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6. Genetic Control of Sex Determination in the Hermaphrodite Germ Line of *Caenorhabditis elegans*

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INTRODUCTION

Sex determination in the *C. elegans* germ line addresses two major problems of biological control. First, any regulation that directs male or female development in all tissues must rely on tissue-specific controls to specify a particular pathway of differentiation (e.g., sperm or oocyte) in a single tissue. The *C. elegans* germ line provides several technical advantages for analyzing sex determination in a single tissue, including powerful genetic selections (Kimble, 1988) and ease of micro-injection (Kimble et al., 1982). Second, the self-fertility of the *C. elegans* hermaphrodite depends upon its transient production of sperm in an otherwise female animal. Analysis of sex determination in the hermaphrodite germ line should therefore shed light on the evolution of hermaphrodites from females. Here, we review our efforts towards identifying the regulatory elements that control the *C. elegans* hermaphrodite germ line to produce sperm and then oocytes.

Normally in *C. elegans, XX* animals are hermaphrodite and *XO* animals are male. The two sexes differ extensively in both soma and germ line. The genetic controls of sex determination and dosage compensation in *C. elegans* that occur at the level of the entire organism have recently been reviewed (Villeneuve and Meyer, 1990; Hodgkin, 1988). Briefly, the primary sex-determining signal is the ratio of *X* chromosomes to sets of autosomes. The *X/A* ratio is interpreted by genes that regulate both the sexual phenotype and dosage compensation. Then one group of genes (*her-1*, the *tra* and *fem* genes) directs sexual differentiation of the animal and a second group (*dpy-21* and other dosage compensation *dpy* genes) sets the level of transcription from the *X* chromosome.

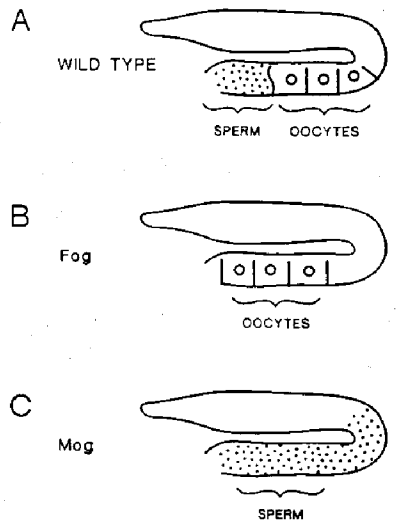

Fig. 1. Schematic diagram of the germ line in an ovotestis of young adult hermaphrodites illustrating phenotypes discussed in this review. **A**: Wild type with the production of sperm then oocytes. **B**: A Fog germ line which produces only oocytes. **C**: A Mog germ line which produces only sperm.

The focus of this review is sex determination in the hermaphrodite germ line. Figure 1A is a simplified diagram of gamete differentiation in a wild-type hermaphrodite ovotestis. Essentially each adult ovotestis is composed of a distal arm containing immature germ line nuclei and a proximal arm containing differentiating gametes. During the fourth larval stage, approximately 40 germ cells in each ovotestis undergo spermatogenesis; then, about at the time the animal molts to become an adult, a switch to oogenesis occurs and oocytes are made continuously throughout the rest of the life of the animal. These oocytes can either be self-fertilized by the hermaphrodite's own sperm or cross-fertilized by male sperm. For a more extensive account of gonadal development and anatomy, see Kimble and Ward (1988).

GLOBAL SEX-DETERMINING GENES

Table I summarizes the loss-of-function (lf) phenotype of those global sex-determining genes discussed in this review. These genes regulate the sexual phenotype in both somatic and germline tissues of *C. elegans,* but do not affect dosage compensation. They fall into two broad classes: those normally required for hermaphrodite development (three *tra* genes) and those normally required for male development (three *fem* genes and *her-1*). *XX* animals lacking the wild-type function of either *tra-1*, *tra-2*, or *tra-3* are masculinized and *XO* animals lacking the wild-type function of *her-1* are transformed into self-fertile hermaphrodites. Loss-of-function mutations in any of the three *fem* genes result in a feminization of both *XX* and *XO* animals. In both *XX* and *XO fem* animals, cells that normally would become sperm are sexually transformed and become oocytes instead. In *XO* males, somatic tissues are sexually transformed from male to female. Therefore, both *XX* and *XO* animals lacking the wild-type function of *fem-1*, *fem-2*, or *fem-3* develop as functional females.

Analysis of the phenotypes of double mutants has been used to deduce the functions of sex-determining genes (e.g., Hodgkin, 1987a). Although the pathways of control are similar in somatic and germline tissues, they differ in a critical way. In both the soma and germ line, the genes act in a cascade of negative regulation. The *her-1* gene acts as a negative regulator of *tra-2* and *tra-3,* and then the *tra-2* and *tra-3* genes act as negative regulators of the *fem* genes. At this point the regulation in the soma diverges from that in the germ line. In the soma, the *fem* genes act as negative regulators of *tra-1* and the state of *tra-1* determines the sexual phenotype (Fig. 2). However, in the germ line, the state of the *fem* genes is epistatic to that of *tra-1* so the *fem* genes determine sex (Fig. 3). Because *tra-1* is a genetically complex gene and its null phenotype in the germ line remains unknown (Hodgkin, 1987b; Schedl et al., 1989), we do not include it in the pathway of regulation in the germ line.

TABLE I.
Globally Acting Sex-Determining Genes

Gene	Loss-of-function phenotype	
	XX	XO
Wild type	Female soma, sperm then oocytes	Male
tra-1[a]	Psuedomale soma, sperm then oocytes	Same as XX
tra-2[b]	Psuedomale soma, sperm only	Male
tra-3[b]	Psuedomale soma, sperm then oocytes	Male
fem-1[c]	Female	Female
fem-2[d]	Female	Female
fem-3[e]	Female	Female
her-1[f]	Female soma, sperm then oocytes	Same as XX

[a]Hodgkin and Brenner (1977); Hodgkin (1987b); Schedl et al. (1989).
[b] Hodgkin and Brenner (1977).
[c]Nelson et al. (1978); Doniach and Hodgkin (1984).
[d]Kimble et al. (1984); Hodgkin (1986).
[e]Hodgkin (1986); Barton, et al. (1987).
[f]Hodgkin (1980); Trent et al., (1988).

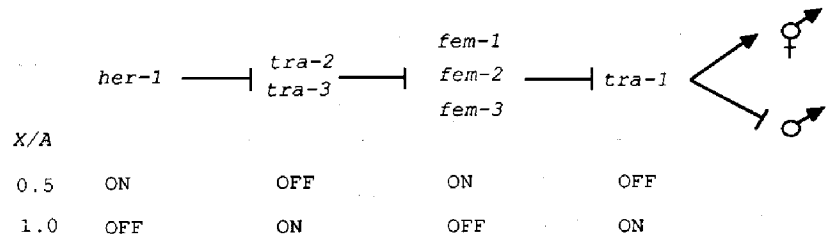

Fig. 2. Pathway for genetic control of sex determination in somatic tissues. The state of the *tra-1* gene is set in response to the *X:A* ratio. When *tra-1* is active hermaphrodite development occurs; when *tra-1* is inactive male development results.

GERM LINE-SPECIFIC SEXUAL REGULATORS

The germ line pathway of sex determination is depicted in Figure 3 as static with the *fem* genes either "on" or "off." In the hermaphrodite germ line, however, the state of the *fem* genes must be modulated. First the *fem* genes must be active to direct male development (spermatogenesis) in an animal with a "female" chromosomal constitution. Then they must be inactivated to achieve the switch from spermatogenesis to oogenesis.

Several genes involved in regulating germ line sex determination have been identified. Mutations with a Fog mutant phenotype (feminization of the germ line) synthesize oocytes in place of sperm; mutations with a Mog mutant phenotype (masculinization of the germ line) synthesize sperm in place of oocytes (Fig. 1). To date five genes have been identified that have specific roles in the regulation of germ line sex (Table II). The genes *fog-1*, *fog-2*, and *mog-1* appear to be limited in their function to the germ line: loss-of-function mutations of *fog-1* and *fog-2* have a Fog phenotype and loss-of-function mutations of *mog-1* are Mog. In addition, the globally acting sex-determination genes, *tra-2* and *fem-3*, are regulated in a germ line-specific manner: *tra-2(gf)* mutations are Fog and *fem-3(gf)* mutations are Mog. The roles that these genes play in the control of germ line sex are discussed below.

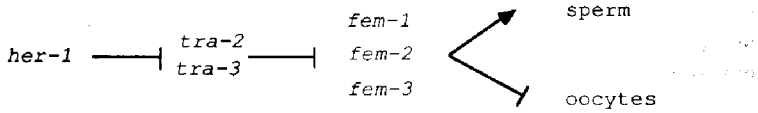

Fig. 3. Pathway for genetic control of germ line sex determination by globally acting sex-determination genes. When the *fem* genes are active sperm are produced; when the *fem* genes are inactive oocytes are produced.

TABLE II.
Germ Line-Specific Sex-Determination Mutations

Class	Gene	Phenotype
Feminizing (Fog)	*fog-1 (lf)*	Males and hermaphrodites make only oocytes
	fog-2 (lf)	Hermaphrodites make only oocytes Males make sperm
	tra-2 (gf)	Hermaphrodites make only oocytes Males make sperm
Masculinizing (Mog)	*mog-1 (lf)*	Males and hermaphrodites make only sperm
	fem-3 (gf)	Males and hermaphrodites make only sperm

The Onset of Hermaphrodite Spermatogenesis: The *fog-2* Gene

In *XX* animals, the *fem* genes are negatively regulated by *tra-2* and *tra-3*. However, the repression of the *fem* genes must be overcome, at least temporarily, in the germ line, to allow a brief period of spermatogenesis. Since gain-of-function (gf) mutations in *tra-2* result in the inability of hermaphrodites to make sperm, it has been proposed that *tra-2* function is modulated to allow *fem* activity (Doniach, 1986). Recently, Schedl and Kimble (1988) have described the *fog-2* gene. Loss-of-function mutations in *fog-2* affect only the hermaphrodite germline and resemble strong *tra-2(gf)* mutations. *XX fog-2(lf)* animals make oocytes in place of sperm; however, *XO fog-2(lf)* animals are normal males. Therefore, a loss of *fog-2* function transforms *C. elegans* from a strain that can reproduce by hermaphrodite self-fertilization to a male/female strain typical of closely related nematode species.

Analysis of double mutant phenotypes places *fog-2* upstream of both the *fem* and *tra* genes in the germ line regulatory pathway. The *fog-2* phenotype and its position in the hierarchy of regulation indicates that its products act to modulate the action of the global sex-determining genes, allowing a male fate to be specified in the germ line of an otherwise female animal. The *fog-2* products may act to inactivate the *tra-2* gene as indicated in Figure 4.

In the male germ line the function of *fog-2* may be redundant with that of *her-1*. The *her-1* gene is thought to keep the *tra-2* gene permanently inactivated in both the male soma and germ line. Since loss of *her-1* function results in the transformation of *XO* animals to hermaphrodites instead of females *fog-2* is active in the germ line of these *XO* animals. As one might expect the *her-1 fog-2* double mutant is female in both *XX* and *XO* animals.

The Switch From Spermatogenesis to Oogenesis: The *mog-1* Gene

After forty or so germ cells undergo spermatogenesis in each ovotestis, a switch to oogenesis occurs. This switch is dependent on *tra-2* activity and inactivation of the *fem* genes. The gene *mog-1* is a candidate for the germ

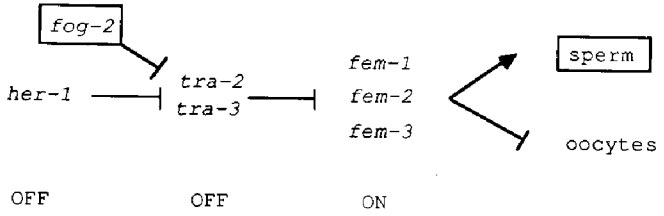

Fig. 4. The *fog-2* gene may control the onset of spermatogenesis by negatively regulating the globally acting genes *tra-2* and *tra-3* in the germ line.

line-specific negative regulation of the *fem* genes. *XX* animals homozygous for a *mog-1(lf)* mutation possess a typical hermaphrodite soma but make sperm continuously (Graham and Kimble, manuscript in preparation). This indicates that *mog-1* is normally required for the specification of germ cells as oocytes. The phenotype of *mog-1* is similar to that described for *fem-3(gf)* mutations (Barton et al., 1987). These *fem-3* mutations are thought to allow *fem-3* to escape a negative regulation necessary for the switch from spermatogenesis to oogenesis. Neither *mog-1(lf)* or *fem-3(gf)* affects *XO* males.

Animals doubly homozygous for *mog-1(lf)* and loss-of-function mutations in any of the three *fem* genes make only oocytes (Graham and Kimble, manuscript in preparation). This indicates that *mog-1* plays a purely regulative role in oogenesis and that its normal function is probably to negatively regulate one or more of the *fem* genes. In contrast the *mog-1(lf); fog-2* double mutant makes only sperm, indicating that *mog-1* acts at some step between *fog-2* and the *fem* genes. Similarly *mog-1* acts downstream of both *her-1* and *tra-3*. Just as loss-of-function mutations of both *tra-2* and *mog-1* alone masculinize the germ line, the double mutant *tra-1; mog-1* makes only sperm. Based on these epistasis experiments, *mog-1* is placed as a negative regulator of the *fem* genes in controlling the sex of the hermaphrodite germ line (Fig. 5).

A Germ Line-Specific Regulator in Both Hermaphrodite and Male Germ Lines: The *fog-1* Gene

Every tissue responds to the *X/A* ratio differently. For example, the somatic gonad of *XX* animals consists of uterine, spermathecal, and oviduct tissue, whereas the somatic gonad of *XO* animals has a seminal vesicle and vas deferens. Sexual differences are also observed in the hypodermis, nerve, muscle, gut, and germ line. How do genes that specify the male or female fate in all tissues direct a particular fate in one tissue? A gene with a mutant phenotype resulting in the sexual transformation of a single tissue might be involved in execution of the tissue-specific fate. The *fog-1* gene has the characteristics of such a gene. Loss of *fog-1* activity results in the production of only oocytes in place of sperm in both *XX* and *XO* animals; furthermore *fog-1* mutations have

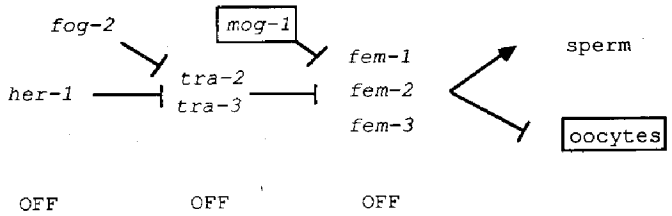

Fig. 5. The *mog-1* gene may control the switch from spermatogenesis to oogenesis by negatively regulating the *fem* genes.

no effect on somatic tissues (Barton and Kimble, submitted). Therefore *fog-1* appears to be required for the specification of spermatogenesis per se.

In double mutant experiments, mutations of *fog-1* behave like mutations in the *fem* genes in the germ line. Animals doubly mutant for *fog-1* and either *her-1*, *tra-1*, *tra-2*, or *tra-3* make only oocytes. Furthermore, a *fog-1(lf); fem-3(gf)* double mutant makes only oocytes. This indicates that *fog-1* acts downstream of *her-1* and the *tra* genes in germ line sexual regulation. Figure 6 shows a model of germ line sexual regulation in which *fog-1* acts along with the *fem* genes to promote spermatogenesis. However, it remains possible that the *fem* genes and *fog-1* act independently or in a network of positive interactions to determine germ cell fate.

Information from temperature-sensitivity studies indicate that the temperature-sensitive period for *fog-1* is later than that for any of the *fem* genes. The temperature-sensitivity periods for the *fem* genes begin well before meiosis and last throughout spermatogenesis (Kimble et al., 1984; Hodgkin, 1986). Temperature-shift experiments with a heat-sensitive allele indicate that *fog-1* acts during or after the last cell division before the entry into meiosis to specify spermatogenesis (Barton and Kimble, submitted). There is a continuous requirement for functional *fog-1* products while germ cells are entering spermatogenesis. The late temperature-sensitive period for *fog-1* suggests that *fog-1* acts after the *fem* genes in the germ line.

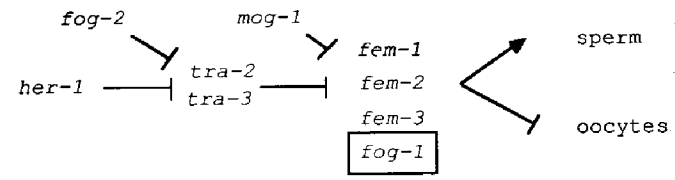

Fig. 6. The *fog-1* gene positively regulates spermatogenesis. *fog-1* mutations behave like *fem* mutations in the germ line.

The *fog-1* gene appears to act as a link between the globally acting sex-determination genes and a germ line-specific response: spermatogenesis. To date no gene has been identified that performs the analogous function for oocyte production. It may be that in the absence of *fog-1* and *fem* activity a germ cell entering meiosis executes oogenesis as a "default" program.

Modulation of *tra-2* and *fem-3* Within the Germ Line

The phenotypes of gain-of-function mutations in both *tra-2* and *fem-3* indicate that each of these global sex-determining genes is regulated in the germ line (Doniach, 1986; Barton et al., 1987). An analysis of the molecular basis of these gain-of-function mutations indicate that each gene is regulated by sequences in the 3′ untranslated region (3′ UTR) to achieve the onset of spermatogenesis and the switch to oogenesis typical of hermaphrodites (Ahringer and Kimble, manuscript in preparation; Okkema and Kimble, manuscript in preparation).

The *fem-3* gene produces a major RNA of 1.55 kb that can encode a 388 amino acid protein (Rosenquist and Kimble, 1988; Rosenquist et al., manuscript in preparation). The amino acid sequence of this *fem-3* protein is not significantly similar to that of any protein in the NBRF data base. The major RNA is expressed at a high level in the germ line of adult hermaphrodites and males, consistent with the requirement for *fem-3* during spermatogenesis and with the packaging of *fem-3* products into oocytes. The major RNA is also present at a high level in early embryos where its size is increased due to additional polyadenylation. By late embryogenesis the level of *fem-3* RNA is very low and remains low until the fourth larval stage in both males and hermaphrodites. It is during the fourth larval stage when spermatogenesis begins. The 1.55 kb RNA is accompanied by a minor message of 1.35 kb that represents 5–10% of *fem-3* RNA; the predicted protein product of this minor message has not yet been determined.

Nine independent gain-of-function mutations have been isolated in *fem-3* (Barton et al., 1987). The *fem-3(gf)* mutations affect only the germ line of hermaphrodites, causing continued spermatogenesis in place of oogenesis. These mutants are therefore defective in the hemaphrodite switch from spermatogenesis to oogenesis. All nine *fem-3(gf)* mutations are temperature sensitive, but vary in severity (two weak, five intermediate, one strong, and one very strong). The temperature-sensitive period for *fem-3(gf)* starts during spermatogenesis and lasts throughout gamete production.

All nine *fem-3(gf)* alleles have been cloned and sequenced. Remarkably these mutations are all associated with changes in the *fem-3* 3′UTR (Fig. 7). Eight of the *fem-3(gf)* mutations alter one of three bases in a small, 7-base-long region in the center of the 3′UTR; further, the alleles of each severity class are due to identical base pair changes. The ninth and strongest allele contains a 114 bp deletion that centers on the 7 base region defined by the other mutations. In addition to the deletion this strong *fem-3(gf)* has a point mutation near the polyadenylation site. Thus, together the *fem-3(gf)* mutations define a site in the 3′UTR involved in the regulation of *fem-3* activity in the germ line. We suggest that this region is required for inactivating *fem-3* to achieve the switch from spermatogenesis to oogenesis.

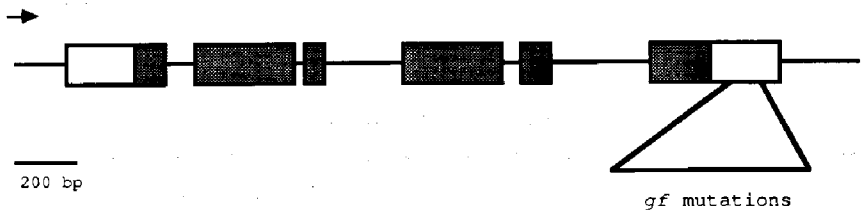

Fig. 7. *fem-3* gain-of-function (gf) mutations are located in the 3′ UTR. This is a schematic drawing of the *fem-3* gene with the exons indicated by boxes and the coding regions indicated by shading. The arrow indicates the direction of transcription. The gf mutations lie within a 114 bp region of the 3′ UTR.

The location of the *fem-3(gf)* mutations in the 3′UTR is surprising in light of their temperature sensitivity. Two *fem-3(gf)* genes sequenced in their entirety had no other alteration and it seems likely that all nine *fem-3(gf)* mutations encode a wild-type *fem-3* protein. There is no simple secondary structure within the 3′UTR or between the 3′UTR and other portions of the *fem-3* sequence. Therefore, the temperature sensitivity of the *fem-3(gf)* mutations may reflect altered interactions with other regulatory molecules. Alternatively, each class of *fem-3(gf)* may result in a different quantity of *fem-3* protein made and the temperature sensitivity may reflect a response of the germ line to more or less *fem 3* product.

Both genetic and molecular studies suggest that the *fem-3(gf)* mutations must interfere with a regulation occurring at the post-transcriptional level that normally decreases or inhibits the activity of *fem-3* RNA in the germ line. First the observed difference in *fem-3* activity between wild-type and *fem-3(gf)* mutant animals is not reflected in a difference in the level of *fem-3* RNA present: about the same amount of *fem-3* RNA is observed in wild-type and *fem-3(gf)* animals (Ahringer and Kimble, manuscript in preparation). Therefore, it is unlikely that sperm are made continuously in *fem-3(gf)* mutants because more *fem-3* RNA is transcribed or because *fem-3* RNA is more stable in those animals. Second, *fem-3* RNA is present in the germ line of wild-type animals; it is produced during oogenesis and is packaged into oocytes (Rosenquist and Kimble, 1988), consistent with genetically characterized maternal effects of *fem-3* (Hodgkin, 1986; Barton et al., 1987). Presumably, production of *fem-3* protein in the germ line would result in inappropriate masculinization of that

tissue. One model for the control of *fem-3* by its 3′UTR is that it inhibits translation. A second model is that the 3′UTR localizes *fem-3* RNA to sites within the germ line that are already determined as female so that production of *fem-3* protein cannot determine the sex.

The *tra-2* gene produces two major RNAs of 4.6 kb and 1.8 kb that are present in the adult hermaphrodite germ line during oocyte production (Okkema and Kimble, manuscript in preparation). This location is expected due to the role of *tra-2* in directing female development. The *tra-2(gf)* phenotype is the elimination of hermaphrodite spermatogenesis. Doniach (1986) described two classes of *tra-2(gf)* mutations. A weak class of *tra-2(gf)* mutations has both a partial loss-of-function character resulting in some masculinization of the hermaphrodite soma and a stronger gain-of-function character that results in germ line feminization. The strong class of *tra-2(gf)* has no loss-of-function character; however, in addition to its gain-of-function effect of feminizing the hermaphrodite germ line, it has a gain-of-function influence on the male soma leading to production of yolk in *XO* animals. Therefore, this second class may be due to an escape from negative regulation occurring both in the hermaphrodite germ line and in the male intestine.

DNA alterations have been found in several alleles of both classes of *tra-2(gf)* mutations (Okkema and Kimble, manuscript in preparation). The defects associated with mutations of the strong class are similar to *fem-3(gf)* mutations in that they affect the 3′UTR (Fig. 8). Within the *tra-2* 3′UTR there is a perfect 28 bp direct repeat that is altered in each strong *tra-2(gf)* mutation examined. The strongest allele deletes both copies of the repeat, while several slightly weaker alleles delete only one copy. Furthermore, each of two spontaneous *tra-2(gf)* mutations are associated with an insertion of the transposable element Tc*1* into one of the direct repeats. As with *fem-3(gf)* mutations the strong class of *tra-2(gf)* mutations are expected to produce a wild-type protein, though the entire *tra-2* gene has not been sequenced for any of these mutants.

The defects associated with mutations of the weak *tra-2* class are found near the end of the coding region (Fig. 8). Though these mutations should cause amino acid changes in the protein, they may also define regulatory regions in either the DNA or RNA.

The *tra-2(gf)* mutations define at least two sites involved in germ line control. Genetically, two negative regulators of *tra-2* have been identified: *her-1* and *fog-2*. Genetic analyses indicate that strong *tra-2(gf)* mutations may interfere with *her-1* control, implicating the 3′UTR in regulation by *her-1* (Okkema and Kimble, manuscript in preparation).

CONCLUSIONS

Studies on sexual regulation in the *C. elegans* hermaphrodite germ line are proving fruitful in the understanding of tissue-specific responses to and modulation of general signals. Two genes, *fog-2* and *mog-1*, have been identified that modulate the globally acting sex-determination pathway in the germ line. The *fog-2* gene directs the sex-determination pathway into the male mode in the germ line in order to produce sperm in an otherwise "female" animal. The *mog-1* gene then acts to switch the pathway back to the female mode to produce oocytes. The globally acting genes *tra-2* and *fem-3* undergo germ line-specific regulation, and both genes contain motifs involved in this regulation that map near the 3′ ends of their RNAs. Both genes normally produce high levels of RNA in the germ line; therefore, the 3′UTR motifs may be involved in negative translational control.

The *fog-1* gene is essential for spermatogenesis but dispensible for all other aspects of male development. Therefore its product may act to link the state of the global sex-determination genes in the germ line to the response of either sperm or oocyte production.

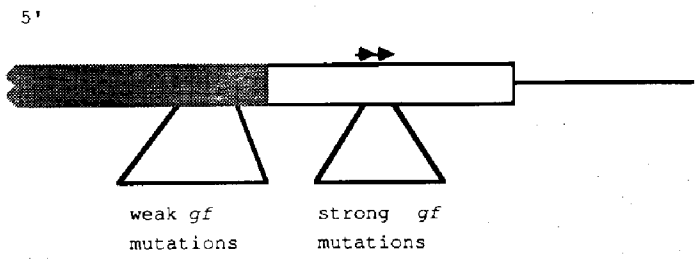

Fig. 8. *tra-2* gain-of-function (gf) mutations lie in two regions near the 3′ end of the gene. Drawn is the 3′ portion of the final *tra-2* exon (boxed) with 3′ flanking DNA indicated by the line. The shaded region indicates the carboxyterminal coding regions and the open region is the 3′ UTR. The double arrow indicates 28 base direct repeats separated by four bases in the 3′ UTR. The strong *tra-2(gf)* mutations affect the 28 base direct repeats, while the weak *tra-2(gf)* mutations are missense mutations within the COOH terminal coding region.

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