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Genetic control of sex determination in the germ line of *Caenorhabditis elegans*

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The nematode *Caenorhabditis elegans* normally exists as one of two sexes: self-fertilizing hermaphrodite or male. Development as hermaphrodite or male requires the differentiation of each tissue in a sex-specific way. In this review, I discuss the genetic control of sex determination in a single tissue of *C. elegans*: the germ line. Sex determination in the germ line depends on the action of two types of genes: – those that act globally in all tissues to direct male or female development and those that act only in the germ line to specify either spermatogenesis or oogenesis. First, I consider a tissue-specific sex-determining gene, *fog-1*, which promotes spermatogenesis in the germ line. Second, I consider the regulation of the hermaphrodite pattern of germ-line gametogenesis where first sperm and then oocytes are produced.

Introduction

One of the great successes of modern developmental genetics has been the identification and characterization of regulatory genes that control sex determination. Sex is normally determined by the chromosomal complement of an individual. For example, in mammals, XX animals are female and XY animals are male. However, in organisms as diverse as worms and mice, mutations have been isolated that override the chromosomal signal and therefore cause sexual differentiation that is independent of the chromosomal constitution of the animal. Genetic studies have emphasized genes that control the sexual phenotype of an entire organism. These 'global' sex-determining genes regulate sexual development in all tissues of the animal. However, the differentation of an animal as male or female involves the regulation of each cell or tissue to differentiate according to a particular sex-specific pathway. In this short review, I discuss progress that has been made in the free-living nematode *Caenorhabditis elegans* to elucidate the genetic regulation of sex determination in a single tissue: the germ line. This regulation depends on both global and tissue-specific sex-determining genes.

Over the past two decades, *C. elegans* has been subjected to intensive genetic and descriptive analyses (see Wood (1988) for review). With its simple anatomy and short life cycle ($3\frac{1}{2}$ days), this tiny worm proves to be particularly useful for studies of developmental regulation. Genetic analyses of sex determination, pioneered by Jonathan Hodgkin at the MRC Laboratory of Molecular Biology in Cambridge, England, have led to the identification and genetic characterization of several 'global' sex-determining genes (see Hodgkin (1987*a*) for review). In addition, genes that control the sexual differentiation of a single tissue, the germ line, have recently been discovered (Doniach 1986*a*; Schedl & Kimble 1988; Barton & Kimble 1988; this laboratory, unpublished results).

Normally in *C. elegans*, XX animals are self-fertilizing hermaphrodites (somatic 'females' that make sperm first and then produce oocytes continuously) and XO animals are males (figure

1). These two sexes differ substantially in morphology, biochemistry, and behaviour (table 1). Oocytes can either be self-fertilized by hermaphrodite sperm or cross-fertilized by male sperm. Sperm and oocytes are distinct cell types: sperm are small cells specialized for motility, whereas oocytes are large cells specialized for embryogenesis (Kimble & Ward 1988).

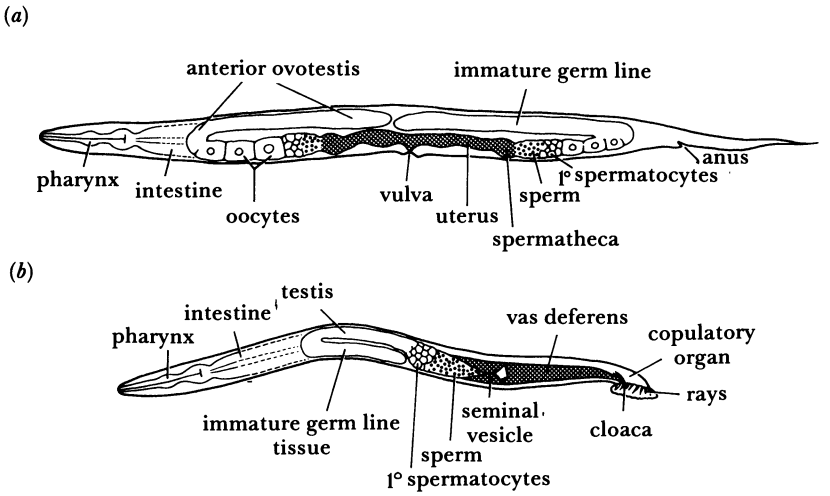

FIGURE 1. Schematic diagrams of (*a*) a young adult hermaphrodite and (*b*) a male. Notice the sexually dimorphic gonads (two ovotestes in the hermaphrodite, one testis in the male) and tails (a whip-like tail in the hermaphrodite, a tail specialized for copulation in the male). The somatic gonad of each is indicated by shading, the rest of the tubular gonad is germ line.

TABLE 1. SEXUAL DIMORPHISM IN *C. ELEGANS*

	hermaphrodite	male
chromosomes	XX; 5AA = 12	XO; 5AA = 11
hypodermis	vulva	copulatory bursa, spicules, rays
nerve	neurons for egg-laying	neurons for mating
muscle	muscles for egg-laying	muscles for mating
intestine	yolk synthesis	—
somatic gonad	two ovotestes, uterus, spermathecae	one testis, seminal vesicle, vas deferens
germ line	sperm, then oocytes	sperm
total somatic nuclei	959	1031
total germ-line nuclei	about 2500	about 1000

The initial signal for sex determination in *C. elegans* is the ratio of X chromosomes to sets of autosomes (A) (Nigon 1951; Madl & Herman 1979). This signal controls the activity of several global sex-determining genes. Certain of these genes, e.g. *sdc-1* (Villeneuve & Meyer 1987), regulate both sexual development and dosage compensation, whereas others, e.g. the *fem* and *tra* genes, regulate only the sexual phenotype (for review see Hodgkin (1987*a*)).

The mutant phenotypes of those sex-determining genes considered further in this review are summarized in table 2. The phenotype of loss-of-function (*lf*) mutations is used to deduce the wild-type function of a gene; the phenotype of gain-of-function (*gf*) mutations may be due to a novel, poisonous, increased, unregulated or inappropriate activity. Although most *lf* mutations are recessive and most *gf* mutations are dominant, among the mutations that regulate *C. elegans* sex determination, both dominant *lf* and recessive *gf* mutations are known.

TABLE 2. PHENOTYPES OF MUTANTS DISCUSSED IN THIS PAPER

	mutant phenotype[a]	
gene	XX	XO
wild type	female soma, sperm then oocytes	male
tra-1(*lf*)[b]	pseudomale soma, sperm, then oocytes	pseudomale soma, sperm, then oocytes
tra-2(*lf*)[c]	pseudomale soma, sperm only	male
tra-3(*lf*)[c]	pseudomale soma, sperm, then oocytes	male
fem-1(*lf*)[d], *fem-2*(*lf*)[e], *fem-3*(*lf*)[f]	female	female
her-1(*lf*)[g]	female soma, sperm, then oocytes	female soma, sperm, then oocytes
fog-1(*lf*)[h]	female	male soma, oocytes only
fog-2(*lf*)[i]	female	male
tra-2(*gf*)[j]	female	male
fem-3(*gf*)[k]	female soma, sperm only	male

[a] 'female' refers to female development in both soma and germline; 'male' refers to male development in both soma and germline
[b] Hodgkin & Brenner (1977); Hodgkin (1987*b*); Kimble & Schedl (1988); T. Schedl, unpublished results.
[c] Hodgkin & Brenner (1977).
[d] Nelson *et al.* (1978); Doniach & Hodgkin (1984).
[e] Kimble *et al.* (1984); Hodgkin (1986).
[f] Hodgkin (1986); Barton *et al.* (1987).
[g] Hodgkin (1980); Trent *et al.* (1988).
[h] Doniach (1986*a*); Barton & Kimble (1988).
[i] Schedl & Kimble (1988).
[j] Doniach (1986*b*); Schedl & Kimble (1988).
[k] Barton *et al.* (1987).

Among the global sex-determining genes of *C. elegans*, some are required for hermaphrodite development and others are required for male development. The phenotype of *lf* mutations in any of three *tra* genes (for sexual *tra*nsformation) is masculinization of XX animals; therefore the *tra* genes normally direct female development. The phenotype of *lf* mutations in any of three *fem* genes (for *fem*inization) is feminization of both XX and XO animals; therefore the *fem* genes normally specify male development in the hermaphrodite germ line (sperm) and in males. The phenotype of *her-1*(*lf*) (for *her*maphroditization) is sexual transformation of XO animals into hermaphrodites; therefore *her-1* normally directs male development.

In addition to global sex-determining genes, there exist germ-line-specific sex-determining genes: *fog* and *mog* genes (for *f*eminization (or *m*asculinization) *o*f the *g*ermline). Mutants that specifically affect germ-line sex determination have been isolated both in mutant screens and by genetic selection (Kimble *et al.* 1986; Doniach 1986*a*; Schedl & Kimble 1988; Barton & Kimble 1988; this laboratory, unpublished results). The idea behind the genetic selections used to isolate germ-line-specific sex determination mutants is shown in figure 2. Basically an XX hermaphrodite is self-sterile if it produces only sperm or only oocytes, but it is self-fertile if sperm and then oocytes are made. Therefore mutants that feminize the hermaphrodite germline so that only oocytes are made (or masculinize it so that only sperm are made) can be used to select for suppressor mutations that reinstate self-fertility. In this way, either masculinizing or feminizing suppressors can be isolated. Mutations in the *fog* and *mog* genes result in sexual transformation in the germ line, but do not affect somatic tissues. The functions of these tissue-specific sex-determining genes are discussed below.

The regulatory relationships among the sex-determining genes have been investigated by examination of the phenotypes of double mutants (see, for example, Hodgkin 1987*a*). In

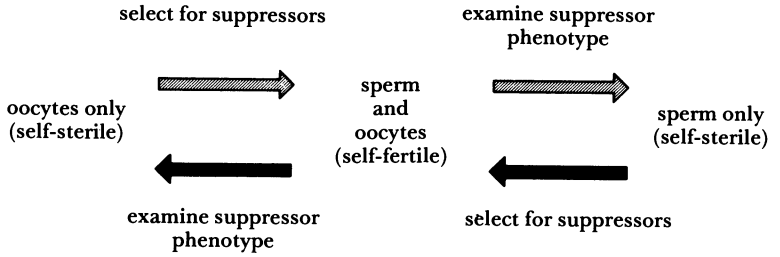

FIGURE 2. Basic idea behind genetic selections for mutants that affect germ-line sex determination. See text for explanation. These selections have been used to isolate mutations in new genes (*fog-1*, Barton & Kimble (1988); *fog-2*, Schedl & Kimble (1988); *fog-3*, M. K. Barton and T. Schedl, unpublished results), to isolate rare gain-of-function mutations in known genes (*tra-1*, M. K. Barton, unpublished results; *tra-2*, Schedl & Kimble (1988); *fem-3*, Barton *et al.* (1987)), and to isolate transposon insertions into sex-determining genes for cloning (*fem-3*, Rosenquist & Kimble (1988); *tra-2*, Okkema & Kimble (1988)).

somatic tissues, a cascade of negative regulators appears to control the activity of a master switch-gene, *tra-1*, to determine the somatic sexual phenotype (Hodgkin 1980, 1987*b*). However, in the germ line, it is the activity of the *fem* genes that plays the critical role in the decision between spermatogenesis and oogenesis (Nelson *et al.* 1978; Doniach & Hodgkin 1984; Kimble *et al.* 1984; Hodgkin 1986). A *fem*(*-1*, *-2 or -3*); *tra-1* double mutant has a male soma but a female germ line. Although the state of *tra-1* can influence sexual fate in the germ line, its role is not understood (Hodgkin 1987*b*; Schedl *et al.* 1988).

The global genes that are central to sex determination in the germ line are *tra-2*, *fem-1*, *fem-2* and *fem*-3. Hodgkin has proposed that *tra-2* negatively regulates the activity of the *fem* genes (Hodgkin 1986). A highly simplified pathway of sex regulation, tailored for consideration of sex determination in the germ line, is provided in table 3*a*. The *tra-1* gene is not included in this pathway because its role in germ-line sex determination has not yet been established. Although *tra-3* is included, its role in germ-line sex determination is variable and probably minor. Therefore emphasis is placed on the role of *tra-2*.

In the simplified pathway shown in table 3*a*, *tra-2* (and *tra-3*) control the activity of the *fem* genes. If *tra-2* is active, then *fem* genes are inactive and female development ensues; conversely if *tra-2* is inactive, the *fem* genes are active and male development occurs. Regulators appear to have evolved to control the *tra-2* gene in response to the X:A ratio or tissue-specific cues. For example, in males the *her-1* gene negatively regulates *tra-2* in response to the X:A ratio; consequently the *fem* genes are active and male development occurs (table 3*b*). Moreover, the *fem* genes may in turn regulate downstream genes in specific tissues. For example, the *fem* genes regulate *tra-1*, which appears to have its primary female-directing function in the soma (Hodgkin 1987*b*; T. Schedl, unpublished results).

In the rest of this review, I consider two questions. Firstly, how do the global male- or female-directing genes specify sexual differentiation of a particular cell type (e.g. sperm)? And secondly, how is male development (spermatogenesis) turned on in the germ line of a somatically female animal and then turned off to permit oogenesis?

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TABLE 3. GENETIC REGULATION OF SEX DETERMINATION IN THE GERM LINE

(See text for explanation. (*a*) Simplified pathway of genetic control of sex determination (adapted from Hodgkin (1986)). (*b*) The onset of male spermatogenesis depends on the X:A ratio, which acts through *her-1* to control *tra-2* on XO males (adapted from Hodgkin (1980)). (*c*) The onset of hermaphrodite spermatogenesis depends on the germ-line-specific regulatory gene, *fog-2*. This may act by controlling *tra-2* in XX (or (XO) hermaphrodites (adapted from Schedl & Kimble (1988)). (*d*) The switch to oogenesis from spermatogenesis in hermaphrodites appears to involve inactivation of *fem-3*. (i) One model for inactivation of *fem-3* invokes a germ-line-specific negative regulator of *fem-3*. (ii) Another model invokes some regulator to inactivate *fog-2* so that *tra-2* might inactivate *fem-3*. That regulator might be an unknown gene, or it might require negative feedback by, for example, *fog-2*.)

(*a*)

tra-2 (*tra-3*) ⊣ { *fem-1*, *fem-2*, *fem-3* }

(*b*)

X:A ratio (0.5) → *her-1* (active) ⊣ *tra-2* (*tra-3*) (inactive) → { *fem-1*, *fem-2*, *fem-3*, *fog-1* } (active) ⇢ sperm

(*c*)

germ line → *fog-2* (active) ⊣ *tra-2* (*tra-3*) (inactive) → { *fem-1*, *fem-2*, *fem-3*, *fog-1* } (active) ⇢ sperm

(*d*)

(i)

fog-2 (active) ⊣ *tra-2* (*tra-3*) (inactive); *mog?* ⊣ { *fem-1*, *fem-2*, *fem-3*, *fog-1* } (inactive) → oocytes

(ii)

mog? ⊣ *fog-2* (inactive); *tra-2* (*tra-3*) (active) ⊣ { *fem-1*, *fem-2*, *fem-3*, *fog-1* } (inactive) → oocytes

GENETIC CONTROL OF CELL TYPE IN THE GERM LINE

The global sex-determining genes direct male or female development in all tissues of the animal. It is clear that specification of a particular male cell type (e.g. sperm, vas deferens) must require additional regulation. One germ-line-specific sex-determining gene, *fog-1*, appears to regulate the male differentiation of germ cells to become sperm (Doniach 1986*a*; Barton & Kimble 1988). In *fog-1* mutants, whether XX or XO, germ cells that would normally differentiate as sperm become oocytes instead. No effect on somatic tissues is observed in *fog-1* mutants. These *fog-1* mutations are semi-dominant. Whereas *fog*-homozygotes make no sperm, *fog-1*/+ heterozygotes make fewer sperm in hermaphrodites and make some sperm and then oocytes in males.

Despite the semidominance of *fog-1* mutations, they are probably due to a loss of *fog-1* function (Barton & Kimble 1988). One argument that *fog-1* mutations are *lf* is based on mutation frequency. After ethyl methane sulphonate (EMS) mutagenesis, the *fog-1* mutations are isolated at the same frequency as *lf* mutations in other genes. A second argument is based on studies in which production of sperm or oocytes was examined in a duplication strain to alter the dose of *fog-1*. The phenotype of an animal carrying one wild-type copy and two mutant

copies of *fog-1* is identical to that of a *fog-1*/+ heterozygote. Also, the phenotype of an animal carrying two wild-type copies and one mutant copy of *fog-1* is identical to that of a wild-type animal. These studies show that the *fog-1* phenotype is dictated by the number of wild-type, rather than mutant, copies of *fog-1*. Therefore the semidominance of *fog-1* is not likely to be due to a novel or unregulated activity. Instead, it seems to indicate a sensitivity of sperm production to the dose of *fog-1*.

The *fog-1* gene is essential to specification of sperm. No sperm are made in double mutants that are homozygous for *fog-1* and either *her-1*(*lf*), *tra-1*(*lf*), *tra-2*(*lf*), *tra-3*(*lf*) or *fem-3*(*gf*) (Doniach, 1986*a*; Barton & Kimble 1988). Indeed, no mutant combination has been found in which sperm are made in an animal homozygous for a *fog-1* mutation. Therefore *fog-1*, like the *fem* genes, must be placed at the end of the pathway of sex-determining regulators in the germ line (table 3). Our working hypothesis is that *fog-1* is a germ-line-specific regulator that directs germ cells to differentiate as sperm when instructed to be male by the *fem* genes.

Control of the onset of spermatogenesis in XX animals and speculation on the evolution of hermaphrodites from females

Spermatogenesis is a male pathway of differentiation. Therefore, production of sperm in XX hermaphrodites requires the activity of male-directing genes in an otherwise female animal. One gene, *fog-2*, appears to regulate the onset of spermatogenesis in hermaphrodites (Schedl & Kimble 1988). All *fog-2* mutations isolated so far are recessive and, after EMS mutagenesis, they arise at a frequency typical of *lf* mutations in other genes. These properties imply that the *fog*-2 mutations cause a loss of the *fog-2* product.

The mutant phenotype of *fog-2* is transformation of XX hermaphrodites into females (table 2.) XX hermaphrodites homozygous for any of 16 mutations in *fog-2* produce only oocytes: cells that normally would have differentiated as sperm become oocytes instead. Mutations in *fog-2* do not alter the normal development of the somatic tissues of XX animals as female. In addition, they do not affect the development of either somatic or germ-line tissues of XO animals as male. The specific effect of *fog-2* mutations on the hermaphrodite germ line suggests that wild-type *fog-2* is required for the onset of spermatogenesis only in the hermaphrodite. It is clear that *fog-2* is not required to specify sperm *per se* because spermatogenesis in XO *fog-2* mutant males occurs normally.

An attractive model for the function of *fog-2* is that it negatively regulates *tra-2* in the germ line to permit the *fem* genes to direct spermatogenesis (figure 3*c*). This suggestion derives from the phenotype of *tra-2*; *fog-2* double mutants. The *tra-2*(*lf*) single mutant causes sexual transformation of XX animals into pseudomales. All tissues of these *tra-2* pseudomales are masculinized, though sexual transformation is not complete. Similarly *tra-2*; *fog-2* double mutants are masculinized. In the germ line, where the activity of *fog-2* might be expected to have some effect, the double mutant makes sperm but not oocytes. Therefore in the absence of *tra-2*, the state of *fog-2* is not critical.

The possibility that *tra-2* is negatively regulated to permit spermatogenesis in hermaphrodites is also suggested by the mutant phenotype of gain-of-function (*gf*) alleles of *tra-2*. XX *tra-2*(*gf*) animals are female, whereas XO animals are male (Doniach 1986*b*; Schedl & Kimble 1988). The *tra-2*(*gf*) phenotype is therefore identical to that of *lf* alleles of *fog-2*. One plausible hypothesis is that *tra-2*(*gf*) is defective in its regulation by *fog-2*.

The activity of *fog-2* does not appear to be sensitive to the X:A ratio. This conclusion comes from the phenotype of *her-1 fog-2* double mutants. The *her-1* single mutant causes sexual transformation of X0 animals into hermaphrodites (Hodgkin 1980). The *her-1 fog-2* double mutant, however, is female (Schedl & Kimble 1988). Therefore, even in XO animals, the state of *fog-2* is critical to the onset of hermaphrodite spermatogenesis.

Both *fog-2*(*lf*) and *tra-2*(*gf*) mutations transform *C. elegans* into a male–female strain. Therefore the evolution of *tra-2* regulation by *fog-2* may have been the primary step in the evolution of *C. elegans* self-fertilizing hermaphrodites from a related *Caenorhabditis* species such as *C. remanei* that reproduces as a male–female strain. It is intriguing that *her-1* and *fog-2* are both on chromosome V. Given that both genes appear to function as negative regulators of *tra-2*, perhaps *her-1* and *fog-2* encode related proteins with *her-1* under regulation by the X:A ratio and *fog-2* under tissue-specific control.

Control of the hermaphrodite switch from spermatogenesis to oogenesis

Once spermatogenesis has been initiated in the hermaphrodite germ line, the switch to oogenesis requires further regulation. The mutant phenotype of *fem-3*(*gf*) alleles may provide insight into the mechanism of this switch.

The wild-type *fem-3* gene is required for specification of the male fate. Both XX and XO mutants lacking *fem-3* activity are sexually transformed into females (hermaphrodites with no sperm) (Hodgkin 1986; Barton *et al.* 1987). Therefore *fem-3* is required both for spermatogenesis in XX hermaphrodites and for male development of all tissues in XO males.

A *fem-3*(*gf*) hermaphrodite makes sperm continuously and in great excess; it makes no oocytes. The somatic tissues of *fem-3*(*gf*) XX animals, however, are not sexually transformed: the soma remains female. This germ-line-specific masculinization by *fem-3*(*gf*) suggests that, in wild-type hermaphrodites, *fem-3* activity is regulated to permit the onset of oogenesis (table 3*d*).

The mechanism by which *fem-3* is inactivated is not known. One possibility is that a germ-line-specific regulator of *fem-3* activity exists (table 3*d*, (i)). Another possibility is that a negative regulator of *fog-2* exists so that *tra-2* can then negatively regulate *fem-3* to permit the switch to oogenesis (table 3*d*, (ii)). Either model predicts the existence of a gene which, when mutant, causes XX animals to produce sperm and to be unable to switch to oogenesis. At least three such genes, the *mog* genes, have been identified (J. Kimble and T. Schedl, unpublished results). However, the *mog* genes have not yet been characterized genetically.

Conclusions

Sex determination in the germ line of the nematode, *C. elegans*, is subject to two kinds of tissue-specific control. These include germ-line-specific regulatory genes, e.g. *fog-1* and *fog-2*, and germ-line-specific regulation of global sex-determining genes as detected by gain-of-function mutations in *fem-3* and *tra-2*. Models are presented by which these genes may regulate the germ line to achieve spermatogenesis and the oogenesis.

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