It ain’t over till it’s ova: germline sex determination in C. elegans

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Summary
Sex determination in most organisms involves a simple binary fate choice between male or female development; the outcome of this decision has profound effects on organismal biology, biochemistry and behaviour. In the nematode C. elegans, there is also a binary choice, either male or hermaphrodite. In C. elegans, distinct genetic pathways control somatic and germline sexual cell fate. Both pathways share a common set of globally acting regulatory genes; however, germline-specific regulatory genes also participate in the decision to make male or female gametes. The determination of sexual fate in the germline of the facultative hermaphrodite poses a special problem, because first sperm then oocytes are produced. It has emerged that additional layers of post-transcriptional regulation have been imposed to modulate the activities of the global sex-determining genes, tra-2 and fem-3; the balance between these activities is crucial in controlling sexual cell fate in the hermaphrodite germ-line. BioEssays 23:596–604, 2001. © 2001 John Wiley & Sons, Inc.

Introduction
The age old question, “which came first, the chicken or the egg?” illustrates the point that there are intrinsic differences between somatic and germline development, owing in part to the immortal nature of the germline. The genetic tractability of the nematode C. elegans, which has facilitated the identification of sex-reversal mutants, has revealed that sexual cell fate decisions are also subject to different controls and modes of regulation between soma and germline. The C. elegans self-fertile hermaphrodite is a specialised female because her soma is essentially female, yet her germline transiently produces a fixed number of male sperm before switching and producing female oocytes. This sexual duality raises a number of questions related not only to how sexual cell fate decisions are differentially regulated between soma and germline, but also how male gametes (sperm) and female gametes (oocytes) can be produced within the same ovotestis.

The genes controlling sexual fate can be separated into global regulators that affect both the soma and germline and tissue-specific regulators that appear to be restricted in function to the germline. Hence, the pathways regulating sex determination in the soma and germline differ because they involve different sets of genes and different patterns of genetic epistasis. In addition, the characterisation of gain-of-function mutations in the global sex-determining genes, tra-2 and fem-3, has led to the identification of post-transcriptional controls that modulate their activities in the hermaphrodite germline. The activity of tra-2 is negatively regulated to allow hermaphrodite spermatogenesis; subsequently, fem-3 is negatively regulated to permit the switch to oogenesis. The apparent need to have separate pathways controlling somatic and germline sexual fate decisions might appear to be a peculiarity of the C. elegans hermaphrodite. Organisms with conventional male/female sex determination, such as Drosophila, however, have also established different mechanisms for determining sexual fate in soma and germline.¹

The somatic sex determination pathway in C. elegans
The X/A ratio is the primary determinant of sex in C. elegans: the decision to develop as a hermaphrodite or male in C. elegans, as in Drosophila, is determined by the ratio of the number of X chromosomes to the number of sets of autosomes.² Diploid XO animals develop as male and XX animals as hermaphrodite. Animals can be partially or completely transformed to the sex opposite that specified by the X/A ratio when they carry mutations in downstream genes controlling sexual fate decisions. Loss-of-function mutations in the genes tra-1, tra-2, or tra-3 (for transformer) masculinise XX animals, but have little or no effect on XO male development.³,⁴ Thus, it was inferred that the normal wild-type function of these genes is to promote female development. Similarly, loss-of-function mutations in the genes her-1 (hermaphroditization), fem-1, fem-2, or fem-3 (feminization) feminise XO animals, suggesting that their normal wild-type function is to promote male development; null mutations in the fem genes also feminise the germline of XX hermaphro-

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These genes are global regulators of both somatic and germline sex determination and their activities have been ordered in a regulatory hierarchy based on genetic epistasis (Fig. 1). For simplicity, the pathway is arranged linearly to illustrate that each gene is responsible for inhibiting the activity of its immediate downstream neighbour, although non-linear interactions between genes have also been shown to exist.

An understanding of the molecular mechanisms underlying the genetic pathway of somatic sex determination has evolved from the molecular identification of genes controlling this process combined with protein interaction studies (Fig. 2). The zinc finger transcription factor, TRA-1, is the master regulator controlling all somatic sexual cell fate decisions. The mab-3 gene is a direct target for TRA-1 transcriptional repression and encodes a protein with sequence and functional similarity to Drosophila.

Figure 1. Somatic pathway of C. elegans sex determination. The X/A ratio is the primary determinant of sex and sets the activity states of the regulatory genes (high or low) controlling sex determination. Genes at the beginning of the pathway control both sex determination and dosage compensation. The interactions between adjacent genes in the pathway are inhibitory in nature. Any significant amino acid similarity to other known proteins, functional motif or predicted function encoded by each gene is indicated. The two-headed arrow linking tra-2 and tra-1 indicates that there is a positive feedback loop whereby TRA-1 appears to upregulate tra-2 mRNA steady-state levels, and TRA-2B or a soluble cytoplasmic region of TRA-2A (TRA-2c) enhances the feminising activity of TRA-1. RRM, RNA recognition motif and NHR, nuclear hormone receptor.

Figure 2. Model of a signal transduction pathway controlling C. elegans somatic sex determination. Left, in XX hermaphrodites a predicted cytoplasmic carboxy-terminal domain of the TRA-2A membrane protein binds to and inactivates FEM-3. In turn, TRA-1 either represses the transcription of genes required for male development or activates the transcription of genes required for the female cell fate. The TRA-3 calpain is predicted to liberate a TRA-2c fragment that translocates to the nucleus and enhances the feminising activity of TRA-1. Right, in XO males, the XO-specific HER-1 ligand inactivates TRA-2A, thereby liberating the FEM proteins and repressing TRA-1.
Dsx and vertebrate Dmrt1.(7,8) So far, mab-3 is the only known gene with a role in sex determination that has been conserved across phyla. This finding supports the hypothesis that sex-determining mechanisms appear to have evolved in reverse order such that a terminal regulator in a hierarchy is more likely to have been conserved than one at an earlier step.(9)

One conclusion from the study of *C. elegans* sex determination is that signal transduction plays a central role in regulating many aspects of somatic sexual cell fate determination (Fig. 2). In XX hermaphrodites, TRA-2A is a membrane protein that binds and inhibits the activity of the male-promoting FEM-3 protein (Fig. 2, left).(10,11) The predicted cytoplasmic carboxy-terminal tail region of TRA-2A contains a domain that directly binds and perhaps sequesters FEM-3.(11) In turn, the activities of the FEM-1, FEM-2, and FEM-3 proteins are inhibited and the TRA-1 zinc finger transcription factor is free to promote female development.(5,6)

Recent studies indicate that the feminising activity of TRA-1 may be enhanced by binding to a soluble carboxy-terminal fragment of TRA-2A, which localises to the nucleus.(12) Such a fragment is predicted to exist naturally in the female germline as TRA-2B, but may also be generated through proteolytic cleavage of TRA-2A by the TRA-3 calpain.(13,14)

In XO males, HER-1 is a predicted extracellular ligand that represses the female-promoting TRA-2A (Fig. 2, right).(15) The *her-1* gene is transcriptionally regulated as *her-1* mRNAs are only detected in XO animals.(16) The inhibition of TRA-2A by HER-1 allows the three FEM proteins to promote male development by inactivating TRA-1 through an unknown mechanism. The identification of FEM-2 as a member of the PP2C family of serine/threonine phosphatases combined with its ability to bind FEM-3 raises the possibility that protein phosphorylation/dephosphorylation is involved in controlling sexual fate; however, a kinase that promotes female development has yet to be identified.(17,18)

Additional upstream genes controlling both sex determination and dosage compensation, including the *fox-1* (feminizing on X) and *sex-1* (signal element on X) numerator elements and the global regulators *sdc-1*, *sdc-2*, and *sdc-3* (sex and dosage compensation) and *xol-1* (XO lethal), have also been extensively studied.(3,19,20) Further discussion of these genes is beyond the scope of this paper, except that it should be noted that the activities of these genes control sex determination because the SDC-2 protein binds to the promoter of *her-1* and represses its transcription in XX hermaphrodites.(21)

**Germ cell proliferation and development**

Germ cells are essential for sexual reproduction. In most organisms, progenitor cells are set aside early in development; in *C. elegans*, germ-line granules (P granules) segregate with germline progenitor cells and may act as germline determinants.(22) P4 is the germline founder cell generated at the fourth cleavage division, which gives rise to all cells of the germline. The germ cells of the *C. elegans* hermaphrodite develop within a pair of tube-like ovotestes, which display proximal/distal polarity (Fig. 3). Most germ cell development is syncytial, so a germ cell refers to a nucleus and its associated cytoplasm surrounded on all but one side by membrane. Although germ cells are syncytial, there is evidence that the membranes partially enclosing each germ nucleus permit autonomous development.(23) Mitotic progenitor germ cells originate at the distal end of the ovotestis (Fig. 3, left). This pool of mitotic cells is maintained by an interaction between the somatic distal tip cell (DTC) and the germline. Laser ablation of

![Figure 3](image)

**Figure 3.** Schematic diagram of the posterior lobe of the hermaphrodite somatic gonad. The distal tip cell (DTC) is located at the distal end of the ovotestis and produces a localised signal that maintains the stem-cell population of mitotic germ cells. As mitotic nuclei divide, those most distal transit through the transition zone, enter meiosis and arrest in pachytene. Germ cells are activated by the RAS/MAPK pathway to exit pachytene and half of the population of germ cells committed to the female fate undergo apoptosis. The surviving female germ cells complete cellularisation and arrest in diakinesis until they are ovulated and fertilised by sperm that were previously made and stored in the spermatheca.
the DTC causes all mitotic cells to cease proliferation and to develop as sperm.\(^{24}\) It has been shown that germline expression of the membrane protein GLP-1, a member of the Notch/LIN-12 family of receptors, and DTC expression of LAG-2, a Delta-like ligand for GLP-1, are both required to maintain proliferation.\(^{25}\) The most proximal (i.e. those furthest from the DTC) mitotic cells undergo a transition and enter meiosis and progress to pachytene. The first 40 cells in each ovotestis to enter meiosis are destined to become sperm; subsequently, oocytes are produced. In the loop region of the germline, germ cells are activated by the RAS/MAPK pathway to exit from pachytene. Laser ablation studies suggest that the signal for pachytene exit may be generated by a somatic sheath cell, which contacts the germline.\(^{26}\) RAS/MAPK-activated female, but not male, germ cells must then make a choice between life and death. It has been estimated that over half of the germ cells adopting the female fate are removed by apoptosis because they have fulfilled their function as nurse cells and, to a lesser degree, because they may have suffered DNA damage.\(^{27,28}\) The surviving female germ cells complete cellularisation and arrest in diakinesis until they are ovulated and fertilised.

### Controlling sexual cell fate in the germline

Against the backdrop of cell proliferation, meiosis, RAS/MAPK signalling and apoptosis, germ cells also acquire a sexual identity. Normally, sperm are produced during the L4 stage of development. Subsequently, adult hermaphrodites switch sex upon encountering a nematode, an event that terminates spermatogenesis. \(^{29–41}\) In the absence of a nematode, sperm are produced and used to fertilise the unfertilised eggs of the hermaphrodite. Thus, hermaphrodites must also decide whether to produce sperm. In the loop region, germline cells are activated by the RAS/MAPK pathway and become destined to develop as sperm or female germ cells. This decision is made by the ETS transcription factor Tra-2, which is negatively regulated to allow the onset of spermatogenesis. \(^{29–33}\) Additional gain-of-function mutations (gf) in tra-2 lead to dominant masculinization or feminization, respectively, of the XX germline, but have little effect on the soma.\(^{30,34,35}\)

Genetic epistasis studies initially indicated that tra-1 was not the terminal regulator of germline sex because fem-1, fem-2, or fem-3, tra-1 double mutants all produced oocytes and not sperm.\(^{5,36}\) Instead, the activity states of the fem-1, fem-2, fem-3, fog-1 and fog-3 genes, which are essential for spermatogenesis in both hermaphrodites and males, determine whether a germ cell developed as a sperm or oocyte.\(^{5,36}\) These results have recently been reinterpreted in light of a study showing that tra-1 transcriptionally regulates fog-3, which was also shown by molecular data to be a terminal regulator of germ cell sexual fate that functions downstream of the fem genes (Fig. 4, right).\(^{37}\) It remains unclear whether the fem genes directly repress tra-1 activity during male germ cell development. Mutations in the fem genes can decrease the level of fog-3 transcripts, however, suggesting that the fem genes may activate fog-3 and also play a direct role in promoting spermatogenesis.\(^{37}\) fog-3 encodes a protein carrying a domain found in the vertebrate Tob and BTG proteins, which are postulated to suppress cell proliferation or promote differentiation.\(^{38}\)

In the hermaphrodite germline, the balance between the feminising properties of tra-2 and the masculinising properties of fem-3 influences whether a germ cell develops as sperm or oocyte (Fig. 4). \(^{39}\) fem-3(gf) mutations cause all germ cells to develop as sperm and tra-2(gf) mutations cause all germ cells to develop as oocytes in XX hermaphrodites. A double mutant carrying both fem-3(gf) and tra-2(gf) mutations develops as a fertile hermaphrodite.\(^{30,34}\) The ability of the hermaphrodite germline to produce first sperm, then oocytes can thus be examined from the perspective of the mechanisms controlling the activities of tra-2 and fem-3.

### Post-transcriptional regulation of tra-2 in the germline

The activity of tra-2 is negatively regulated to allow the onset of spermatogenesis (Fig. 4A). In XO males, repression of TRA-2 by HER-1 is probably sufficient to inactivate tra-2 activity and to promote spermatogenesis. In the XX hermaphrodite germline, her-1 mRNAs are absent, and there is evidence that tra-2 activity is inhibited by two post-transcriptional controls (Fig. 4A). One post-transcriptional control of tra-2 activity is mediated through a pair of 28 bp elements that form a direct repeat (DRE) found in the tra-2 3’UTR. Gain-of-function mutations that disrupt one or both of the DREs feminize the hermaphrodite germline.\(^{35,39}\) Evidence that tra-2 mRNAs may be translationally regulated has been obtained by showing that tra-2 mRNAs isolated from tra-2(gf) mutants are associated with larger polysome fractions than tra-2 mRNAs isolated from wild-type animals.\(^{39}\) It has also been reported that TRA-2 protein levels appear to be higher in tra-2(gf) animals based on immunocytochemical detection of TRA-2 protein using antibodies.\(^{40}\) Gel mobility shift assays indicate that a factor(s) called DRF (for Direct Repeat Factor) binds to the tra-2 DRE.\(^{40}\) The laf-1 gene has been postulated to encode a component of DRF activity, but the molecular identity of laf-1 remains to be determined.\(^{41}\)

A known factor that binds to the tra-2’3’UTR is GLD-1; this interaction was identified in a yeast three-hybrid screen.\(^{40}\) gld-1 encodes an RNA-binding protein containing a KH domain RNA-binding motif and is a member of a family of proteins carrying a STAR domain.\(^{42,43}\) Mutations in gld-1...
affect sex determination, meiotic progression in the hermaphroditic and proliferation in both the male and hermaphrodite germlines.\(^{(42,44,45)}\) The diverse range of phenotypes displayed by \(\text{gld-1}\) mutants can be explained by postulating that \(\text{GLD-1}\) acts as a translational repressor that masks multiple germline mRNAs and that its functions are modulated by interactions with other proteins. Recent studies have shown that \(\text{GLD-1}\) directly interacts with \(\text{FOG-2}\).\(^{(46)}\) The \(\text{fog-2}\) gene encodes an F-box-containing protein.\(^{(46)}\) Genetically, \(\text{fog-2}\) acts as a germline-specific repressor of \(\text{tra-2}\) activity that promotes hermaphrodite spermatogenesis; unlike \(\text{fog-1}\) and \(\text{fog-3}\), \(\text{fog-2}\) is not required for XO male spermatogenesis.\(^{(30)}\) It is speculated that \(\text{fog-2}\) may play a hermaphrodite-specific role in repressing \(\text{tra-2}\) activity, whereas \(\text{GLD-1}\) may play a more global role in mediating translational repression.

A second control involved in regulating the activity of \(\text{tra-2}\) was identified through the analysis of a distinct class of \(\text{tra-2}\) gain-of-function mutants that have been renamed \(\text{tra-2(mx)}\) (for mixed character). These mutations feminise the germline by repressing spermatogenesis; however, they also lead to a recessive partial masculinisation of somatic tissues.\(^{(30,35)}\) Unlike the previously described \(\text{tra-2(gf)}\) mutations, the \(\text{tra-2(mx)}\) mutations appear to identify a post-translational regulatory mechanism, because they each change a single amino-acid found within a cluster of 22 amino acids present in the carboxy-terminal region of \(\text{TRA-2A}\) and \(\text{TRA-2B}.\)\(^{(13)}\) \(\text{TRA-2B}\) corresponds to a germline-specific protein encoded by the \(\text{tra-2}\) locus, which shares the same sequence as the carboxy-terminal region of \(\text{TRA-2A}.\) The region corresponding to this carboxy-terminal sequence, which is present in \(\text{TRA-2B}\) and as a soluble form derived from \(\text{TRA-2A}\) (discussed below), will be collectively referred to as \(\text{TRA-2c}.\) The \(\text{TRA-2c}\) region contains not only the MX site, but also a distinct FEM-3 binding site. It has been shown that \(\text{TRA-2A}\) provides the feminising activity of the \(\text{tra-2}\) locus primarily through its role in binding \(\text{FEM-3}.\)\(^{(11,47)}\) The sequence of \(\text{TRA-2c}\) was initially predicted to be cytoplasmic because it does not contain any membrane-spanning domains.\(^{(1)}\)

From the genetic properties of \(\text{mx}\) mutations, it was postulated that the MX domain identified a binding site for a repressor of \(\text{tra-2}\) germline activity. Surprisingly, it was found by far western analysis that \(\text{TRA-2c}\) was capable of binding to the transcription factor \(\text{TRA-1};\) moreover, this interaction required the MX region, but not the FEM-3-binding domain.\(^{(12)}\) Mutations in the MX region were shown to disrupt the interaction between \(\text{TRA-2c}\) and \(\text{TRA-1}.\)\(^{(12)}\) It was also shown

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**Figure 4.** Pathway of *C. elegans* germline sex determination. A balance between the levels of fem-3 and tra-2 determines whether spermatogenesis or oogenesis ensues. **A:** Spermatogenesis occurs during the fourth larval stage and requires the inhibition of \(\text{tra-2}\) to achieve high levels of fem-3 activity. In XO animals, the global activity of her-1 inhibits \(\text{tra-2}\), so males produce sperm continuously. In XX animals, her-1 activity is absent and \(\text{tra-2}\) is negatively regulated post-transcriptionally through its 3’ UTR by \(\text{gld-1}\) and \(\text{fog-2}\) to permit the onset of hermaphrodite spermatogenesis. It is postulated that the \(\text{laf-1}\) gene controls the activity of \(\text{tra-2}\) in the hermaphrodite germline. **B:** The onset of oogenesis occurs in adults, although the commitment to the oocyte fate is probably made earlier. Post-transcriptional regulation of fem-3 is required to permit the switch to oogenesis. The \(\text{tra-1}\) gene is also involved in the transcriptional repression of fog-3, which is essential for the male germ cell fate. The dotted lines indicate that, although a high fem-3/tra-2 ratio promotes male germ cell development and a high tra-2/fem-3 ratio promotes female germ cell development, it is not known how the individual levels of tra-2 and fem-3 are regulated temporally.
that a GFP-tagged TRA-2c localised to the nucleus indicating that it has the opportunity to interact with TRA-1. This interaction is likely to occur in both the germline and soma, since it has been shown that the TRA-3 calpain protease is capable of cleaving TRA-2A and generating a product comparable in sequence to the germline TRA-2B.\(^{14}\) It seems likely that the soluble TRA-2c peptide acts to enhance the feminising activity of TRA-1 and the partial somatic masculinisation observed in tra-2(mx) mutants is consistent with this. It thus remains unclear why the tra-2(mx) mutations, which cause dissociation of the TRA-2c/TRA-1 complex, would lead to germline feminisation. One simple model, however, predicts that there exists a germline repressor that recognises the TRA-2c/TRA-1 complex, but not the separate components. Thus, because of the dosage sensitivity of the germ line, deregulated TRA-1 and TRA-2c could lead to female development.\(^{12}\)

**Post-transcriptional regulation of fem-3 in the germline**

The switch from hermaphrodite spermatogenesis to oogenesis is achieved by negatively regulating the activity of fem-3 (Fig. 4B). Post-transcriptional regulation of fem-3 activity has been shown to be mediated by a 5 bp cis-acting element found in the fem-3 3’ UTR, named the PME (for point mutation element), and additional trans-acting factors. The PME sequence was delineated because it is disrupted in all 17 fem-3(gf) alleles.\(^{48}\) Overexpression of an RNA composed of a wild-type fem-3 3’UTR sequence leads to germline masculinisation; this result was interpreted to indicate that fem-3 activity could be deregulated by titration of a negative regulator.\(^{48}\) A yeast three-hybrid screen has since led to the identification of two RNA-binding proteins that are capable of interacting with wild-type, but not mutant, fem-3 PME sequences.\(^{49}\) The genes encoding this binding activity are 92% identical at the nucleotide level and have been named fbf-1 and fbf-2 (for fem-3 binding factor). FBF-1 and FBF-2 are homologues of the Drosophila Pumilio protein, which mediates translational inhibition of maternal hunchback mRNA by binding to nanos response elements contained in the hunchback 3’UTR.\(^{50}\) When the activities of both fbf-1 and fbf-2 were simultaneously inhibited by RNA-mediated interference (RNAi) a Mog phenotype similar to that of fem-3(gf) mutants was observed, confirming that these genes appear to play a role in controlling sex determination in the germline.\(^{49}\) Because the fbf genes appear to have redundant activities, it is unlikely that they would be easily identified by forward genetic screens.

Two additional proteins, NOS-3 and CPB-1, were identified in a yeast two-hybrid screen searching for cytoplasmic components capable of interacting with FBF. NOS-3 is a homologue of the Drosophila Nanos protein.\(^{51}\) In Drosophila, Nanos functions as an accessory to Pumilio to promote translational repression of hunchback.\(^{52}\) Two other C. elegans nos genes have been identified, nos-1 and nos-2; these genes appear to play a role in primordial germ cell development and survival.\(^{51,53}\) The CPB-1 protein is related to cytoplasmic polyadenylation element binding (CPEB) proteins found in *Xenopus* and other organisms.\(^{64}\) The CPEB proteins play diverse roles, including the translational regulation of specific mRNAs. One of the other Ce-cpb genes has been shown to correspond to fog-1, a gene already known to be essential for spermatogenesis.\(^{29,54,55}\) Further studies are required before the roles of the CPEB proteins in regulating germ cell sex determination are understood.

The components necessary for fem-3 3’UTR-mediated post-transcriptional regulation are also present in the soma based on assays that are capable of monitoring the effect fem-3 3’UTR sequences have on lacZ reporter expression in somatic tissues.\(^{56}\) Gel mobility shift assays also indicate that a PME-binding factor other than FBF, which is germline-specific, may be present in somatic tissues.\(^{56}\) It was further shown that each of six mog genes is required to promote post-transcriptional downregulation of fem-3 activity. Three of the mog genes have now been cloned and have been shown to encode members of the DEAH-box RNA helicase family.\(^{57,58}\) Members of this family of RNA helicases play diverse roles in RNA metabolism, including translation and splicing. It remains to be determined whether the MOG proteins interact directly or indirectly with FBF and NOS-3 to repress fem-3 activity. Because there is a maternal requirement for mog gene activity during embryogenesis, these genes may play additional roles in regulating RNA metabolism that are not limited to sex determination.\(^{52,53}\) Although many of the trans-acting factors mediating the post-transcriptional regulation of fem-3 3’germline activity are homologues of proteins involved in translational regulation in other organisms, it still remains to be determined whether post-transcriptional regulation of fem-3 germ line activity occurs at the level of translational regulation, RNA stability or architecture. At this point, the nature of the sperm/oocyte switch in the hermaphrodite germline is also unknown. One simple model is that one or more of the MOG and FBF proteins is initially limiting, but gradually accumulates with the growth of the germline, eventually titrating and repressing the fem-3 transcripts. Oogenesis would then ensue.

**Why are there differences between somatic and germline sex-determining mechanisms?**

The controls regulating germ line sex are likely to have arisen, in part, because of the intimate relationship between the pathways regulating the proliferation, development and physiology of a germ cell and its acquisition of sexual identity. For example, one can speculate that the importance of translational regulation in controlling germ cell sexual fate may result from the need to retain maternal mRNAs in a quiescent state until fertilisation.\(^{59}\) The involvement of the *C. elegans* CPEB proteins is consistent with this notion.
Finally, it has been observed that the sex-determining genes are rapidly evolving. A comparison of genes found in both *C. elegans* and its close relative *C. briggsae*, which is estimated to have diverged from *C. elegans* between 15 and 50 million years ago, has shown that *tra-1* and *tra-2* have evolved most rapidly of all the genes so far compared between the two species.\(^{60,61}\) A *tra-2* homologue has been cloned from *C. remanei*, the closest gonochoristic relative of *C. elegans*.\(^{62}\) Sequence comparisons indicate that the *C. elegans* TRA-2A protein is equally distant (43–44% ID) from its *C. briggsae* homologue as it is from its *C. remanei* homologue.\(^{61,62}\) It also appears that both of the post-transcriptional controls regulating *tra-2* germline activity, the MX region and DRF-binding activity of the 3′ UTR, are conserved in the *C. remanei tra-2* gene products. Thus, the gonochoristic nature of *C. remanei* is not explained by the simple absence of regulatory domains controlling *tra-2* germline activity.\(^{62}\) It is possible that a repressor of *tra-2* germline activity, such as *fog-2* may be absent in *C. remanei*.\(^{30}\)

Alternatively, recent phylogenetic studies indicate that hermaphroditism may have evolved independently several times from gonochoristic ancestors (D.H. Fitch, personal communication). If gonochorism is the ancestral state, then androdioecious development is likely to be more complex because both quantitative and qualitative changes in the interactions involving sex-determining genes have probably co-evolved to allow the production of both male and female gametes within the same gonad. In this scenario, the post-transcriptional control domains present in *C. remanei* may be insufficient to promote spermatogenesis because these domains either lack interacting partners or because their partners may have suboptimal binding affinities. Moreover, the ability to undergo spermatogenesis is only half of the hermaphrodite story — sperm production must also be limited to allow the switch to oogenesis.

Presently, the whole genome of *C. briggsae* is being sequenced, so it is conceivable that the genome sequence of *C. remanei* may also be obtained. The availability of whole genome sequences will facilitate the direct phylogenetic sequence comparisons of all genes known to play a role in sex determination in *C. elegans* and perhaps provide additional clues regarding the evolution of sex-determining mechanisms.

It is possible that the sensitivity of the germline to changes in the dose of masculinising and feminising activities allows the hermaphrodite to respond to her environment and to make appropriate adjustments in brood sizes. Since the number of sperm produced by the hermaphrodite controls brood size, this is an important life history trait and appears to have been optimised for maximal population growth.\(^{63}\) It is interesting to note in this regard that post-transcriptional control exerted through the *fem-3* 3′ UTR is a temperature-sensitive process, which superficially transforms fecundity into an environmentally determined trait.\(^{34}\) In some reptiles, sex determination is dependent on the temperature of the environment.\(^{64}\)

### Summary and future perspectives

Forward genetic screens to identify mutants and the molecular identification of genes that control sexual cell fate have led to an extensive understanding of the mechanisms underlying the control of sex determination in *C. elegans*. It is apparent, however, from protein interaction studies that there are still additional genes affecting sexual fate that have not been identified by conventional genetic screens. Some of these genes, such as *fbb-1* and *fbb-2* may have been missed because they are genetically redundant. The availability of the entire *C. elegans* genome sequence allows the use of functional genomic techniques to identify these additional genes. RNAi is a particularly powerful tool for gaining insights into the function of novel genes, and has played an important role in validating the involvement of genes identified on the basis of protein interaction studies.\(^{65}\) In order to learn more about the genes and processes that regulate sexual fate decisions, DNA microarrays are also proving to be an invaluable tool for generating new hypotheses. For example, a recent study has identified many transcripts that are enriched in the germline, and these have been further categorised into those preferentially associated with oogenesis, spermatogenesis or simply intrinsic to the germline.\(^{66}\) Global clustering analysis should further help to reveal how these gene activities change in response to differences in organismal physiology and sexual state.

In summary, the analysis of *C. elegans* sex determination has provided a rich source of information about post-transcriptional and post-translational controls regulating gene activity. It is anticipated that the powerful combination of genetics, protein interaction analyses, and other functional genomic tools will uncover answers to the questions remaining about mechanisms controlling sexual cell fate in *C. elegans*.

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