

Enigma variations: control of sexual fate in nematode germ cells

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Abstract

A new study showing that neither FEM-2 nor FEM-3 is required for spermatogenesis in *Caenorhabditis briggsae*, unlike in *Caenorhabditis elegans*, implies that the sex-determination pathway in these species is evolving rapidly, and supports the proposal that they evolved hermaphroditism independently.

Years ago, French pop star Patrick Juvet raised a question that evolutionary biologists are pondering anew in the aftermath of a paper about nematode sex determination from Eric Haag, David Pilgrim and their colleagues published recently in *Developmental Cell* [1]: “Où sont les femmes?” - Where are the *fems*? By showing that the *fem* genes, which are essential for spermatogenesis in *Caenorhabditis elegans*, are dispensable in *Caenorhabditis briggsae* germ cells, they proved that the regulatory pathways in these species have undergone recent and dramatic change.

Regulation of sexual traits evolves rapidly

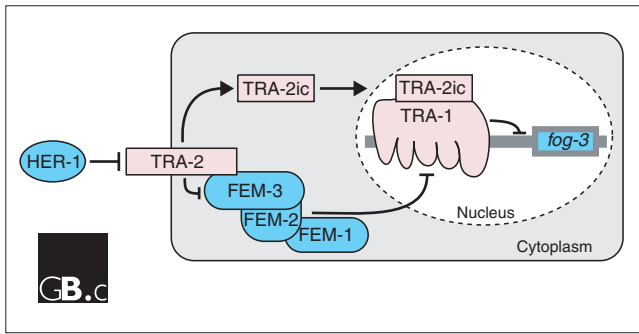
During the 1980s and 1990s, researchers cloned many of the genes that control sexual development in nematodes and fruit flies, and found that none of them resembled each other (reviewed in [2]). Since many other regulatory pathways were conserved, these results took everyone by surprise. Later, Raymond *et al.* [3] found that a single downstream gene, *mab-3/doublesex*, had been conserved between nematodes and insects. Taken together, these data implied that the regulatory pathways that control sexual development are derived from a common ancestor, but have been evolving rapidly.

As an example of just how rapid this process can be, consider the nematode family Rhabditidae. In most of its species, XO animals are male and XX animals are female. Some species, however, feature XO males and XX hermaphrodites. Furthermore, all these hermaphrodites are essentially female animals that make their own sperm during larval development,

which they use for self-fertilization. Surprisingly, self-fertile hermaphrodites have evolved independently many times in the Rhabditidae [4]. Even during recent evolution, these mating systems have changed multiple times within a small subgroup of the genus *Caenorhabditis* [5,6]. Thus, these nematodes provide a terrific model for studying the rapid evolution of sexual traits, and the recent work by Hill *et al.* [1] is the first major advance in this developing field.

The *fem* genes promote male sexual fates in *C. elegans*

Genetic analysis of *C. elegans* revealed that male sexual fates are coordinated by a secreted protein, HER-1, that binds to and inactivates the receptor protein TRA-2 (reviewed in [7]). Three intracellular proteins, FEM-1, FEM-2, and FEM-3, help transmit this inhibitory signal to the transcription factor TRA-1, which controls cell fate (Figure 1). Inactivation of any of these *fem* genes has two effects: all somatic cells choose female fates; and all germ cells choose oogenesis. How the FEM proteins work is unclear. FEM-1 contains ankyrin repeats [8], which often mediate interactions with other proteins. *In vitro* assays show that it binds FEM-2 [9]. FEM-2 is a PP2C-type protein phosphatase [10,11], but its targets remain unknown. FEM-3 is a novel protein [12] that can bind FEM-2 [10] and TRA-2 [13]. Perhaps FEM-3 forms the core of a complex that promotes male development by inhibiting TRA-1 activity (see Figure 1). In the soma, the three FEM proteins appear to be the major pathway for information flow between the receptor TRA-2 and the transcription factor

**Figure 1**

Two routes to the nucleus in the germline cell-fate pathway in *C. elegans*. HER-1 is a secreted protein that specifies male cell fates in *C. elegans*, including spermatogenesis. It binds the transmembrane receptor TRA-2 and inhibits its activity in XO animals, causing male development. The inactivation of TRA-2 permits three interacting cytoplasmic proteins - FEM-1, FEM-2 and FEM-3 - to direct male fates by inhibiting the transcription factor TRA-1. When TRA-1 is inactive, genes like *fog-3* are free to specify spermatogenesis. (Although XX hermaphrodites do not produce HER-1, the FOG-2 and GLD-1 proteins prevent the production of TRA-2 during larval development, allowing the FEM proteins to direct spermatogenesis for a brief time in a female gonad.) Surprisingly, a fragment cleaved from TRA-2 (TRA-2ic, for intracellular) can also interact directly with TRA-1. Mutations that block this interaction cause oogenesis. In the figure, male-promoting factors are shown in blue, female ones in pink, and all proteins that touch are known to interact.

TRA-1. Protein-protein interaction studies using the yeast two-hybrid system showed, however, that an intracellular fragment derived from TRA-2 (TRA-2ic; see Figure 1) can bind directly to TRA-1 [14,15]. Furthermore, mutations that disrupt this interaction cause all germ cells to choose oogenesis [14-17]. These findings raise several questions. Why are there two information paths? How did this system arise? And are parallel, redundant pathways stable during evolution? The logical place to turn for answers was the other hermaphroditic species in the *Elegans* genus, *C. briggsae*.

The role of the *fem* genes in *C. briggsae*

Both *tra-1* and *tra-2* are conserved in *C. briggsae*, and both regulate sexual development much as in *C. elegans* [18,19]. A third gene, *fog-3*, is a major target of TRA-1 in *C. elegans* [20] (see Figure 1), and its promoter, coding sequence and function are conserved in *C. briggsae* [21]. This result suggested that the entire sex-determination pathway might be the same in these nematodes, so Nayak *et al.* [22] used the *C. briggsae* genome sequence to search for the other factors. And this is where the surprises started. They found no clear homolog of *fog-2*, a gene that regulates *tra-2* translation in *C. elegans* hermaphrodites. They had suspected that this might be so, as *fog-2* seemed to have evolved recently in *C. elegans* from a duplicated F-box gene [23]. And Nayak *et al.* [22] also showed that the partner of FOG-2 in *C. elegans*, an RNA-binding protein called GLD-1, has an entirely different function in *C. briggsae* - it promotes

oogenesis rather than spermatogenesis. Given these findings, and the likelihood that *C. briggsae* and *C. elegans* had evolved self-fertile hermaphroditism independently, Hill *et al.* [1] focused on the germline, and the role of the three *fem* genes, which are required for spermatogenesis in both sexes of *C. elegans*.

Initial experiments using RNA interference (RNAi) suggested that the *fem* genes were not required for spermatogenesis in *C. briggsae* [24,25]. RNAi usually lowers but does not eliminate gene activity, however, so the meaning of these results remained in doubt. To resolve this dilemma, Hill *et al.* [1] decided to look for null alleles of both genes, using two clever approaches. First, they screened for deletions of *fem-2* and *fem-3* using the same PCR-based methods that had been developed for *C. elegans*. Despite the effort involved, this method is ideal for finding null alleles. Second, they screened for suppressors of *tra-2*, a method that yields lots of mutations in the *fem* genes in *C. elegans*.

Having isolated deletion mutants of both *fem-2* and *fem-3* in *C. briggsae*, Hill *et al.* [1] found that mutant XX animals were able to make sperm just fine, unlike the analogous mutants in *C. elegans*. Since *C. elegans* hermaphrodite development depends on a competition between TRA-2 and FEM-3 activity (reviewed in [7]), these results showed that something fundamentally different must be happening in *C. briggsae*. Given these findings from XX hermaphrodites, one might imagine that the FEM pathway plays no role at all in the *C. briggsae* germline. But Hill *et al.* [1] went on to show that *C. briggsae* XO males require both *fem-2* and *fem-3* to continue producing sperm. Mutations in either gene cause males to switch to oogenesis late in life. And although *tra-2* mutants normally produce only sperm, the addition of a *fem* mutation caused these animals to switch to oogenesis later in life, suggesting that in hermaphrodites too the FEM proteins are required to maintain the ability to produce sperm. The FEM proteins are therefore active in the germlines of both sexes in *C. briggsae*. But where in the developmental pathway? And how?

If the role of the *fem* genes in primary sex determination had been supplanted by new genes in *C. briggsae*, one might expect to find mutations in those genes among the pool of *tra-2* suppressors. A large screen for *tra-2* suppressors did not, however, yield any mutations that caused *C. briggsae* XX *tra-2* mutants to develop as females [1]. Thus, the sex-determination pathway appears to work differently in *C. briggsae* and *C. elegans*.

What are the alternatives?

The new results raise many questions. First, how does TRA-2 interact with TRA-1? By showing that the FEM proteins have a different role in the *C. briggsae* germline than expected, Hill *et al.* [1] underscore the importance of the direct

interaction between TRA-2 and TRA-1, as this represents the other known pathway from receptor to nucleus (Figure 1). Wang and Kimble [14] have shown that this interaction is conserved in *C. briggsae*. However, the exact nature and function of this interaction remain unknown in either species, so much work remains to be done.

Second, has the germline sex-determination pathway recruited somatic genes? Were the *fem* genes originally somatic regulators that were expressed in the germline only because maternal product was needed in embryos? In this scenario, the *fem* genes once played no role in spermatogenesis, as suggested by RNAi experiments that show no requirement for them in *Caenorhabditis remanei* males [24]. One could imagine that ectopic expression of *fem* transcripts in the maternal germline led first to a small role for FEM proteins in spermatogenesis (as in *C. briggsae*) and later to an absolute requirement for FEMs for male sex determination (as in *C. elegans*). This type of change could also have worked in reverse.

Third, what constitutes the switch that controls spermatogenesis and oogenesis in *C. briggsae*? That *C. briggsae fem-2(lf)*, *fem-3(lf)* and *tra-1(lf)* mutants (where *lf* indicates loss of function) all produce sperm when young and oocytes when old (D. Keller and E. Haag, personal communication) suggests that the activity of the genes that specify spermatogenesis or oogenesis changes naturally during aging. If so, the sex-determination pathway modulates this rate of change to produce males or females, and the ground state would be hermaphrodite development.

Finally, could binary pathways, such as those that regulate sex, be inherently unstable? Since *C. elegans* has two pathways that transmit information from TRA-2 to the nucleus, the requirement for the *fem* genes might have arisen recently from a subsidiary role like that in *C. briggsae*. If so, in other nematode species the TRA-2/TRA-1 interaction might have become completely superfluous, and been lost. As the output of a binary pathway is always one of two states (like male or female), it is easy to imagine upstream regulators constantly being added to or subtracted from binary pathways during evolution. Perhaps that feature explains why the downstream regulator *mab-3/doublesex* is the only sex-determination gene conserved between worms and flies.

Thus, recent studies comparing sex-determination pathways in *C. elegans* and *C. briggsae* have raised numerous fascinating questions, many of which can be answered by studying other *Caenorhabditis* species. Since the genomes of many of these species are now being sequenced, the future should be exciting.

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