Sex Determination in *Caenorhabditis elegans*[1]

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Abstract: In *Caenorhabditis elegans,* the decision to develop as a hermaphrodite or male is controlled by a cascade of regulatory genes. These genes and other tissue-specific regulatory genes also control sexual fate in the hermaphrodite germline, which makes sperm first and then oocytes. In this review, we summarize the genetic and molecular characterization of these genes and speculate how they mutually interact to specify sexual fate.

Key words: Caenorhabditis elegans, hermaphrodite, nematode, sex determination.

Caenorhabditis elegans is a free-living soil nematode easily maintained in laboratory culture. As an experimental organism, *C. elegans* is favored by many researchers because of its simple genetics, anatomy, and cell biology (6). These attributes have resulted in the identification of key genes that regulate sexual fate in the nematode. Current research is aimed at understanding how these genes function and mutually interact at the molecular level.

This review is intended to provide a brief overview of sex determination in *C. elegans.* First, an outline of the genetics of *C. elegans* sex determination will be presented. The remainder of the review will highlight recent advances in understanding the molecular basis of sex determination. Although molecular analysis is still at an early stage, transcriptional regulation and signal transduction appear to be two important controls of sex determination.

C. ELEGANS HAS TWO SEXES: HERMAPHRODITE AND MALE

Normally, adult *C. elegans* are either males or hermaphrodites. Hermaphrodites are essentially somatic females that first produce sperm and then oocytes. Males and hermaphrodites differ extensively in morphology, biochemistry, and behavior. The cell lineage of *C. elegans* de-

velopment has been documented in its entirety (30). Approximately 30% of tissues in the nematode are sexually dimorphic. Hermaphrodites have a spiked tail and a pair of ovotestes that meet centrally near the vulva. The males have a fan-shaped posterior that is specialized for copulation and a single-lobed gonad. The hermaphrodite germline is composed of a small pool of mitotically dividing nuclei that provide descendants that enter meiosis and differentiate into sperm or oocyte nuclei. The meiotic cells produced first become sperm; only oocytes are formed subsequently.

The initial sex-determining signal of *C. elegans* is based on the ratio of X chromosomes to sets of autosomes, the X:A ratio (21). In response to this signal, the regulatory pathway that controls sexual fate is set into one of two modes. Diploid animals with two X chromosomes (XX, X:A = 1.0) develop as hermaphrodites, whereas animals with a single X chromosome (XO, X:A = 0.5) become males (Fig. 1). Polyploids frequently arise in parasitic nematode species that reproduce by mitotic parthenogenesis (34). In *Heterodera glycines,* tetraploidy has been shown to exist within an amphimictic or cross-breeding population (36). In *Strongyloides papillosus,* a nematode that has both a free-living and parasitic life cycle, females arise through mitotic or meiotic parthenogenesis, whereas males appear to result from chromatin diminution following mitotic parthenogenesis (3,35).

IDENTIFICATION OF SEX-DETERMINING GENES

Genes that regulate the decision to follow hermaphrodite or male development

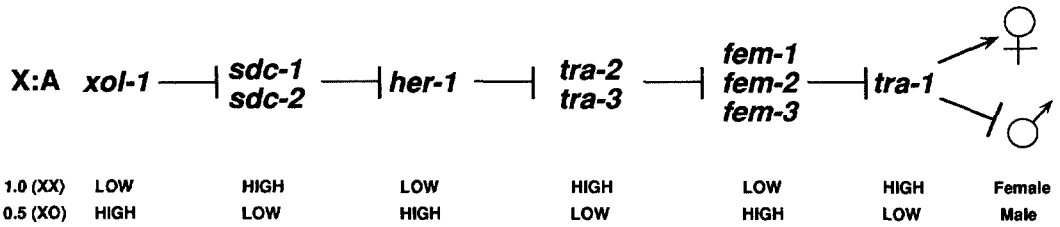|	xol-1	sdc-1 / sdc-2	her-1	tra-2 / tra-3	fem-1 / fem-2 / fem-3	tra-1	
1.0 (XX)	LOW	HIGH	LOW	HIGH	LOW	HIGH	Female
0.5 (XO)	HIGH	LOW	HIGH	LOW	HIGH	LOW	Male

FIG. 1.   The regulatory pathway of sex determination in *C. elegans*. The X:A ratio is interpreted by the genes *xol-1*, *sdc-1*, and *sdc-2*. In XX animals, the *sdc* proteins are speculated to repress the *her-1* gene at the transcriptional level. Because the *her-1* gene product is absent, the *tra-2* and *tra-3* gene products repress *fem* gene activity, possibly through a protein–protein interaction. Thus, the *tra-1* protein is free to promote female development, perhaps by transcriptionally activating female differentiation genes. In XO animals, *her-1* is transcribed and its protein product represses the activity of the *tra-2* and *tra-3* proteins. In turn, the *fem* proteins may prevent the proper intracellular localization or function of the *tra-1* protein and male development occurs. These models should be considered plausible, but speculative.

have been identified by mutations that can completely transform an embryo destined to become one sex to an adult of the other sex. The normal wild-type function of each of these genes has been determined from its loss-of-function or absence phenotype. For example, a mutation that abolishes *xol* (for *X*O *l*ethal) (22), *her* (for *her*maphroditization) (11,32), or *fem* (for *fem*inization) (2,9,12,17,23) gene activity feminizes XO males. Therefore, these genes must normally promote male development. By similar logic, the masculinization of XX hermaphrodites by *sdc* (for *s*ex and *d*osage *c*ompensation) (25,37) or *tra* (for *tra*nsformer) (13,15,18,26) loss-of-function mutations indicates that these genes normally promote female development. The *xol* and *sdc* genes control both sex determination and dosage compensation. Dosage compensation is a process that equalizes the level of X-linked transcription between XX and XO animals. Further discussion of this process is beyond the scope of this review. The remaining *her*, *tra*, and *fem* genes control only sex determination. Of course, additional, undiscovered genes could also be involved in sex determination or dosage compensation.

Relationships among the different sex-determining genes have been analyzed by examining the phenotype of double mutant combinations. For example, a *her-1; tra-2* double mutant has a *tra-2* and not a *her-1* phenotype (11). Thus, the activity of *tra-2* is epistatic to that of *her-1*. Genetic epistasis experiments (14,38) have been extremely useful in defining a proposed pathway of regulatory interactions (Fig. 1). As indicated, the pathway consists of a series of on–off (or high–low) switches, in which the activity of a gene or its product depends on the state of genes immediately preceding it. In this pathway, *tra-1* is the master regulator that determines whether an animal develops a male or hermaphrodite soma (14). It is not possible to deduce the molecular basis of regulation from such a pathway derived from genetic epistasis experiments.

MOLECULAR CHARACTERIZATION OF SEX-DETERMINING GENES

Many of the genes involved in the regulation of sex determination have now been cloned. A number of methods are available to identify *C. elegans* genes molecularly. One method relies on gene tagging by transposon insertion (7). A second method involves injecting cloned DNA into the hermaphrodite syncytial gonad to identify a sequence that is capable of rescuing the mutant phenotype of the endogenous gene (10). The small size of the *C. elegans* genome, about 100,000 kilobases (29), combined with extensive physical and genetic map data, contribute to the efficiency of gene identification.

A picture is emerging in which transcriptional control and signal transduction

are key components in the regulation of sexual fate. The *sdc-1* gene, a negative regulator of *her-1,* encodes a putative protein that contains zinc-finger motifs (24). These motifs occur in other proteins that function as transcriptional regulators. The *her-1* gene appears to be transcriptionally regulated, as a high mRNA level occurs in XO males and a low or undetectable level in XX hermaphrodites (31). Therefore, in a simple model, *sdc-1* might negatively regulate *her-1* at the transcriptional level. At the end of the model pathway, *tra-1* encodes two putative proteins also with zinc-finger motifs (D. Zarkower and J. Hodgkin, pers. comm.). Thus, *tra-1* may transcriptionally regulate downstream genes involved in somatic differentiation.

Recent genetic analysis suggests that cell interactions are also involved in the control of sexual fate. In *C. elegans,* free duplications are used to generate genetically mosaic animals. These duplications provide wild-type gene activity to rescue an otherwise homozygous mutant. Mosaic animals result when the duplication is mitotically lost in some cells and not in others. It is often possible to distinguish cells carrying the duplication from those that do not. If a gene product functions cell autonomously (e.g., a receptor), then a cell that has lost the duplication will show a mutant phenotype. If a gene product functions cell nonautonomously (e.g., a ligand), then a cell that has lost the duplication might be wild-type because it is rescued by a gene product produced by neighboring cells. In the case of the *her-1* gene, which normally promotes XO male development, mosaic analysis indicates the *her-1* gene does not act cell autonomously (16). In mosaic animals, XO cells that are *her-1*($-$) can be masculinized and XO cells that are *her-1*($+$) can be feminized. This result indicates that the sexual fate of a cell can be influenced by the gene activity in its neighbor. Molecular characterization of the *her-1* gene indicates that it encodes a putative secretory protein of low molecular weight (M. Perry and W. B. Wood, pers. comm.). Immediately downstream of *her-1*

in the regulatory hierarchy, *tra-2* probably encodes a membrane protein, based on its sequence (19). Thus, in one simple model, the putative *tra-2* membrane protein mediates cell–cell communication by being a receptor for the *her-1* molecular signal.

In XO males, if the putative *tra-2* membrane protein is inactivated by binding the *her-1* molecular signal, then the *fem* proteins would be free to promote male development. Although the *fem-1* (28) and *fem-3* (2) protein sequences have been predicted from cDNA sequences, it is unknown how they negatively regulate *tra-1.* The *fem-1* protein contains *cdc10*/SWI6 repeats (also known as ankyrin repeats) (28), which have been implicated in mediating protein–protein interactions (20). Thus, in one potential model, *fem-1* may negatively regulate *tra-1* through a protein–protein interaction mediated through the *cdc10*/ SWI6 repeats. This interaction could alter *tra-1* protein localization or activity.

In XX hermaphrodites, the repression of *fem* genes by *tra-2* allows *tra-1* to promote female development. Because all three *fem* genes are required for male development, only one needs to be inactivated. Thus, the binding of the *tra-2* protein to one or more *fem* proteins could inhibit their activity and thereby allow *tra-1* to promote female development, perhaps by transcriptional activation of female differentiation genes. All of these discussed models are plausible, but readers should consider them speculative.

Germline Regulation

Germline sex, or the decision to become sperm or oocyte, is controlled by global and tissue-specific sex-determining genes (Fig. 2). The global genes are thought to have similar functions in both the germ line and soma. However, the regulation by global genes in the germ line differs from that of the soma because the last gene in the hierarchy is not *tra-1* (compare Fig. 1 to Fig. 2). Instead, the *fem* genes dictate whether sperm or oocytes will be produced (14,38). Although *tra-1* can affect germline

fog-2   mog-1

> ⟍   ⟍
            ⟍ fem-3
her-1 ──┤ tra-2 ──┤ fem-2
        (tra-3)   fem-1
                     fog-1
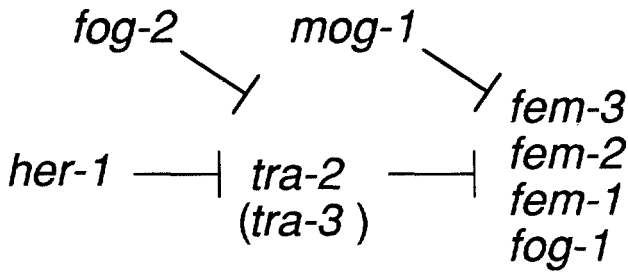
XO Male	ON	OFF	ON	SPERM
XX Hermaphrodite	OFF	OFF	ON	SPERM
	OFF	?	OFF	OOCYTES

FIG. 2. The regulatory pathway of germline sex determination. Germline sex is controlled by the same genes that regulate somatic sex (*her-1*, *tra-2,3*, and *fem-1,2,3*) and by tissue-specific regulatory genes that have virtually no effect on somatic sex determination (*mog-1*, *fog-1*, and *fog-2*). Although *tra-1* affects germline sex determination, it is not the final gene in the pathway, and its role remains unclear. Instead, the activities of the *fem* and the *fog-1* genes determine whether a germ cell develops as a sperm or oocyte. In XO males, the *her-1* protein represses the *tra* proteins and sperm are made because the *fem* and *fog-1* gene products are active. In XX hermaphrodites, spermatogenesis is initiated because the *tra-2* protein is negatively regulated by the *fog-2* gene product, and the *fem* and *fog-1* gene products are active. The subsequent switch to oogenesis occurs when *fem-3* is negatively regulated, perhaps by *mog-1*, in an interaction that may involve translational control. These models are speculative, and molecular models await cloning of these genes.

sex determination, its position in the pathway is unclear. The tissue-specific sex-determining genes affect only germline sex determination and have virtually no effect on somatic sex. These genes have been named *fog* (for *f*eminization *o*f *g*ermline) and *mog* (for *m*asculinization *o*f *g*ermline) based on their loss-of-function phenotype. The *fog-1* gene is required alone with the *fem* genes for spermatogenesis in both XX and XO animals (5). The *fog-2* (27) and *mog-1* (P. Graham and J. Kimble, unpubl.) genes appear to regulate the onset of hermaphrodite spermatogenesis and the subsequent switch to oogenesis. The *fog* and *mog* genes have not yet been cloned, so their molecular products are unknown.

Hermaphroditism may have evolved as a secondary specialization that allows a female to support both spermatogenesis and oogenesis in the same germline. In *C. elegans*, gain-of-function mutations have been identified that disrupt hermaphrodite self-fertility, but they have virtually no effect on somatic development. This finding indicates that the hermaphrodite germ line may have evolved tissue-specific controls to regulate the activities of global sex-determining genes.

Because gain-of-function *tra-2* mutants make oocytes but not sperm (8,27), *tra-2* normally is inactivated to allow the onset of spermatogenesis in the hermaphrodite germline. The *fog-2* gene is a candidate for this negative regulator, because loss-of-function mutations in *fog-2* also prevent the initiation of spermatogenesis in the hermaphrodite germline (27) (Fig. 2).

Because gain-of-function *fem-3* mutants normally make sperm but not oocytes (4), *fem-3* normally is regulated in the hermaphrodite germline to induce the switch from spermatogenesis to oogenesis. Recent molecular analyses of *fem-3* gain-of-function mutations reveal that each involves a single base change within a 5 nucleotide sequence in the 3' untranslated region (3' UTR) (1). Because these mutations have no detectable effect on *fem-3* mRNA steady-state levels, it was speculated that the 5 nucleotide sequence in the 3' UTR might identify a binding site for a translational repressor (1). A genetic candidate for this repressor is *mog-1* (P. Gra-

ham and J. Kimble, unpubl.), which has a germline phenotype similar to that of gain-of-function *fem-3* mutations (Fig. 2).

FUTURE PERSPECTIVES

We are just beginning to understand how sexual fate is regulated in *C. elegans*. Many questions remain. How do the gene products of the sex-determining genes interact with one another biochemically? Are these interactions direct or do they involve other previously unidentified genes that may be redundant or pleiotropic? How conserved are sex-determining mechanisms among different nematode species?

The molecular identification of sex-determining genes in *C. elegans* now makes it possible to identify gene homologs in other nematode species. This applies not only to sex-determining genes, but to any gene for which there is sequence information. Thus, developmental processes can be analyzed in nematodes that are not amenable to genetic dissection. Comparing *C. elegans* DNA and predicted amino acid sequences to the sequences of homologs found in other nematodes should identify conserved domains that may be important for gene activity and regulation. For example, understanding how germline sex is regulated in hermaphrodite and male-female species will provide insights into how a hermaphrodite sex may have evolved from a female sex. Furthermore, knowledge gained from understanding mechanisms that control sexual fate might be applied to develop strategies, based on sexual transformation or germline sterility, to control populations of parasitic nematodes, as was originally proposed nearly 20 years ago (33).

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