Sex determination in the germ line*

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Abstract

Sexual identity is one of a few basic parameters that specify how development should proceed. Although sex determination has profound effects on many tissues, its most ancient and fundamental role is ensuring that some germ cells become sperm, and others become oocytes or eggs. Spermatocytes and oocytes are usually produced in male and female animals, respectively, but *C. elegans* is uniquely suitable for studying the control of these cell fates because both types of cells are made from a common pool of progenitors in XX hermaphrodites. Extensive genetic and molecular studies have shown that the sexual fate of germ cells in *C. elegans* is controlled by the same genes that regulate sexual identity in other parts of the animal. However, this regulatory pathway has additional features that are unique to the germ line. First, several genes, like the three *fogs*, act only in germ cells. Second, the three *fem* genes act in concert with targets of *tra-1* to control germ cell fates, but do not act this way in the soma. Third, translational repression of *tra-2* is essential for hermaphrodite spermatogenesis. Fourth, translational repression of *fem-3* is needed for oogenesis. In this review, we present genetic and molecular models for how these processes work, and summarize the evidence upon which they are built.

1. Sex determination in germ cells

In *C. elegans* there are two sexes. Animals with two X chromosomes (XX) and two sets of autosomes have a female soma and a hermaphrodite germ line, and so are called hermaphrodites. Animals with a single X chromosome (XO) and two sets of autosomes have a male soma and germline (see Somatic sex determination). Hermaphrodite self-fertility is achieved by a transient period of spermatogenesis in the L3 stage followed by a switch to oogenesis in the L4. Males begin spermatogenesis in the L3/L4 stage and this continues through adulthood. Although one might expect that the regulation of sexual identity in germ cells would be just like female/male choices in the soma, studies in both worms and flies show that it is more complex. Several features that make the germ line unique might contribute to this complexity. For example, the germ line is the most ancient tissue in animals, and the only sexually dimorphic tissue common to all species. Thus, it has had a long evolutionary history and many opportunities to accumulate new regulatory circuits. In addition, oocytes or eggs need to be packed with regulatory molecules that will direct early embryonic development, including sex determination, and some of these molecules must be repressed so that they don't affect the germ line itself. Finally, in species like *C. elegans*, where the germline of a single individual can produce both male and female germ cells, additional layers of regulation are required to ensure plasticity in sexual fate.

The core pathway that regulates somatic sexual fate forms a central part of the pathway that controls germline sexual fate (Figure 1; Goodwin and Ellis; 2002; see Somatic sex determination and X-chromosome dosage compensation). It is composed of seven genes that are each necessary for sexual identity in germ cells and somatic cells. These genes function in a negative regulatory hierarchy to control TRA-1 activity (Hodgkin, 1987, see Somatic sex determination). However, TRA-1 is not the terminal regulator of sexual fate in the germ line, and germline specific genes and regulatory interactions are superimposed on this core pathway to allow hermaphrodite development. Below, we briefly describe the core pathway and then the additional genes and layers of regulation that act in germline sex determination.

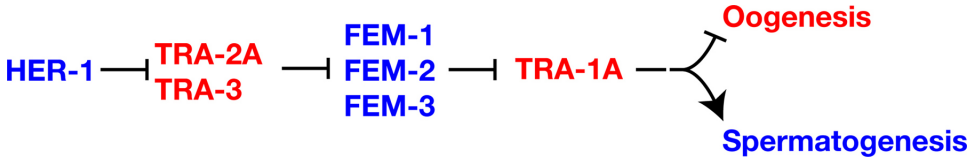

Figure 1. The core sex determination pathway. Proteins are shown in capital letters, and are indicated when the regulatory interactions are known to take place at this level; in all other cases, gene names are used and are shown in italics. Positive interactions are indicated with an arrow, and negative ones by a "--|". Genes that promote spermatogenesis are blue, and those that promote oogenesis are red. Not shown are sex-determination and dosage compensation genes that act upstream of HER-1, which also form part of the "global" sex determination pathway (see Somatic sex determination and X-chromosome dosage compensation).

2. The soma acts through HER-1 to regulate the sexual fate of germ cells

The *her-1* gene acts in XO animals to promote male somatic development and continuous spermatogenesis (Hodgkin, 1980). This *her-1* activity is restricted to XO animals by the SDC (sex determination and dosage compensation) genes, which repress *her-1* transcription in XX animals (Chu et al., 2002; for details see Somatic sex

determination and X-chromosome dosage compensation). HER-1 plays a pivotal role in coordinating sexual fate throughout the animal, as several lines of evidence indicate that HER-1 transmits a masculinizing signal between cells. This evidence includes sequence analyses indicating that HER-1 is a small, secreted protein (Perry et al., 1993), genetic mosaic analyses indicating that HER-1 acts non-autonomously (Hunter and Wood, 1992), and ectopic expression of HER-1 in the muscle of XX animals, which masculinizes somatic tissues and causes germ cells to become sperm (Perry et al., 1993). Based on mosaic analysis, HER-1 activity in the intestine and somatic gonad exerts the greatest influence on germ cell fates (Hunter and Wood, 1992).

HER-1 promotes spermatogenesis by binding to and inactivating the transmembrane protein TRA-2 (Kuwabara and Kimble, 1995; Kuwabara et al., 1992; Okkema and Kimble, 1991). Likely HER-1 sites required for this interaction have been identified by X-ray analysis of HER-1 crystals (Hamaoka et al., 2004), and the TRA-2 receptor site by genetic analysis (Kuwabara, 1996). TRA-2, in turn, inactivates the FEM proteins (Hodgkin, 1986). The cytoplasmic domain of TRA-2 can bind FEM-3 (Mehra et al., 1999). Thus, a simple model is that HER-1 binding to the extracellular domain of TRA-2 disrupts the ability of the intracellular domain of TRA-2 to bind and inactivate FEM-3. FEM-1 contains ankyrin repeats, FEM-2 is a PP2C phosphatase, and FEM-3 is novel (Ahringer et al., 1992; Pilgrim et al., 1995; Spence et al., 1990). TRA-1 is a transcription factor related to the *Drosophila* Ci and vertebrate GLI proteins (Zarkower and Hodgkin, 1992). The FEM proteins inactivate TRA-1 by a mechanism that is not yet understood.

In *Drosophila*, germ cell sexual fate is also controlled by somatic signals, and these signals interact with processes intrinsic to the germ line (reviewed by Oliver, 2002). In flies, additional genes act only in the germ line to control sexual fate, but the genetic pathways involved have been hard to unravel. By contrast, several well-characterized regulatory circuits have been identified in the nematode germ line; in general, these circuits modulate the activity of sex-determination genes to allow for hermaphrodite spermatogenesis.

3. In germ cells, the sex determination pathway acts through FOG-1 and FOG-3

In both males and hermaphrodites, sperm production requires *fog-1*, *fog-3* and the three *fem* genes (Barton and Kimble, 1990; Doniach and Hodgkin, 1984; Ellis and Kimble, 1995; Hodgkin, 1986). Mutations in any of these genes cause all germ cells to differentiate as oocytes.

3.1. *fog-1* and *fog-3*

Two facts imply that *fog-1* and *fog-3* control sexual fate in germ cells and probably act at the end of the sex-determination pathway. First, mutations in *fog-1* or *fog-3* cause germ cells to differentiate as oocytes rather than sperm, but have no effect on other parts of the body. Second, genetic analyses indicate that mutations in *fog-1* and *fog-3* act downstream of mutations in all other genes in the pathway (Figure 2). Thus, these two genes might be directly responsible for initiating spermatogenesis.

The *fog-1* gene produces two major transcripts (Jin et al., 2001; Luitjens et al., 2000). The large transcript is necessary for *fog-1* function and is sufficient to rescue *fog-1(q253)* mutants, but the small one has no detectable activity (Jin et al., 2001a; Jin et al., 2001b). Other sex-determination genes, like *her-1*, *tra-1* and *tra-2*, also make minor transcripts of unknown function. Here we discuss only the essential products of each gene.

Figure 2. FOG-1, FOG-3 and the FEM proteins promote spermatogenesis. Proteins are shown in uppercase, and genes in lowercase italics, to indicate that TRA-1 controls transcription of the *fog-1* and *fog-3* genes. Positive interactions are indicated with an arrow, and negative ones by a "--|". Genes that promote spermatogenesis are blue, and those that promote oogenesis are red.

Sequence analysis reveals that FOG-1 is a Cytoplasmic Polyadenylation Element Binding (CPEB) protein. All CPEB proteins contain two RNA Recognition Motifs (RRMs) and a C-H domain that binds zinc and interacts with RNA (reviewed by Mendez and Richter, 2001). Because other CPEB proteins regulate the translation of specific messages, FOG-1 probably regulates the translation of mRNAs that control sexual fate in the germ line. Interestingly, FOG-1 can bind the *fog-1* message itself (Jin et al., 2001a), so it might be auto-regulatory. Other targets of FOG-1 have not yet been identified.

Although three other CPEB proteins are also expressed in the germ line (Luitjens et al., 2000), only FOG-1 regulates sexual fate. How is this specificity achieved? Analyses of FOG-1 homologs from other *Caenorhabditis* species show that FOG-1 has a conserved insertion in the C-H domain that is not found in other CPEB proteins (Cho et al., 2004); this domain might specify FOG-1's unique function. One possibility is that FOG-1 binds specific elements found only in mRNAs that act in sex determination. Alternatively, FOG-1 might interact with other proteins that confer the ability to regulate sexual fate.

FOG-3 is a member of the Tob family of proteins (Chen et al., 2000). These proteins are found in all animals, and share a common amino-terminal domain of 115 amino acids. This family has been implicated in the control of cell division and differentiation, but so far little is know about their biochemical functions. Tob itself has both nuclear import and export signals in the conserved amino terminus (Kawamura-Tsuzuku et al., 2004; Maekawa et al., 2004), and associates with several Smad transcription factors during osteogenesis (Yoshida et al., 2000), so perhaps FOG-3 also regulates transcription.

TRA-1 appears to control germ cell fate by regulating the transcription of *fog-1* and *fog-3*. Each gene has three to six potential TRA-1 binding sites in its promoter; more than are found in any other gene in the genome (Chen and Ellis, 2000; Jin et al., 2001). Furthermore, the *fog-3* promoter can bind TRA-1 in gel shift assays, and this binding requires intact TRA-1 sites (Chen and Ellis, 2000). However, analyses of promoter mutations show that some of the TRA-1 binding sites mediate activation of *fog-3* transgenes, whereas studies of *fog-3* expression imply that at least one site mediates repression. Furthermore, *tra-1(null)* mutants usually make sperm early in life and oocytes later on (Hodgkin, 1987; Schedl et al., 1989). One explanation for these complex observations is that TRA-1 acts on the *fog-3* promoter to repress spermatogenesis in some circumstances, and to activate it in others.

3.2. *fem-1*, *fem-2* and *fem-3*

Just as in the soma, the *fem* genes regulate the activity of *tra-1* in germ cells. For example, quantitative RT-PCR studies of *fem*; *tra-1* double mutants show that the FEM proteins act through TRA-1 to control the level of *fog-3* transcripts (Chen and Ellis, 2000). However, these double mutants make oocytes instead of sperm, which shows that the FEM proteins also act downstream of TRA-1 (Figure 2, Hodgkin, 1986). Perhaps the FEM proteins directly activate FOG-1 or FOG-3, or regulate an unknown target.

4. Special regulatory modules promote hermaphrodite spermatogenesis

How do hermaphrodites undergo a brief period of spermatogenesis, given that XX animals do not express the HER-1 masculinizing non-autonomous signal? Genetic screens have revealed three regulatory modules that play a central role in this process.

4.1. Translational regulation of *tra-2* by GLD-1 and FOG-2

Gain-of-function mutations in *tra-2* cause XX animals to make only oocytes, but have little effect on males (Doniach, 1986). Sequence analysis showed that these *tra-2(gf)* mutations disrupt sequences in the 3' UTR known as TGEs (Goodwin et al., 1993). In wild-type animals, these elements bind a protein complex that includes GLD-1 (Goodwin et al., 1993; Jan et al., 1999). Three observations imply that GLD-1 represses translation of *tra-2* (Figure 3). First, GLD-1 is required for spermatogenesis in hermaphrodites but not in males (Francis et al., 1995; Francis et al.,1995). Second, somatically expressed GLD-1 can repress translation of somatic transgene RNAs that contain TGEs (Jan et al., 1999). Third, TRA-2 levels are increased in *gld-1* null mutant germ lines, based on *in situ* antibody staining (Jan et al., 1999).

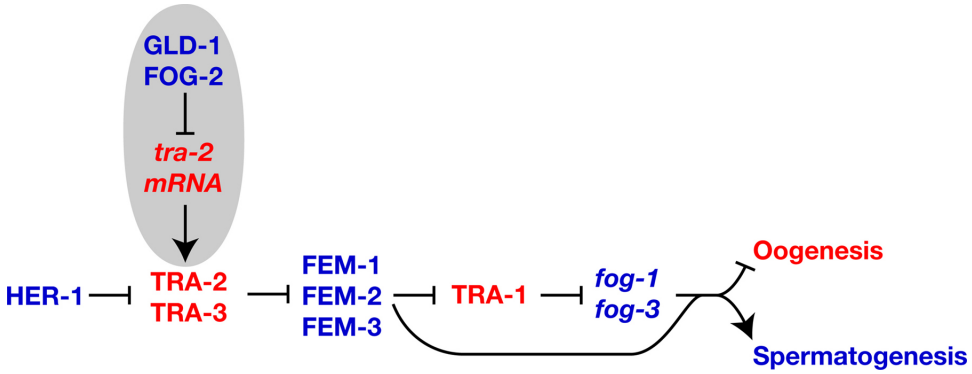

Figure 3. Regulatory modules that promote spermatogenesis. Proteins are shown in uppercase, and genes or mRNAs in lowercase italics. Positive interactions are indicated with an arrow, and negative ones by a "--|". Genes that promote spermatogenesis are blue, and those that promote oogenesis are red. Regulatory circuits that promote hermaphrodite spermatogenesis are highlighted in gray.

GLD-1 is a member of the STAR family of proteins, which contain a maxi-KH RNA binding domain and two other conserved domains, called QUA1 and QUA2 (Jones et al., 1996). *In vitro* studies indicate that GLD-1 acts as a dimer (Ryder et al., 2004). Because GLD-1 has pleiotropic roles in germline development (Francis et al., 1995; Francis et al., 1995) it must regulate other messages in addition to *tra-2*. Some of these mRNA targets have been identified (Lee and Schedl, 2001; 2004; Marin and Evans, 2003; Mootz et al., 2004). Recently, *in vitro* biochemical studies implicated a hexanucleotide sequence as part of the GLD-1 binding site, at least for some RNA targets (Ryder et al., 2004).

fog-2 is required specifically for hermaphrodite spermatogenesis (Schedl and Kimble, 1988). FOG-2 binds GLD-1 in the yeast two-hybrid assay and *in vitro*, which implies that they interact to regulate *tra-2* (Figure 3; Clifford et al., 2000). Genetic data support this model, since epistasis analyses place *fog-2* upstream of *tra-2*, and loss of *fog-2* activity causes the same phenotype as *tra-2(gf)* mutations (Schedl and Kimble, 1988). The C-terminus of FOG-2, which mediates its interaction with GLD-1, has been evolving rapidly (Nayak et al., 2004). The N-terminus of FOG-2 contains an F-box (Clifford et al., 2000) and binds the Skp1-related protein SKR-1 (Nayak et al., 2004); in other proteins, this would imply an interaction with the SCF ubiquitin ligase machinery. However, FOG-2 cannot target GLD-1 for degradation, since both promote spermatogenesis. Perhaps it recruits other proteins to the *tra-2* 3'UTR to block translation.

Some data suggest that *laf-1* also regulates the translation of *tra-2* messages (Goodwin et al., 1997). However, definitive tests of epistasis have not been possible, because *laf-1* alleles cause recessive lethality as well as dominant feminization.

4.2. Direct interactions between TRA-2 and TRA-1

Several missense mutations in the intracellular domain of TRA-2 prevent spermatogenesis in hermaphrodites but not in males (Doniach, 1986; Kuwabara et al., 1998). Although this feminizing effect is dominant, these mutations also cause recessive masculinization in the soma, so they were named *mixomorphic* (*mx*) alleles. Surprisingly, these alleles alter a region of TRA-2 that normally binds TRA-1, and disrupt this interaction (Lum et al., 2000; Wang and Kimble, 2001). What remains unclear is the purpose of this TRA-2/TRA-1 interaction. One possibility is that the TRA-2/TRA-1 complex directly promotes oogenesis and other female fates; this model is consistent with the fact that *tra-2(mx)* alleles behave as if they cause increased wild-type activity (Doniach, 1986; Schedl and Kimble, 1988). Alternatively, the TRA-2/TRA-1 complex might promote spermatogenesis, as implied by the fact that blocking this interaction leads to oogenesis.

Since TRA-2 is a membrane protein, and TRA-1 is a transcription factor, how do they find each other? Lum et al. (2000) showed that the intracellular portion of TRA-2 is imported to the nucleus. Since *tra-2* requires *tra-3* to promote female development, and *tra-3* encodes a calpain protease (Barnes and Hodgkin, 1996), TRA-3 might cleave TRA-2 to release this intracellular fragment. This interaction has been confirmed *in vitro* (Sokol and Kuwabara, 2000). Although germ cells produce a small transcript, *tra-2B*, that encodes a similar intracellular fragment, this transcript is associated with oogenesis rather than spermatogenesis (Kuwabara et al., 1998), and is unlikely to act in this regulatory process.

4.3. Export of TRA-1 from the nucleus

Since TRA-1 is a transcription factor, it needs to be in the nucleus to control cell fates. Surprisingly, TRA-1 binds sequences in the 3'-UTR of *tra-2* messages (Graves et al., 1999). Furthermore, a mutation in *tra-2* that blocks this interaction increases the levels of nuclear TRA-1 (Segal et al., 2001). This mutation also promotes oogenesis, though this effect is not unexpected since it deletes both TGEs, which are needed for translational repression of *tra-2*. Taken together, these results suggest that *tra-2* messages might negatively regulate TRA-1 activity. The export of *tra-2* messages requires CRM-1, as well as a group of genes that prevent export by the alternative NXF-1 pathway (Kuersten et al., 2004). Perhaps these complex interactions between *tra-2* and TRA-1 help modulate TRA-1 activity during hermaphrodite development.

5. Regulation of *fem-3* translation is required for hermaphrodite oogenesis

Hermaphrodites are only fertile if they eventually stop making sperm and begin to produce oocytes. By the mid-L4 stage, all germ cells that will form sperm are in pachytene (or later stages) and about the same number of cells have begun oogenesis, based on expression of the yolk receptor transcript (Figure 4). Thus, the decision to switch from spermatogenesis to oogenesis must have occurred at some earlier, yet to be determined, point. Three lines of evidence suggest that a crucial factor for determining how long hermaphrodites make sperm is the ratio of TRA-2 activity to FEM-3 activity. First, the *tra-2* gain-of-function mutations described above increase the production of TRA-2, which causes XX animals to make oocytes instead of sperm (Doniach, 1986). Second, *fem-3* gain-of-function mutations increase the production of FEM-3, which causes XX animals to make sperm instead of oocytes (below, Barton et al., 1987). Third, these *tra-2(gf)* and *fem-3(gf)* mutations can mutually suppress each other, restoring production of both sperm and oocytes (Schedl and Kimble, 1988). Because germ cell fates are so sensitive to the ratio of TRA-2 to FEM-3 activity, it is not surprising that additional regulatory modules control the production of FEM-3 protein from *fem-3* messenger RNAs.

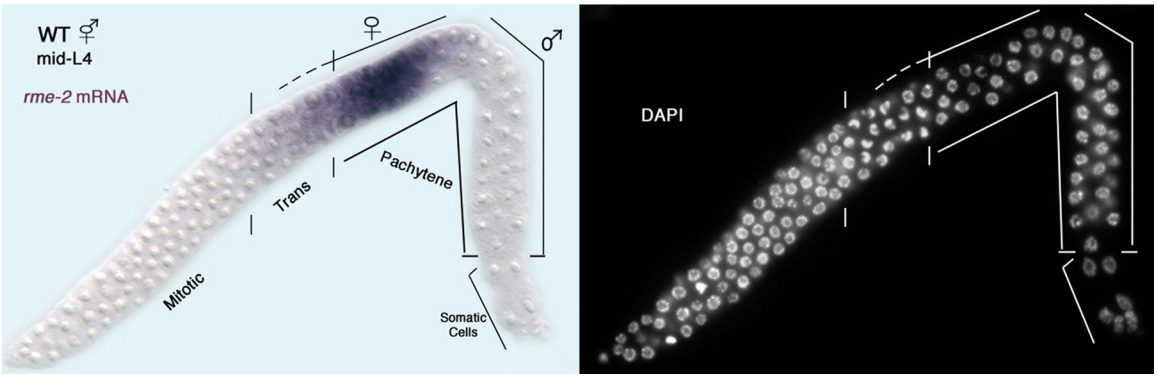

Figure 4. An early point following the switch from hermaphrodite spermatogenesis to oogenesis. *In situ* hybridization of the *rme-2* yolk receptor mRNA to a dissected L4 hermaphrodite gonad: left, DIC image with *rme-2* staining in purple; right, fluorescence microscopy image of DAPI staining to visualize chromosome morphology; distal tip on the lower left. Shown are the meiotic prophase stages leptotene and zygotene (indicated 'trans' for transition zone) as well as pachytene. Germ cells in the proximal end of the gonad are undergoing spermatogenesis and do not express the *rme-2* yolk receptor mRNA. Germ cells located just distally, from the transition zone to the loop (bend in gonad) are undergoing oogenesis and express *rme-2* mRNA (M. H. Lee and T. Schedl, unpublished results). Thus, at this time point, there are both male and female pachytene stage germ cells.

5.1. Repression of *fem-3* translation by FBF-1, FBF-2 and NOS-3

Hermaphrodites cannot switch from the production of sperm to that of oocytes unless *fem-3* activity is repressed by a "Point Mutation Element," or PME, located in the 3'UTR (Ahringer and Kimble, 1991; Ahringer et al., 1992; Rosenquist and Kimble, 1988). Gain-of-function mutations in *fem-3* alter this PME, resulting in increased *fem-3* activity and constitutive spermatogenesis. Since these mutations don't change the level of *fem-3* transcripts, but appear to increase the length of the poly(A) tail, they probably increase *fem-3* translation.

Using the yeast three-hybrid assay, Zhang et al. (1997) identified two proteins, called FBF-1 and FBF-2, that bind the *fem-3* PME. These proteins are remarkably similar in amino acid sequence, and have overlapping functions. The gain-of-function mutations in the PME prevent the FBF proteins from binding the *fem-3* 3'-UTR. Moreover, inactivation of the *fbf* genes by RNA-mediated interference recapitulates the phenotype of these *fem-3(gf)* mutants — the XX animals produce sperm instead of oocytes. Thus, FBF-1 and FBF-2 repress the translation of *fem-3*

messages by binding the PME (Figure 5). Although *fbf-1* and *fbf-2* single mutants are usually wild type, recent results suggest that they are not completely redundant, but have slightly different roles in the control of sexual fate and cell proliferation (Lamont et al., 2004).

The FBF proteins are homologs of the *Drosophila* translational regulator Pumilio, which interacts with Nanos to inhibit translation of *hunchback* messages. Similarly, the *C. elegans* FBF proteins bind NOS-3 in the yeast two-hybrid system and *in vitro* (Kraemer et al., 1999). Furthermore, inactivation of NOS-3 and the two other NOS homologs by RNAi causes many XX animals to produce sperm instead of oocytes (Kraemer et al., 1999). Thus, NOS-3 is likely to act with the FBF proteins to repress translation of *fem-3*.

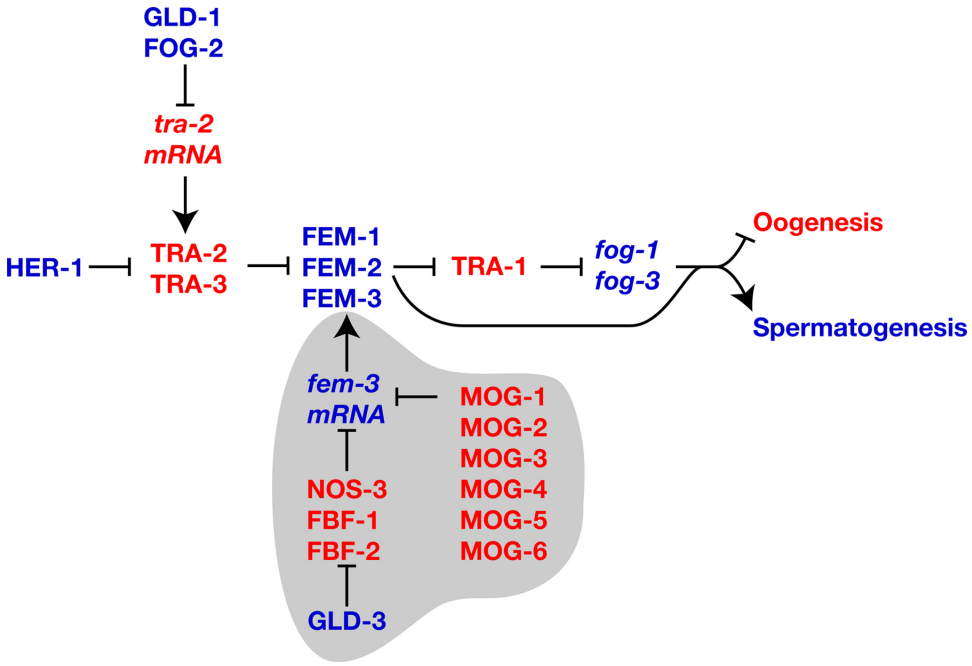

Figure 5. Regulation of *fem-3* translation. Proteins are shown in uppercase, and genes or mRNAs in lowercase italics. Positive interactions are indicated with an arrow, and negative ones by a "--|". Genes that promote spermatogenesis are blue, and those that promote oogenesis are red. Molecules that regulate translation of *fem-3* messages are highlighted in gray.

The same yeast two-hybrid screen that identified NOS-3 also showed that the GLD-3 protein interacts with FBF-1 and FBF-2 (Eckmann et al., 2002). GLD-3 is a homolog of Bicaudal-C from *Drosophila*. Mutant analysis showed that GLD-3 plays several roles in germline development, one of which is the promotion of spermatogenesis — in both *gld-3(null)* males and hermaphrodites, some cells that should have become sperm instead develop as oocytes. This *gld-3* phenotype is suppressed by mutations in the *fbf* genes, which implies that GLD-3 negatively regulates FBF activity (Figure 5).

5.2. *mogs* and friends

The activity of *fem-3* mRNA is also controlled by a second group of genes. Recessive mutations in *mog-1*, *mog-2*, *mog-3*, *mog-4*, *mog-5* or *mog-6* cause hermaphrodites to make sperm instead of oocytes, and also affect the size of the germ line and the viability of embryos (Graham and Kimble, 1993; Graham et al., 1993). By using reporter assays, Gallegos et al. (1998) showed that *mog* gene activity can also be detected in the soma, where they act through the *fem-3* 3'UTR to repress reporter constructs. However, mutations in the *mog* genes don't cause masculinization of the soma in hermaphrodites. One possible explanation is that maternal *mog* activity is sufficient for normal development. Alternatively, the level of *fem-3* transcripts in the soma of hermaphrodites might be outside the range that is responsive to repression by the *mog* genes.

Four of the *mog* genes have been cloned; *mog-1*, *mog-4*, and *mog-5* encode DEAH-box proteins that are similar in sequence to the yeast proteins PRP16, PRP2, and PRP22, respectively (Puoti and Kimble, 1999; Puoti and Kimble, 2000), and *mog-6* encodes an unusual nuclear cyclophilin (Belfiore et al., 2004). All four proteins bind the MEP-1 zinc-finger protein (Belfiore et al., 2002; Belfiore et al., 2004), which is also located in the nucleus, but has

essential functions not shared by the *mog* genes. At this time, the manner in which the MOG complex controls target messages remains unknown.

Two other genes have similar phenotypes. First, *mag-1* is the homolog of the *Drosophila mago nashi* gene, which regulates several aspects of germline development. In *C. elegans*, *mag-1(RNAi)* causes hermaphrodites to produce sperm instead of oocytes, and affects embryonic viability (Li et al., 2000). Although these phenotypes suggest that *mag-1* functions in the same process as the *mog* genes, the *mag-1(RNAi)* phenotype is suppressed by mutations in *fog-2* (Li et al., 2000), whereas the *mog-1* phenotype is not (Graham and Kimble, 1993). Second, *atx-2(RNAi)* causes hermaphrodites to make sperm instead of oocytes, in addition to numerous other defects in germline development (Ciosk et al., 2004; Maine et al., 2004).

6. Crucial questions for the future

Although this field has reached a mature stage, in which a genetic regulatory process of great complexity has been identified and dissected into component steps, many new lines of research have opened up in the past several years and some old questions remain unsolved.

6.1. How do hermaphrodites initiate the switch from spermatogenesis to oogenesis?

We suggested above that the crucial factor controlling whether germ cells become sperm or oocytes is the ratio of TRA-2 activity to FEM-3 activity, and described several regulatory pathways that influence these activities. However, we do not yet know why hermaphrodites make sperm early in life, and oocytes later on, since we don't know which regulatory activities change at the appropriate time. For example, GLD-1 and FOG-2 are expressed in both larval and adult hermaphrodites, so it seems unlikely that repression of *tra-2* ends when animals reach sexual maturity, thus allowing the switch to oogenesis, although it remains possible that the activity of a third factor, or a modification to either FOG-2 or GLD-1, might be responsible for the switch.

As proposed by Goodwin and Ellis (2002), three explanations might account for this puzzle. First, one of these regulatory proteins might be modified to change its activity in a manner that could not be detected by RNA or protein cytological analysis. Second, the switch might be caused by a change in the activity of a gene that has not yet been discovered. Third, the increasing size and cell number in the developing germ line might slowly change the relative activities of the *tra-2* and *fem-3* regulators, so that the ratio of *tra-2* to *fem-3* activity automatically flips during late larval development.

6.2. How is the regulation of sexual fate integrated with the control of proliferation?

In theory, these two decisions might be completely independent, so that germ cells entering meiosis merely check the status of the sex-determination pathway to learn if they should begin spermatogenesis or oogenesis. However, many of the genes that act in one pathway also regulate the other. For example, the FBF proteins and GLD-1 control both processes. Furthermore, genes that control progression through the pachytene stage of meiosis also promote spermatogenesis. Are these pleiotropic effects accidental, resulting only because many genes have pleiotropic roles in development? Or do these dual activities help coordinate the overall development of the germ line? Furthermore, do these data shed any light on the developmental point at which germ cells commit to one sexual fate or the other?

6.3. What other influences control germ cell fate?

The ablation of a single sheath/spermathecal precursor cell in the somatic gonad can completely feminize the germ line in XX animals (McCarter et al., 1997). This result suggests that a second somatic signal might influence germ cell fates, since *her-1* is not expressed in XX animals. However, no ligand or receptor has been identified, and it is possible that these ablations exert their influence indirectly, by reducing the size of the germ line. Indeed, direct ablation of a subset of germ cells at the L2/L3 molt also completely feminizes some XX animals (McCarter et al., 1997).

There is also a potential signal transduction pathway that promotes spermatogenesis, but whose mechanism of operation remains unknown. Genes of the ERK MAP Kinase pathway are required for several different germ cell fates, including the progression of cells through the pachytene stage of meiosis I (Church et al., 1995; Hsu et al., 2002). Partial loss of function of *lin-45* Raf, *mek-2* MEK and *mpk-1* ERK can causes hermaphrodites to make only oocytes instead of sperm. Furthermore, complete inactivation of these genes causes germ cells to arrest at the

pachytene stage of meiosis and produce oocyte-specific transcripts, even in males (M. Ohmachi, M. H. Lee, E. Lambie and T. Schedl, personal communication). Thus, this pathway normally promotes spermatogenesis. However, the source of the signal, or even whether these genes act in their familiar mode as transducers of a signal, remains unknown.

6.4. How did this sex determination system evolve?

Genes that regulate sexual fate in germ cells also determine if XX animals develop as females or hermaphrodites. Since new phylogenies show that hermaphroditism likely has evolved independently in *C. elegans* and *C. briggsae* (Cho et al., 2004 Kiontke et al., 2004), these nematodes provide an ideal subject for comparative evolutionary studies. In particular, we need to know how the sex-determination pathway is modified over time to create new mating systems (see The evolution of nematode sex determination).

6.5. Is *C. elegans* a good model for germline development in other animals?

Although we have a solid working model of sex-determination in the *C. elegans* germ line, the picture from *Drosophila* and mammals remains confusing. However, homologs of many genes that act in the *C. elegans* germ line are expressed in the germ cells of other animals. Do any of these genes play conserved roles in germline development, like *mab-3* and *doublesex* do in the soma?

6.6. What technical innovations could revolutionize this field?

A major problem facing research on the *C. elegans* germ line is the difficulty in making reliable reporter constructs. Often, transgenic reporters are silenced in the germ line, while expression in the soma is not disrupted. This problem is at least partially caused by the ability of the germ line to shut down transcription of repetitive regions of the genome, such as are often created by transgenes. Despite the use of high-complexity DNA (Kelly et al., 1997) and particle bombardment (Praitis et al., 2001) to counter this problem, these transgenes generally take much more work to produce than similar ones in somatic tissues. New methods to simplify this process, or the identification of related nematodes with less stringent controls on expression in the germ line, could revolutionize the field.

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