

# Molecular genetics of sex determination in *C. elegans*

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Sex determination poses a number of fundamental questions in developmental regulation. How are cells initially committed to one fate? How is that commitment executed and maintained? And how are groups of cells coordinated to adopt the same fate? In the nematode *Caenorhabditis elegans*, genetic and molecular dissection of the mechanisms of sex determination is beginning to provide a glimpse of how sexual fates are specified.

In this review, we begin by introducing the key sex-determining genes of *C. elegans*, which have been recently reviewed<sup>1,2</sup>. We then turn to the molecular regulation of sexual commitment. Many genes in the sex determination cascade have now been cloned, their transcripts identified, and their protein products predicted from cDNA sequences (Table 1; Box 1). Although the molecular characterization of this regulatory hierarchy is still in its infancy, the picture that is emerging suggests that both transcriptional regulation and transmembrane signalling play major roles in determining the sex of this small worm. Furthermore, we speculate that transmembrane signalling might provide a mechanism to ensure unified sexual development throughout the worm.

## Regulatory circuitry for *C. elegans* sex determination

*C. elegans* can develop as either of two sexes. The ratio of X chromosomes to sets of autosomes (the X:A ratio) establishes the regulatory hierarchy of sex determination in one of two modes<sup>3</sup>. Embryos with two X chromosomes and a diploid set of autosomes (X:A = 1) become hermaphrodites, whereas embryos with only one X chromosome develop as males (X:A = 0.5). Hermaphrodites are somatic females with a specialized germ line that first produces sperm and

*Sexual fate in the nematode Caenorhabditis elegans is controlled by a group of genetically well-characterized genes. Several of these sex-determining genes have now been analysed at the molecular level. Transcriptional regulation is likely to control both commitment to a single sexual fate and maintenance of that decision; in addition, intercellular signalling appears to coordinate the sexual fates of cells throughout the animal to adopt a single sexual fate.*

then oocytes. The X:A ratio also controls dosage compensation, with the result that X-specific transcript levels are equalized in XX and XO animals<sup>2</sup>.

Genes that control whether a *C. elegans* embryo becomes a hermaphrodite or a male have been identified by isolating mutations that transform the entire animal from one sex to the other (Table 1). The normal functions of these genes have been deduced from their loss-of-function phenotypes. For example, mutations that reduce or abolish *sdc* or *tra* gene activity masculinize XX embryos (see Box 1 for derivation of gene names). Therefore, the *sdc* and *tra* genes normally direct hermaphrodite differentiation in XX embryos. Similar logic argues that the *xol*, *ber* and *fem* genes direct male differentiation in XO embryos. Three genes – *xol-1*, *sdc-1* and *sdc-2* – control both sex

TABLE 1. Genes that influence sex determination<sup>a</sup>

Gene	Sex specified	Function	Molecular identity	References
<i>tra-1</i>	Hermaphrodite	Soma: terminal regulator Germ line: negative regulator of <i>fem-1</i> , <i>fem-2</i> , <i>fem-3</i> , <i>fog-1</i>	Zinc finger protein	– <sup>b</sup>
<i>tra-2</i>	Hermaphrodite	Negative regulator of <i>fem</i> genes	Membrane protein	22
<i>tra-3</i>	Hermaphrodite	Negative regulator of <i>fem</i> genes	?	–
<i>fem-1</i>	Male	Soma: negative regulator of <i>tra-1</i> Germ line: terminal regulator	<i>cdc10</i> /SWI6 repeats	30
<i>fem-2</i>	Male	Soma: negative regulator of <i>tra-1</i> Germ line: terminal regulator	?	–
<i>fem-3</i>	Male	Soma: negative regulator of <i>tra-1</i> Germ line: terminal regulator	Novel protein, probably soluble	31
<i>ber-1</i>	Male	Negative regulator of <i>tra-2</i>	Novel protein, probably secreted	– <sup>c</sup>
<i>sdc-1</i>	Hermaphrodite	Negative regulator of <i>ber-1</i>	Zinc finger protein	17
<i>sdc-2</i>	Hermaphrodite	Negative regulator of <i>ber-1</i>	?	–
<i>xol-1</i>	Male	Negative regulator of <i>sdc</i> genes	?	–

<sup>a</sup>References for identification of genes listed and genetic analyses leading to their proposed functions can be obtained in Refs 1 or 2.  
<sup>b</sup>D. Zarkower and J. Hodgkin, pers. commun.  
<sup>c</sup>M. Perry and W.B. Wood, pers. commun.

**Box 1. *C. elegans* sex-determining genes**

Sex-determining genes in *C. elegans* are named according to their loss-of-function phenotypes.

- xol*: XO lethal
- sdc*: Sex and dosage compensation
- ber*: Hermaphroditization
- tra*: Transformer
- fem*: Feminization

determination and dosage compensation, whereas seven other genes – *ber-1*, *fem-1*, *fem-2*, *fem-3*, *tra-1*, *tra-2* and *tra-3* – control only sex determination (see Ref. 2 for references to individual genes). Double mutant epistasis experiments suggest that the sex-determining genes of *C. elegans* function as a series of alternating on/off (or high/low) switches, with the activity of each gene controlled by the state of one or more upstream genes<sup>1,2</sup> (Fig. 1). The final gene in the pathway of somatic sex determination, *tra-1*, acts as the master regulator that determines whether an animal develops as a hermaphrodite or as a male<sup>4,5</sup>.

The roles of most of the globally acting sex determination genes, as described above, are the same in the soma and the germ line. However, *tra-1* is not the final regulator of sexual fate in the germ line and its role in germ-line sex determination remains unclear<sup>5,6</sup>. Instead, the *fem* genes and a germ-line-specific gene, *fog-1* (for feminization of the germ line; Ref. 7), are the terminal regulators in the germ line. The activities of all four of these genes are required for spermatogenesis in both XO males and XX hermaphrodites. Understanding how the XX hermaphrodite germ line can circumvent the X:A ratio to allow spermatogenesis and then switch to oogenesis poses a unique problem in sex determination. Genetic analyses of *tra-2* (Ref. 8) and *fem-3* (Refs 9, 10) gain-of-function mutants suggest that germ-line-specific regulation of these two globally acting genes is key to controlling these processes (germ-line sex determination reviewed in Ref. 1).

The initial commitment to a particular sexual fate is made during embryogenesis. Several components of the sex-determining machinery are maternally contributed to the embryo<sup>2</sup>: *sdc-1*, *fem-1*, *fem-2*, *fem-3* and *tra-3*. Thus, the zygote is poised to respond to its X:A ratio immediately after fertilization. The *sdc-1* gene has a brief temperature-sensitive period that begins and ends during embryogenesis<sup>11</sup>. Since the temperature-sensitive period indicates when *sdc-1* might be acting, the fact that the *sdc-1* sensitive period occurs early in development suggests it has a role in sexual commitment but not maintenance. Sex-determining genes downstream of *sdc-1* in the regulatory hierarchy execute and maintain sexual commitment. For example, the timing of the temperature-sensitive period of *ber-1* suggests that it has a maintenance function in adult males to prevent yolk expression (P. Schedin, PhD thesis, University of Colorado, Boulder, 1988).

Cell interactions play a critical role in *C. elegans* sex determination. Mosaic analyses suggest that two early acting genes, *sdc-1* (Ref. 11) and *ber-1* (Ref. 12), do not act cell-autonomously. If a cell lacks either *sdc-1* or *ber-1*, its sexual fate can be influenced by the activity of the wild-type gene in adjacent cells. In addition, when the X:A ratio is ambiguous, as in triploid animals (3X:2A), the sexual fate of a cell can be influenced by its position along the anteroposterior axis, rather than its lineage<sup>13</sup>. Taken together, these lines of evidence indicate that an early acting component of the pathway (the X:A ratio, *sdc-1* or *ber-1*) may influence sex determination by controlling the generation of a signal that mediates cell-cell interactions. By contrast, the *tra-1* gene acts cell-autonomously and is likely to be part of the receiving mechanism<sup>14</sup>. The other genes in the pathway have not been analysed in genetic mosaics.

**Multiple levels of transcriptional control**

Transcriptional regulation appears to control several aspects of sex determination in *C. elegans* (Fig. 1). A high level of *ber-1* mRNA is found in XO males, whereas XX hermaphrodites contain low or undetectable levels<sup>15</sup>. The *sdc-1* gene, one of the proposed negative

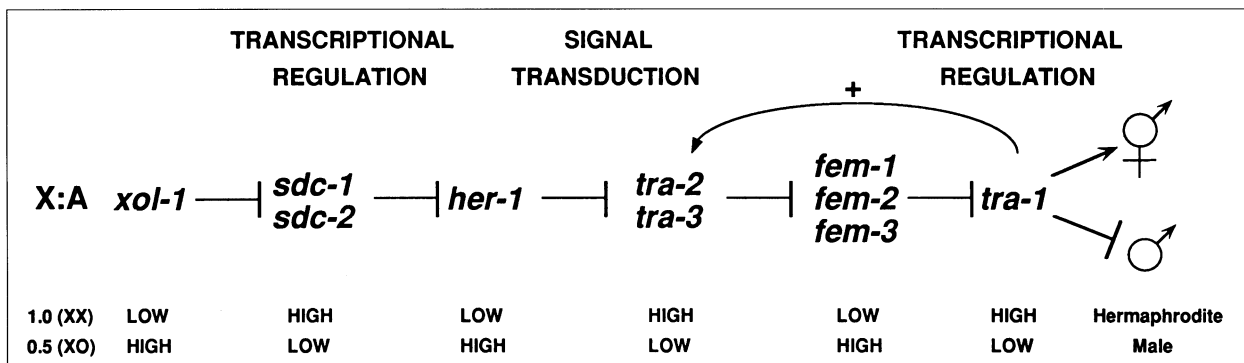


FIG 1

Regulatory pathway of sex determination in somatic tissues<sup>1,2</sup>. The *tra-1* gene is the terminal regulator of sex determination in the soma. In XX embryos, the *sdc* genes are active and inhibit *ber-1*; therefore *tra-2* and *tra-3* are active and repress the *fem* genes; this frees *tra-1* to promote hermaphrodite development. Commitment to the hermaphrodite pathway of differentiation is proposed to be reinforced by a positive feedback loop in which *tra-1* activates *tra-2*. In XO embryos, *xol-1* inhibits *sdc* genes and *ber-1* negatively regulates *tra-2* and *tra-3*; in turn, the *fem* genes repress *tra-1*, leading to male development. Although both *tra-2* and *tra-3* are equivalently positioned in the sex determination pathway, *tra-3* is postulated<sup>21</sup> to be a partly dispensable positive cofactor of *tra-2* and hence is omitted from further discussion.

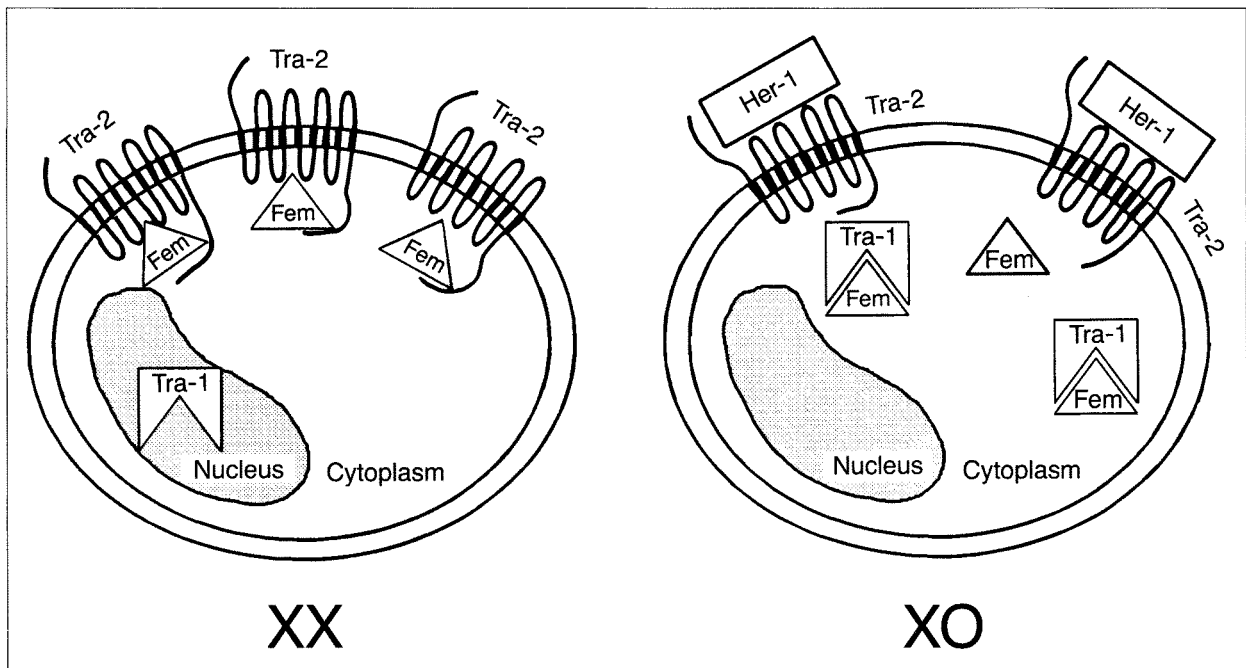


FIG 2

A speculative model for the molecular basis of somatic sex determination. In this model, we consider knowledge of the molecular identity of the sex-determining genes together with our understanding of their functional interrelationships and their behavior in genetic mosaics (see text, Fig. 1, and Table 1 for background). In XX hermaphrodites, the Tra-2 membrane protein sequesters one or more of the Fem proteins, thus allowing Tra-1 to enter the nucleus and direct hermaphrodite development. In XO males, the *her-1* product binds Tra-2 and thereby frees the Fem proteins to interfere with Tra-1 activity. Although this mechanism is consistent with available data, other mechanisms remain possible. Reproduced from Ref. 22.

regulators of *her-1*, is predicted to encode a protein with seven zinc finger motifs<sup>16,17</sup>. Therefore, a simple model is that the *her-1* gene is transcriptionally regulated, perhaps by *sd-1*. Similarly, *tra-1*, which is predicted to encode two proteins with zinc finger motifs (D. Zarkower and J. Hodgkin, pers. commun.), may transcriptionally regulate female differentiation genes, such as the vitellogenins. A high level of vitellogenin mRNA is found in XX hermaphrodites, whereas a low or undetectable level is seen in XO males<sup>18</sup>. Therefore, *tra-1* might itself direct vitellogenin gene transcription or it might act indirectly by turning off *mab-3*, a putative negative regulator of vitellogenin gene expression<sup>19</sup>.

Transcriptional regulation may also be critical to amplification within the pathway and commitment to the female fate. mRNA from the *tra-2* gene is at least 15-fold more abundant in adult XX hermaphrodites than in adult XO males<sup>20</sup>. However, XX *tra-1* mutants that have been transformed from hermaphrodites into males due to a lack of *tra-1* have a low, male-specific amount of *tra-2* mRNA<sup>20</sup>. Because *tra-1* acts downstream of *tra-2* in the genetic pathway (Fig. 1), the activity of *tra-1* would not be expected to affect the level of *tra-2* mRNA. One simple explanation is that *tra-1* might control *tra-2* transcription by means of a positive feedback loop that reinforces the commitment of an XX animal to the hermaphrodite fate (Fig. 1).

#### Transmembrane signalling and sex determination

Signal transduction may bridge the beginning and end of the *C. elegans* sex determination pathway (Fig. 1). Genetic mosaic analysis indicates that *her-1* activity

is not cell-autonomous<sup>12</sup>. The *her-1* gene is thought<sup>21</sup> to promote male development in XO animals by acting as a negative regulator of *tra-2*. Thus, when XO animals lack *her-1*, all cells are transformed to follow the hermaphrodite rather than the male fate. In animals mosaic for *her-1*, some XO cells that are *her-1*(-) are masculinized and some XO cells that are *her-1*(+) are feminized<sup>12</sup>. The predicted product of *her-1* is a low molecular weight secreted protein (M. Perry and W.B. Wood, pers. commun.). Intriguingly, the predicted product of *tra-2*, which acts immediately downstream of *her-1*, is an integral membrane protein with multiple membrane-spanning domains<sup>22</sup>. Therefore, in XO animals the Tra-2 protein may mediate cell-cell communication by receiving and transducing the *her-1* signal. Although the role of *tra-2* is to promote female development in XX animals, *tra-2* mRNAs are found in both XX and XO animals<sup>20</sup>. Hence, Tra-2 protein is likely to be similarly expressed. A simple, though speculative model is shown in Fig. 2. According to this model, the Her-1 protein negatively regulates *tra-2* in XO males by binding to the Tra-2 protein. This binding interaction might inhibit Tra-2 activity by stimulating the endocytosis of Tra-2 protein or by disrupting other interactions involving Tra-2. Inactivation of Tra-2 by binding to Her-1 is also predicted to override the germ-line-specific controls that allow both sperm and oocytes to be produced. In the absence of Her-1, the XO germ line produces both sperm and oocytes. The putative Her-1 protein shows no similarity to other proteins (M. Perry and W.B. Wood, pers. commun.), while the Tra-2 protein sequence<sup>22</sup> reveals marginal similarity to the product of the

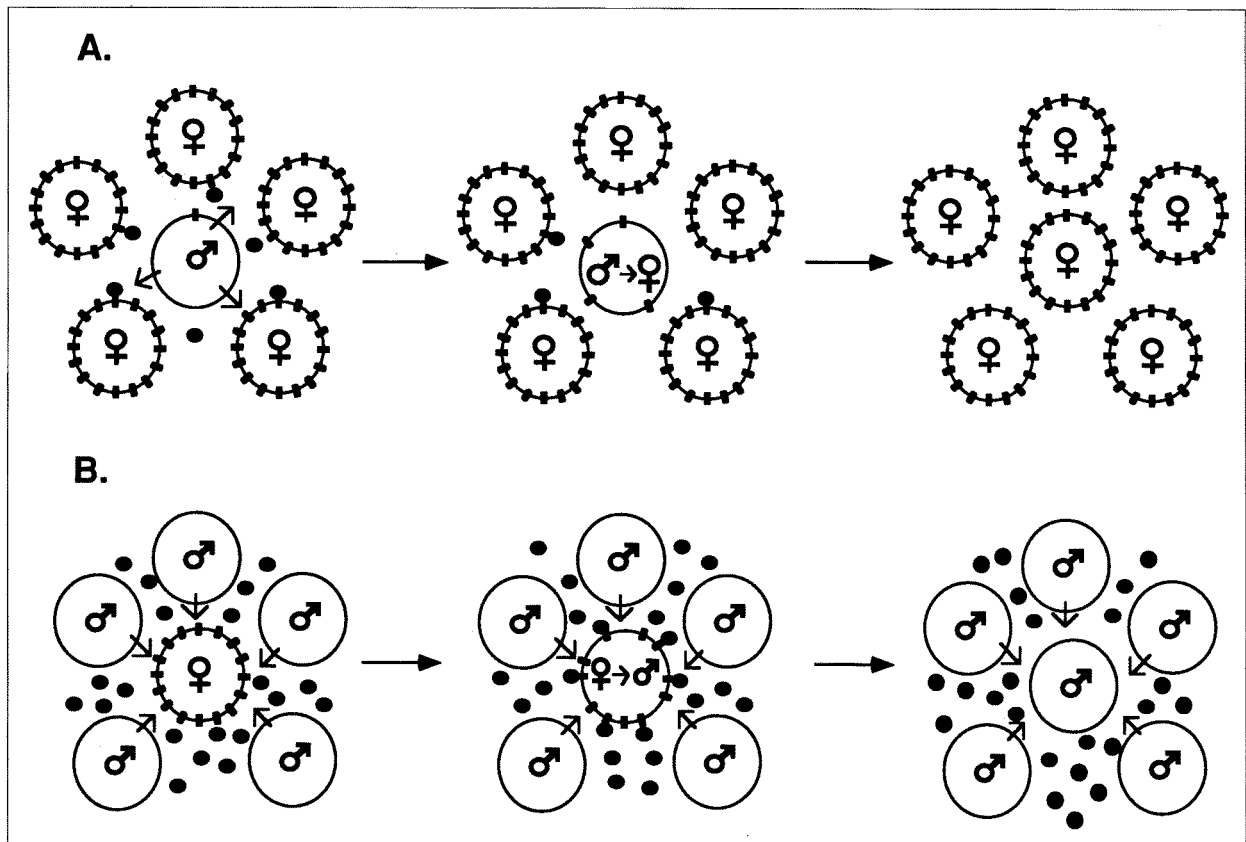


FIG 3

A model for the coordination of cells to adopt a single sexual fate. This model is based on several speculations about the Her-1 and Tra-2 proteins. (1) Tra-2 is abundant in female cells but rare in male cells. This idea relies on the difference in abundance of *tra-2* mRNA in XX and XO animals<sup>20</sup>. (2) The *her-1* gene encodes a secreted protein and *tra-2* encodes a membrane protein. These ideas are based on the nonautonomy of *her-1* (Ref. 12), the sequence of the predicted *her-1* product (M. Perry and W.B. Wood, pers. commun.) and the sequence of the predicted Tra-2 protein<sup>22</sup>. (3) Her-1 binds and thereby inactivates Tra-2. This idea is based on genetic arguments: *her-1* negatively regulates *tra-2* (Ref. 21), and no genes have been identified between *her-1* and *tra-2* in the sex determination pathway. (A) One male cell in a field of female cells is recruited to the female fate by the activity of its neighbors. Left: A single male cell secretes Her-1 (filled circles), which binds to the excess Tra-2 (filled rectangles) on its neighbors. Middle: Because little Her-1 remains after it binds to neighboring cells, Tra-2 on the male cell is activated. Therefore, more Tra-2 protein is made because of a positive feedback loop on *tra-2* expression, and the cell transforms from male to female. Right: All cells are committed to the female fate. (B) One female cell in a field of male cells is recruited to the male fate by the activity of its neighbors. Left: A single female cell produces abundant Tra-2, which binds and is inactivated by the excess of secreted Her-1 protein. Middle: Because Tra-2 is inactivated, less Tra-2 protein is made in the female cell, and the cell transforms from female to male. Right: All cells are committed to the male fate. Modified, with permission, from Ref. 22.

*Drosophila* gene *patched*<sup>23,24</sup>, which is involved in positional signalling.

What advantage might cell communication have for sex determination? One idea is that cell interactions could ensure uniform sexual development. During worm development, most sexual structures are formed by the descendants of more than one precursor cell<sup>25-27</sup>. Hence, cells must be coordinated to follow the same sexual fate. A *her-1/tra-2*-mediated transmembrane signalling pathway might provide a mechanism that can coordinate the sexual fate decision among neighboring cells. Figure 3 describes how cell communication mediated by *her-1* and *tra-2*, coupled to a positive feedback loop on *tra-2* expression, could direct single sex development. As shown in Fig. 3A, a male cell might be driven to the female fate if there is insufficient Her-1 protein to bind and inactivate Tra-2. Failure to inactivate the low levels of Tra-2 associated with males may activate a positive feedback loop on

*tra-2* expression. This proposed amplification of Tra-2 protein levels is predicted to drive a male cell to the female fate. Conversely, as shown in Fig. 3B, we propose that a female cell can be driven to the male fate if there is sufficient Her-1 protein produced by the surrounding male cells to bind and inactivate the Tra-2 protein of the single female cell.

A second possible role for cell communication within the sex determination pathway is to correct errors in the initial reading of the X:A ratio. Such a correction function might occur by feedback from the sex-determining pathway to the dosage compensation pathway. In *Drosophila*, improperly specified cells die and correctly specified cells compensate for these deaths<sup>28</sup>. In *C. elegans*, there are relatively few cells and little capacity for replacement of dying cells during development<sup>29</sup>. Therefore, a mechanism that corrects errors without destroying improperly determined cells could be particularly important in nematodes.

### The final steps of specifying somatic sex

The activity of *tra-1* determines whether an animal develops a female or male soma<sup>4,5</sup>. On the basis of our understanding of the genetic pathway of sex determination and the molecular characterization of sex-determining genes, we suggest that the final state of *tra-1* activity may be controlled post-translationally. We speculate that, in XX animals, the Tra-2 protein inhibits *fem* gene activity by binding one or more of the *fem* products (Fig. 2). Such an interaction might involve the carboxy-terminal tail of Tra-2, which is proposed to be intracellular and necessary for Tra-2 activity<sup>22</sup>. The proteins encoded by *fem-1* and *fem-3* are also predicted to be intracellular<sup>30,31</sup>. Since each *fem* gene is essential, one plausible model is that each gene encodes a component of a multisubunit Fem protein. Consequently, inactivation of any one of the three *fem* gene products would be sufficient to allow *tra-1* to promote female development. In XO animals, we speculate that the activity of *tra-1* might be negatively regulated by a protein-protein interaction involving one or more of the *fem* gene products. Consistent with this idea, the predicted protein sequence<sup>30</sup> of *fem-1* contains six repeats of the cdc10/SWI6 motif, which has been implicated in mediating protein-protein interactions<sup>32</sup>. Two proteins that bind transcription factors also contain cdc10/SWI6 repeats, notably IκB<sup>33</sup>, the negative regulator of NF-κB, and the β subunit of the GA-binding protein<sup>34</sup>. Therefore, a direct interaction between Tra-1 and Fem-1, mediated by the cdc10/SWI6 motif found in Fem-1, might alter the intracellular localization of Tra-1 or disrupt its activity.

### Conclusion

Many of the genes that control sexual fate in *C. elegans* have been cloned and their sequences analysed. It is now possible to speculate about the molecular mechanisms underlying the established genetic pathway. The identification of zinc finger motifs in proteins encoded by *sdcc-1* (Ref. 17) and *tra-1* (D. Zarkower and J. Hodgkin, pers. commun.) suggests that transcriptional regulation may control both the initial steps in commitment to a single sexual fate and the ultimate expression of differentiation-specific genes. The *tra-1* gene may also reinforce a commitment to the female fate by means of a positive feedback loop that acts through *tra-2* (Ref. 22). We speculate that the coordination of sexual fate throughout the animal is accomplished by a transmembrane signal transduction pathway that is mediated by *her-1* and *tra-2*. The presence of cdc10/SWI6 repeats in the Fem-1 protein sequence<sup>30</sup> and the association of this motif with other proteins that bind transcription factors suggests that Fem-1 might negatively regulate Tra-1. As a next step, biochemical analyses will be required to confirm the identities of these conceptual proteins and to determine how they interact.

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