crittenden \textit{et al.}, 2002; Thompson \textit{et al.}, 2005). In addition, PUF-8 acts redundantly with the translational regulator MEX-3 to keep germ cells in mitosis (Ariz, Mainpal and Subramaniam, 2009), and ATX-2 also promotes mitotic proliferation (Ciosk, DePalma and Priess, 2004; Maine \textit{et al.}, 2004).

Some of the genes that regulate sex determination also act at later stages during meiosis. For example, GLD-1 is needed to maintain germ cells in oogenesis, since oocytes return to mitosis and form tumours in \textit{gld-1(null)} mutants (Francis \textit{et al.}, 1995a). PUF-8 plays an analogous role in the male germline, preventing spermatocytes from returning to mitosis and forming tumours (Subramaniam and Seydoux, 2003). And DAZ-1 is required for germ cells to progress beyond the pachytene phase of oogenesis (Karashima, Sugimoto and Yamamoto, 2000).

1.8.1 Translational regulators define zones within the germline syncytium

Why do so many translational regulators control both the sperm/oocyte decision, and the entry into meiosis? The germline is a long tube in which cell fates are arranged from a stem cell niche at the distal end to fully differentiated germ cells at the proximal end (Figure 1.1). Since much of the tube is a syncytium, perhaps translational regulators promote the localized production of target proteins, thus dividing the syncytium into zones, each with germ cells at a different stage of development.

A few observations support this model. The interactions between different translational regulators appear to set up zones of protein expression, with FBF activity high near the distal tip, and FOG-1 and GLD-1 high proximally (Figure 1.4). Furthermore, experiments with transgenes show that most genes in the germline are controlled in large part by their 3′UTRs, rather than their promoters (Merritt \textit{et al.}, 2008), confirming the importance of translational regulation. By contrast, most proteins that mediate signal transduction do not play dual roles. For example, none of the proteins that form the HER-1 to TRA-1 signal transduction pathway regulate mitosis or meiosis. Instead, the
only members of this pathway that influence the cell cycle are the translational regulator FOG-1 and its partner FOG-3. Similarly, the GLP-1 signal transduction pathway controls mitosis without influencing the sperm/oocyte decision, whereas many of the translational regulators it influences do both (reviewed by Kimble and Crittenden, 2007).

1.8.2 The sperm/oocyte decision is likely to occur near the entry into meiosis

Since primordial germ cells and germ cells in the early stages of meiosis look the same in both sexes, it has been hard to identify the point at which each cell decides between spermatogenesis and oogenesis. Several early markers of spermatogenesis are first detected in pachytene germ cells during prophase I (Jones, Francis and Schedl, 1996). By contrast, some early markers of oogenesis are found more distally along the tube, in cells that are making the transition to meiosis, and even in some mitotic cells. Since distal cells express female markers but more proximal ones do not (M.H. Lee and T. Schedl, shown by Ellis and Schedl, 2006), these transcripts might accurately reflect the sexual fates of individual nuclei in the germline syncytium. Thus, the sperm/oocyte decision probably occurs between late mitosis and the pachytene phase of meiosis I.

Although many translational regulators control both the sperm/oocyte decision and the entry into meiosis, there is no simple correlation between these two fates (Table 1.1). Some genes promote spermatogenesis and mitosis. Some promote spermatogenesis and meiosis. Some promote oogenesis and mitosis, and others promote oogenesis and meiosis. One simple model to explain this complex pattern is that the two decisions are made at almost the same point in the germline tube. Thus, each of these translational regulators might originally have been expressed in this region because of its role in either sex determination or the entry into meiosis, but was eventually recruited into the other pathway to tighten the control of target messages.

This hypothesis is supported by temperature-shift experiments conducted using fog-1, which specifies sexual fate, and glp-1, which promotes mitosis (Barton and Kimble, 1990). These studies indicate that fog-1 is needed continually to promote spermatogenesis, and that temperature shifts that affect both fog-1 and glp-1 alter both decisions, as if the genes were acting on cell fates at roughly the same time.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sperm/oocyte decision</th>
<th>Mitosis/meiosis decision</th>
<th>Biochemical function</th>
</tr>
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<tr>
<td>atx-2</td>
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<td>Promotes mitosis</td>
<td>Ataxin family</td>
</tr>
<tr>
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<td>Promotes oogenesis</td>
<td>Promotes mitosis</td>
<td>PUF translational regulator</td>
</tr>
<tr>
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<td>Promotes mitosis</td>
<td>PUF translational regulator</td>
</tr>
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<td>Promotes meiosis</td>
<td>Nanos translational regulator</td>
</tr>
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<td>Promotes meiosis</td>
<td>Translational regulator</td>
</tr>
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<td>Promotes meiosis</td>
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</tr>
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</tr>
<tr>
<td>fog-3</td>
<td>Promotes spermatogenesis</td>
<td>Promotes mitosis</td>
<td>Tob protein</td>
</tr>
</tbody>
</table>
1.8.3 Some translational regulators are also essential for embryogenesis

Translational regulators might also be crucial for repressing transcripts that are essential for the future embryo, but which would be harmful in developing oocytes. For example, all three of the *fem* genes show maternal effects, which suggests that their transcripts are packaged into oocytes to help control the sex of the future embryo (Hodgkin, 1986). The phenotype of *fem-3(gf)* mutants implies that high levels of FEM-3 protein cause spermatogenesis, so translational inhibitors like the FBF proteins might play a critical role in preventing *fem-3* transcripts from blocking oogenesis. As discussed above, other translational regulators are essential, apparently because they control targets involved in sex determination, as well as targets needed for embryogenesis.

1.9 The future

Although we now understand a great deal about how the sperm/oocyte decision is made in *C. elegans*, this information has opened up a new set of questions for the future; questions that should dominate the next several years of research.

1.9.1 What are the primary targets controlled by the sperm/oocyte decision?

Although we know that *fog-1* and *fog-3* act at the end of the sex-determination pathway to promote spermatogenesis, we do not know what their targets are. Possible candidates include *fog-1* and *fog-3* themselves, and genes like *cpb-1*, that act early in spermatogenesis. Furthermore, we do not know what genes are activated early in oogenesis by the absence of *fog-1* and *fog-3* activity. Identifying these targets and working out how the action of *fog-1* and *fog-3* controls their activities is critical for understanding the sperm/oocyte decision. Over the next few years, our focus should move from studying the sex-determination process per se to elucidating the mechanics of cell fate determination in the germline.

1.9.2 How has the sperm/oocyte decision changed during evolution?

This question entails two very different lines of research. The first concerns whether there has been broad conservation of genes involved in the sperm/oocyte decision. Although *fog-1* and *fog-3* have homologues in all animals, some of which are expressed in germ cells, it is not known if any of these homologues regulates germ cell fates. Furthermore, the possible conservation of genes downstream of *fog-1* and *fog-3* remains a complete mystery.

The second line of enquiry concerns how the sperm/oocyte decision changes during evolution. Comparative analysis of nematode species is beginning to provide some answers to this question. Genes of the core pathway are conserved in structure and function amongst relatives of *C. elegans*, and most show only subtle differences
between species. For example, *fem-2* and *fem-3* mutations always cause oogenesis in *C. elegans*, but only cause oogenesis under some conditions in *C. briggsae* (Hill et al., 2006). By contrast, genes that modulate the core pathway seem to be evolving rapidly. For example, *fog-2* and *gld-1* are needed for hermaphrodite spermatogenesis in *C. elegans*, but not in *C. briggsae*, which lacks a *fog-2* gene (Nayak, Goree and Schedl, 2005). However, *C. briggsae* has recruited a different member of the F-box family of proteins, SHE-1, to specify hermaphrodite development (Guo, Lang and Ellis, 2009). Finally, both *fog-2* and *she-1* control *tra-2* activity, and knocking down *tra-2* function in the male/female species *C. remanei* can help create self-fertile animals (Baldi, Cho and Ellis, 2009).

1.9.3 How does the somatic gonad influence the sperm/oocyte decision?

So far, we know that the distal tip cells signal to nearby germ cells to remain in mitosis, and that a variety of somatic cells in the male act through HER-1 to cause germ cells to adopt male fates and begin spermatogenesis. However, the selective killing of cells in the hermaphrodite gonad showed that there might be additional signals (above). Furthermore, a surprising genetic experiment supports this hypothesis: mutations in the *fshr-1* gene, which acts in the somatic gonad, promote spermatogenesis over oogenesis (Cho, Rogers and Fay, 2007). How *fshr-1* works, and what additional interactions occur between the soma and germline remain mysterious. Since the somatic gonad is critical for the development of germ cells in most animals, these studies could open up entirely new avenues for research.

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