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Control of germ cell differentiation in *Caenorhabditis elegans*

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Abstract. The germline of *Caenorhabditis elegans* is organized in a linear fashion—the most distal germ cells remain in mitosis, those in the middle enter meiosis and proximal cells differentiate as sperm or oocytes. Two signal transduction pathways control germ cell fates. The *glp-1* gene mediates a signal that promotes mitosis and the genes of the sex determination pathway mediate a signal that determines if germ cells will develop as sperm or oocytes. Information from these pathways acts through terminal regulators to specify cell fate. For example, *fog-1* and *fog-3* are required to initiate spermatogenesis and *gld-1* appears to be required to initiate oogenesis. Study of these terminal regulators suggests that the decisions about sexual identity and mitosis are linked in germ cells. We propose a tripartite interaction that forces germ cells to adopt one of only three fates—mitosis, spermatogenesis or oogenesis.

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In most animals, germ cells must either proliferate by dividing mitotically or enter meiosis and differentiate as sperm or oocytes. How these decisions are made is not understood fully for any organism. In this paper, we review current knowledge about the regulation of germ cell fate in *Caenorhabditis elegans*. In addition, we propose a model for how the terminal regulators of mitosis, spermatogenesis and oogenesis interact in the developing germline.

In *C. elegans*, two germline founder cells divide mitotically to generate more than 1000 descendants. Most of these descendants are part of a large syncytium. For simplicity, we call each germline nucleus with its surrounding cytoplasm and membranes, a 'germ cell', although each 'cell' is continuous with the central core of the germline. In males, the germ cells are arranged in a single U-shaped testis. By contrast, hermaphrodites have two U-shaped ovotestes (Fig. 1) that each produce sperm and oocytes, allowing the animals to self-fertilize. Within each testis or ovotestis, distal germ cells remain in mitosis, those in the middle enter meiosis and proximal germ cells differentiate. In males, all differentiating germ cells form sperm, whereas in hermaphrodites, the first germ cells to differentiate form sperm and later ones become oocytes (Fig. 1).

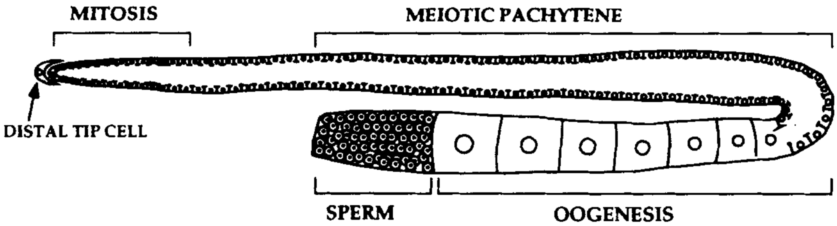

FIG. 1. A single hermaphrodite ovotestis. The ovotestis is a U-shaped tube. At the distal end, *glp-1* mediates a signal from the distal tip cell that promotes mitosis. In the middle, germ cells enter meiotic pachytene. The first germ cells to complete meiosis form sperm and are located at the proximal end of the ovotestis. The remaining germ cells differentiate as oocytes.

To understand how the fate of germ cells is controlled, we have focused on three questions. How do germ cells determine whether they should divide mitotically or enter meiosis? How does a germ cell decide between spermatogenesis and oogenesis? How are these two regulatory processes linked? So far, results indicate that these decisions involve a diverse but interlocking set of regulatory mechanisms.

The decision between mitosis and meiosis

At the distal end of each gonadal arm is a somatic cell, the distal tip cell, which is adjacent to the region where germ cells proliferate. If the distal tip cell is killed with a laser microbeam, germ cells stop dividing mitotically and enter meiosis (Kimble & White 1981). Thus, a signal from the distal tip cell is required for germ cells to continue mitosis.

The *glp-1* gene is predicted to encode a receptor that receives the signal from the distal tip cell. Four lines of evidence support this model. First, *glp-1* is required for normal germline proliferation: in mutants lacking *glp-1*, all germ cells enter meiosis early in larval development (Austin & Kimble 1987). Second, studies of genetic mosaics demonstrate that *glp-1* functions within germ cells, but is not needed in the distal dip cell (Austin & Kimble 1987). Thus, *glp-1* acts to receive rather than to transmit a signal. Third, the sequence of *glp-1* suggests that it encodes a receptor protein, similar to those encoded by the *lin-12* and *Notch* genes (Yochem & Greenwald 1989, Austin & Kimble 1989). Fourth, antibody staining reveals that *glp-1* protein (GLP-1) is found on the membranes of germ cells that are proliferating mitotically, exactly where a receptor is expected to function. After germ cells have entered meiosis they no longer need *glp-1*; antibody staining confirms that GLP-1 disappears from meiotic germ cells (S. Crittenden, personal communication).

How does *glp-1* promote mitosis in the germline? More than half of GLP-1 consists of tandemly repeated elements. Ten EGF-like repeats (which are similar in sequence to epidermal growth factor) and three LNG repeats (found in *lin-12*, *Notch* and *glp-1*) are located in the extracellular domain; six ANK repeats (found in ankyrin) are located in the intracellular domain (Yochem & Greenwald 1989, Austin & Kimble 1989). Sequence analysis of loss-of-function mutations revealed that each set of repeated elements is crucial for GLP-1 function (Kodoyianni et al 1992). The extracellular domain is likely to interact with a ligand, but nothing more is known about its function. Because the ANK repeats are located intracellularly, they might interact with other proteins to promote mitosis. Indeed, in transgenic animals, overexpression of a construct consisting primarily of the ankyrin repeats provides deregulated GLP-1 activity (Roehl & Kimble 1993). This result suggests that the ANK repeats are the signalling part of GLP-1 and that removal of the extracellular region makes these repeats constitutively active.

Because GLP-1 cannot act alone to control germ cell fate, both an upstream ligand and a downstream target must exist. These interacting proteins have not yet been identified. The best candidates are the products of *lag-1* and *lag-2*, two genes required for both *glp-1* and *lin-12* to function (Lambie & Kimble 1991). Two other genes whose products might interact with *glp-1* have been identified by mutations that cause unregulated proliferation of germ cells. One of these genes, *gld-1*, is described below. A mutation in the second gene causes excess proliferation in both male and hermaphrodite germlines; this gene might help suppress mitosis (L. Kadyk, personal communication).

The decision between spermatogenesis and oogenesis

Sex determination in the soma

The genes that regulate sexual fate in the soma of *C. elegans* also determine which germ cells will develop as sperm and which as oocytes, a process in which several genes that act only in the germline also participate. Thus, to understand the regulation of sex determination in the germline, we must first examine how the sexual identity of somatic tissues is controlled.

In *C. elegans*, sex determination is controlled initially by the ratio of X chromosomes to sets of autosomes (Madl & Herman 1979). Animals that are 1X:2A develop as males, whereas animals that are 2X:2A develop a female body and hermaphrodite germline. The X:A ratio determines the activity of the *xol-1*, and *sdc-1*, *sdc-2* and *sdc-3* genes, which in turn control two processes—dosage compensation and sex determination (reviewed by Villenueve & Meyer 1990). The next step appears to be secretion of a signal that coordinates sexual identity among different cells. Genetic analysis (Hodgkin 1980, Hunter & Wood 1992) and the sequences of the *her-1* and *tra-2* genes (Perry et al 1993, Kuwabara et al 1992) suggest that *her-1* encodes this signalling molecule and that *tra-2* encodes its receptor. In contrast to most receptor systems, the *her-1* product is predicted to inactivate the *tra-2* product, its putative receptor.

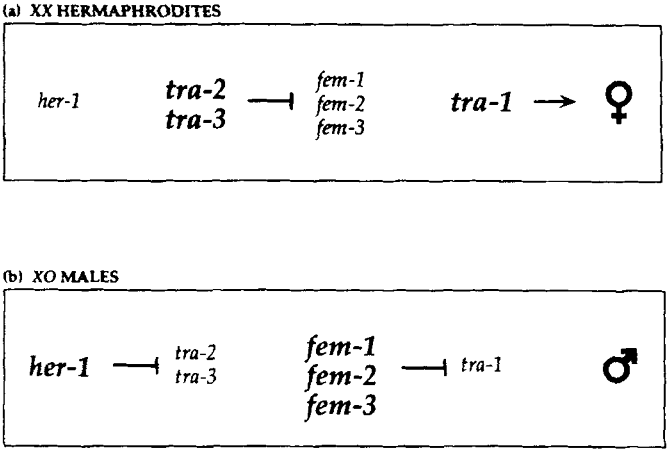

FIG. 2. Sex determination in somatic tissues. These diagrams represent the state of the sex determination genes in the soma of (a) XX hermaphrodites and (b) XO males. Active genes are shown in bold type and inactive genes in small type. Arrows indicate positive regulation and blunt lines indicate negative regulation. See text for further explanation of these regulatory pathways.

The specification of a cell as male or female depends on genes downstream of the signal mediated by *her-1* and *tra-2* (Fig. 2). In XX animals, *tra-2* and *tra-3* inhibit three *fem* genes, allowing *tra-1* to direct female cell fates (Fig. 2a, Hodgkin 1986). In XO animals, *tra-2* and *tra-3* are inhibited by *her-1*. This allows the *fem* genes to suppress *tra-1*, permitting male development (Fig. 2b). Two criteria indicate that *tra-1* is the terminal regulator of sexual fates in somatic tissues. First, *tra-1* acts at the end of the sex determination pathway in the soma (Fig. 2). Second, *tra-1* is essential for female development in somatic tissues: if *tra-1* is inactive, male development occurs. The *tra-1* gene probably controls sexual fate at the transcriptional level, since sequence analysis suggests it encodes a zinc finger protein (Zarkower & Hodgkin 1992).

Sex determination in the germline

Regulation of the choice between spermatogenesis and oogenesis differs in two ways from regulation of the choice between male and female cell fates in somatic tissues (compare Fig. 2 with Fig. 3). First, *tra-1* is not the terminal regulator of germline fate, because animals lacking *tra-1* activity can make both sperm and oocytes (Hodgkin 1987, Schedl et al 1989). Instead, the genes *fog-1*, *fog-3* and *gld-1* are likely to be terminal regulators for the germline (see below, Fig. 3a,b). Since mutations in *tra-1* do influence germ cell fate, *tra-1* might act in the germline to modulate transcription of other sex determination genes.

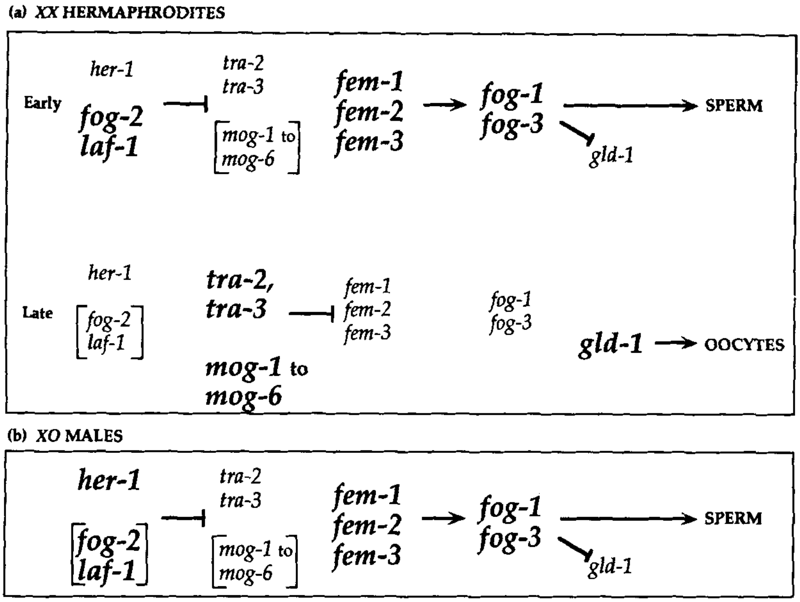

FIG. 3. Sex determination in the germline. These diagrams represent the state of the sex determination genes in the germline of (a) XX hermaphrodites and (b) XO males. Active genes are shown in bold type and inactive genes in small type. Arrows indicate positive regulation and blunt lines indicate negative regulation. Genetic analysis has not revealed if the genes *laf-1*, *fog-2* and *mog-1* to *mog-6* are active outside the times at which they are required. These genes are enclosed in brackets at those times when their activity is unknown. See text for further explanation of these regulatory pathways.

Second, the production of sperm and then oocytes in hermaphrodites requires regulation of the sex determination pathway to allow transient male development in an otherwise female body. This regulation involves inhibition of *tra-2* activity to allow spermatogenesis (Doniach 1986, Schedl & Kimble 1988) and inhibition of *fem-3* activity to permit the change from spermatogenesis to oogenesis (Barton et al 1987).

The suppression of *tra-2* to permit hermaphrodite spermatogenesis has two components (Fig 3b). First, the translation of *tra-2* mRNA is regulated by two direct repeats found in its 3′ untranslated region (Goodwin et al 1993). If these sequences are disrupted, *tra-2* transcripts become more heavily loaded with ribosomes, implying that extra *tra-2* protein is synthesized. The increased *tra-2* activity causes production of oocytes rather than sperm in young hermaphrodites, transforming them into true females. One candidate for the translational repressor of *tra-2* is defined by the *laf-1* gene (E. Goodwin, personal communication). Because deletion of the *tra-2* direct repeats also has minor effects on male sex determination, this translational regulation is not specific to the hermaphrodite germline. Second, *tra-2* activity is inhibited by the *fog-2* gene (Schedl & Kimble 1988). Since *fog-2* is required only in the hermaphrodite germline, it is unlikely to regulate *tra-2* translation by interacting with the direct repeats of the 3′ untranslated region. Instead, *fog-2* might regulate activity of the *tra-2* protein.

The suppression of *fem-3* to allow the change from spermatogenesis to oogenesis also appears to involve translational control. Several mutations in *fem-3* disrupt a regulatory element in the 3′ untranslated region of the transcript; these mutations increase *fem-3* activity and cause all differentiating germ cells to become sperm, even in hermaphrodites (Barton et al 1987, Ahringer & Kimble 1991). Several genes exist that might encode a translational repressor of *fem-3*: these are called *mog-1* to *mog-6* (Graham & Kimble 1993, P. Graham, personal communication). Mutations in each *mog* gene cause germ cells that would normally differentiate as oocytes to become sperm.

In hermaphrodites, the relative activity of the *tra-2* and *fem-3* genes is a crucial factor in determining what sexual fate germ cells adopt. Too much *tra-2* activity causes all germ cells to differentiate as oocytes, whereas too much *fem-3* activity causes them all to form sperm. Furthermore, an increase in *tra-2* activity can be compensated for by an increase in *fem-3* activity, and vice versa (Schedl & Kimble 1988).

Terminal regulators of germ cell fate

Which genes act at the end of the regulatory pathways that control germ cell fate to initiate spermatogenesis, oogenesis or mitosis? To date, there are five genes that might act as terminal regulators of spermatogenesis and one gene that might specify oogenesis. By contrast, no gene that is essential for germ cells to enter mitosis has been identified.

The initiation of spermatogenesis requires five genes: the three *fem* genes, which also act in somatic sex determination and two genes that function only in the germline: *fog-1* (Barton & Kimble 1990) and *fog-3* (R. E. Ellis & J. Kimble, unpublished results). These five genes are absolutely required, in both sexes, to specify that germ cells differentiate as sperm. If any one of them is inactivated by mutation, all differentiating germ cells form oocytes. Because *fog-1* and *fog-3* act only on the germline, whereas the three *fem* genes are needed in all tissues, we speculate that *fog-1* and *fog-3* are the terminal regulators for spermatogenesis (as indicated in Figs. 3 and 4). However, any of the *fem* genes might instead play this role, or all five genes might encode components of a single complex that specifies spermatogenesis.

What is the terminal regulator for oogenesis? The best candidate is the gene *gld-1* (R. Francis, M. K. Barton, J. Kimble & T. Schedl, unpublished results). Animals lacking *gld-1* activity are unable to form oocytes under any known conditions. Instead, germ cells that would normally differentiate as oocytes enter meiosis, but subsequently return to mitosis and proliferate, forming a tumorous mass of cells (T. Schedl, personal communication). This defect is not caused by a general failure of meiosis, because males that lack *gld-1* make sperm normally. Thus *gld-1* is required for the initiation of oogenesis. One counter-intuitive result is that hermaphrodites with only a single active copy of *gld-1* sometimes make more oocytes than the wild-type, rather than less as expected (T. Schedl, personal communication). Perhaps there are complex regulatory controls of *gld-1* activity and perturbing *gld-1* dosage causes over-compensation by these controls.

A model for the control of germ cell fate

How is germ cell fate controlled? The simplest model involves two binary switches—one that specifies mitosis or meiosis and one that determines sex. This model accommodates what is known about the *glp-1*, *fog-1* and *fog-3* genes: in the absence of *glp-1*, mitotic germ cells enter meiosis and in the absence of *fog-1* or *fog-3*, germ cells differentiate as oocytes rather than as sperm. However, the *gld-1* gene does not fit neatly into this picture. In *gld-1* loss-of-function (lf) mutants, germ cells that would normally differentiate as oocytes enter mitosis. Therefore, the *gld-1* gene appears to link the decisions controlling sexual identity and mitosis.

Figure 4 presents our working model for how the decision between mitosis and meiosis is linked to the decision between spermatogenesis and oogenesis. We propose that germ cell fate is controlled by interactions among the terminal regulators of spermatogenesis, oogenesis and mitosis. In Fig. 4, the terminal regulators are placed at the corners of a triangle and their interactions are represented by arrows on the sides of that triangle. Central to this model is the ability of each terminal regulator to specify one fate while inhibiting at least one of the other fates. These cross-regulatory interactions prevent the activation of two fates at once.

The genetic interactions proposed in Fig. 4 are based on several lines of evidence. First, hermaphrodites or males lacking either *fog-1* or *fog-3* make oocytes, a process that depends on *gld-1*. We therefore propose that *fog-1* and *fog-3* inhibit *gld-1* to allow spermatogenesis. Second, the absence of *gld-1* does not drive germ cells into spermatogenesis. Because spermatogenesis is not activated by the loss of *gld-1*, there is no evidence that *gld-1* inhibits *fog-1* or *fog-3*. Instead, the activities of *fog-1* and *fog-3* are controlled by the sex determination pathway. Third, in animals lacking both *gld-1* activity and *fog* activity, germ cells do not become stuck in early meiosis, but instead return to mitosis. On the basis of this result, we postulate that both *gld-1* and *fog-1* and *fog-3* inhibit genes that promote mitosis. Fourth, germ cells that have returned to mitosis remain in mitosis and do not form sperm or oocytes. Thus, the terminal regulator for mitosis might inhibit *gld-1*, *fog-1* and *fog-3*. Finally, we propose

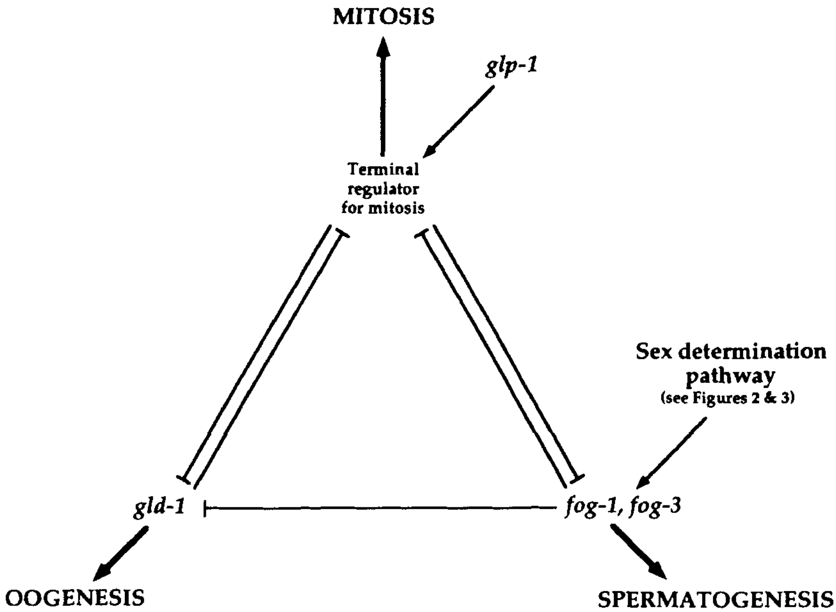

FIG. 4. A model of the control of germ cell fate. This model shows how the terminal regulators for spermatogenesis, oogenesis and mitosis might interact to determine cell fate. We propose that these interactions occur in germ cells in early meiosis, which retain full developmental potential. For simplicity, *fog-1* and *fog-3* are shown as the only terminal regulators for spermatogenesis, although it is possible that the *fem* genes act with them. Arrows indicate positive regulation and blunt lines indicate negative regulation.

that the interactions shown in Fig. 4 occur when a germ cell first enters meiosis in response to declining *glp-1* activity, because at this point it can still adopt any of the three normal germ cell fates: spermatogenesis, oogenesis or mitosis.

Two lines of evidence from double mutants support particular aspects of our model. First is the male phenotype of *gld-1(lf) fog-1* and *gld-1(lf) fog-3* double mutants (T. Schedl, personal communication, R. E. Ellis, unpublished results). Differentiating germ cells form sperm in wild-type males and in *gld-1(lf)* males, whereas they make oocytes in *fog-1* or *fog-3* males. By contrast, in *gld-1 fog-1* or *gld-1 fog-3* double mutants, differentiating germ cells make neither sperm nor oocytes, but instead return to mitosis and proliferate. These results support the assertion that *fog-1* and *fog-3*, like *gld-1*, can prevent germ cells from returning to mitosis (as indicated in Fig. 4). The second line of evidence involves rare mutations in *gld-1*, called *gld-1(Mog)* alleles, that cause hermaphrodites to make sperm rather than oocytes (T. Schedl & J. Kimble, unpublished results). As described above, germ cells in *gld-1(lf) fog-3* males return to mitosis and proliferate. By contrast, germ cells in *gld-1(Mog) fog-3* males not only fail to make sperm or oocytes, but also fail to return to mitosis (R. E. Ellis, unpublished results). We infer that the product of this *gld-1*(*Mog*) allele has lost its ability to specify oogenesis, but retains the ability to repress mitosis.

How is the spatial pattern of germ cell fates achieved in the adult testis and ovotestes? The distal tip cell is essential for this spatial regulation in both males and hermaphrodites. After ablation of the distal tip cells during early larval development, all germ cells enter meiosis and differentiate as sperm (Kimble & White 1981). We speculate that the distal tip cell is crucial for stimulating the activity of *glp-1*, and that *glp-1* promotes mitosis in the distal region of the germline at the expense of both spermatogenesis and oogenesis. According to our model, the terminal regulators of spermatogenesis and oogenesis are both inhibited in this region. As germ cells move away from the distal tip cell, *glp-1* activity declines and the germ cells escape its influence. This escape allows entry into meiosis. In both males and hermaphrodites, the first cells that enter meiosis commit to spermatogenesis. We propose that *tra-2* is inhibited at this time during germline development, allowing *fog-1* and *fog-3* to promote spermatogenesis and inhibit the genes required for oogenesis or mitosis. In males, this is the end of the story: spermatogenesis continues throughout adulthood. In hermaphrodites, *fog-1* or *fog-3* eventually become inactive, perhaps by repression of *fem-3*. This permits *gld-1* to direct oogenesis as later germ cells enter meiosis.

In summary, genetic and molecular analyses have revealed two signal transduction pathways that regulate germ cell fate, one controlling the decision to remain in mitosis and the other the sexual identity of germ cells. We have proposed that the terminal regulators of these pathways interact in a tripartite decision, so that germ cells can decide between three cell fates: spermatogenesis, oogenesis or mitosis. Furthermore, we have proposed that the outcome of these interactions changes during development, which accounts for the spatial arrangement of cell fates in the germline.

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References

Ahringer J, Kimble J 1991 Control of the sperm–oocyte switch in *Caenorhabditis elegans* hermaphrodites by the *fem-3* 3′ untranslated region. Nature 349:346–348

Austin J, Kimble J 1987 *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. Cell 51:589–599

Austin J, Kimble J 1989 Transcript analysis of *glp-1* and *lin-12*, homologous genes required for cell interactions during development of C. elegans. Cell 58: 565–571

Barton MK, Kimble J 1990 *fog-1*, a regulatory gene required for specification of spermatogenesis in the germ line of *Caenorhabditis elegans*. Genetics 125: 29–39

Barton MK, Schedl TB, Kimble J 1987 Gain-of-function mutations of *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. Genetics 115:107–119

Doniach T 1986 Activity of the sex-determining gene *tra-2* is modulated to allow spermatogenesis in the *C. elegans* hermaphrodite. Genetics 114:53–76

Goodwin EB, Okkema PG, Evans TC, Kimble J 1993 Translational regulation of *tra-2* by its 3′UTR controls sexual identity in C. elegans. Cell 75:329–339

Graham PL, Kimble J 1993 The *mog-1* gene is required for the switch from spermatogenesis to oogenesis in *Caenorhabditis elegans*. Genetics 133:919–931

Hodgkin J 1980 More sex-determination mutants of *Caenorhabditis elegans*. Genetics 96:649–664

Hodgkin J 1986 Sex determination in the nematode *C. elegans*: analysis of *tra-3* suppressors and characterization of *fem* genes. Genetics 114:15–52

Hodgkin J 1987 A genetic analysis of the sex-determining gene, *tra-1*, in the nematode *Caenorhabditis elegans*. Genes & Dev 1:731–745

Hunter CP, Wood WB 1992 Evidence from mosaic analysis of the masculinizing gene *her-1* for cell interactions in *C. elegans* sex determination. Nature 355: 551–555

Kimble JE, White JG 1981 On the control of germ cell development in *Caenorhabditis elegans*. Dev Biol 81:208–219

Kodoyianni V, Maine EM, Kimble J 1992 Molecular basis of loss-of-function mutations in the *glp-1* gene of *Caenorhabditis elegans*. Mol Biol Cell 3:1199–1213

Kuwabara PE, Okkema PG, Kimble J 1992 *tra-2* encodes a membrane protein and may mediate cell communication in the *Caenorhabditis elegans* sex determination pathway. Mol Biol Cell 3:461–473

Lambie EJ, Kimble J 1991 Two homologous regulatory genes, *lin-12* and *glp-1*, have overlapping functions. Development 112:231–240

Madl JE, Hermann RK 1979 Polyploids and sex determination in *Caenorhabditis elegans*. Genetics 93:393–402

Perry MD, Li W, Trent C et al 1993 Molecular characterization of the *her-1* gene suggests a direct role in cell signaling during *Caenorhabditis elegans* sex determination. Genes & Dev 7:216–228

Roehl H, Kimble J 1993 The ankyrin repeat region of GLP-1 is sufficient to direct cell fate in *C. elegans*. Nature 364:632–634

Schedl T, Kimble J 1988 *fog-2*, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics 119:43–61

Schedl T, Graham PL, Barton MK, Kimble J 1989 Analysis of the role of *tra-1* in germline sex determination in the nematode *Caenorhabditis elegans*. Genetics 123: 755–769

Villenueve AM, Meyer BJ 1990 The regulatory hierarchy controlling sex determination and dosage compensation in *Caenorhabditis elegans*. Adv Genet 27:117–188

Yochem J, Greenwald I 1989 *glp-1* and *lin-12*, genes implicated in distinct cell–cell interactions in C. elegans, encode similar transmembrane proteins. Cell 58: 553–563

Zarkower D, Hodgkin J 1992 Molecular analysis of the C. elegans sex determination gene *tra-1*. Cell 70:237–249

DISCUSSION

St Johnston: In several other systems, mRNAs that are translationally repressed have very short poly(A) tails which then become longer at the time of translational activation. Do you see any such changes in poly(A) tail length that correlate with the translational regulation of the mRNAs that you have described?

Kimble: We've looked at the polyadenylation of both *fem-3* and *tra-2* mRNAs. We see an increase of 50–100 adenylate residues in the translationally active mRNAs. We have not yet looked at *glp-1*.

Nöthiger: You showed that *glp-1* is *necessary* for maintaining the mitotic activity near the distal tip cell. Can you express *glp-1* farther down in the gonad? Is *glp-1* also *sufficient* to maintain mitotic activity throughout the gonad if you express it ectopically?

Kimble: That experiment has not been done. We have looked at various mutants that have ectopic germline mitoses. These include the *gld-1* tumorous germline and the *lin-12* loss-of-function mutants that have an over-proliferative germline. What we find is that GLP-1 protein is present in the ectopic mitotic germ cells. Expression of *glp-1* in the germline is limited by the lack of a good inducible germline promoter. We have expressed *glp-1* in the soma and analysed its function there, but we haven't been able to express it in the germline. The way we are trying to get around that problem is to do RNA injections. One of the questions we will ask is whether *glp-1* RNA injected into the meiotic pachytene region can drive those germ cells back into mitosis.

Fritz: At what stage of development of the germ cell does the ligand for GLP-1 inhibit the commitment to meiosis?

Kimble: Our only knowledge of the GLP-1 ligand is based on analogy with *Drosophila*. A gene called *Delta* has been proposed to encode a ligand for Notch (Fehon et al 1990). We have recently cloned a gene, *lag-2*, that has sequence similarity to *Delta*. Therefore, LAG-2 may be the signal for GLP-1. I think this is a very interesting possibility.

Strome: Is there any evidence for a specialized population of germline stem cells that remain in the distal tip region, as opposed to a homogeneous population of dividing germ cells, some of which get pushed far enough from the distal tip to begin meiosis and differentiation?

Kimble: I use the term 'stem cell' in a broad sense to mean a cell that is continuing with mitosis and producing cells that will move out of the region and differentiate.

Mahowald: Perhaps another way of asking the same question is: how quickly after you knock out the distal tip cell do the germ cells go into meiosis?

Kimble: One can take a temperature-sensitive *glp-1* mutant that is wild-type at permissive temperature and shift it to restrictive temperature. After six hours at restrictive temperature, when they do not look like they are in meiotic pachytene, if you shift the temperature down, they are irreversibly committed to going into meiotic pachytene. The best experiment to address that question was done by Henry Roehl in my lab (unpublished results). When he shifted a *glp-1* temperature-sensitive mutant from permissive to restrictive temperature, germ cells were irreversibly committed to meiosis within six hours. When he shifted animals back to permissive temperature within six hours, the germ cells remained mitotic. Therefore, our estimate is that commitment from mitosis to meiosis takes about six hours.

McLaren: Do we know anything about what the transition from mitosis into meiosis involves? The prophase of meiosis is very strange and distinctive, but what about the previous DNA replication and the previous cell division—are they different? Where does the transition lie?

Kimble: The simple answer is that we know nothing. The more complicated, but not much more informative, answer is that we know that there's a mitotic zone that extends along the distal–proximal axis about 20 germ cell diameters down from the distal tip. Meiotic pachytene starts at about 35 diameters down from the distal tip. From 20–30 diameters along the axis, there is a transition zone. We postulate that the three-way decision between mitosis, spermatogenesis and oogenesis is being made in that transition zone. But Tim Schedl has evidence that in *gld-1* mutants, germ cells can enter meiosis and then return into mitosis (R. Francis, M. Barton, J. Kimble & T. Schedl, unpublished results).

Schedl: Null mutations in the *C. elegans* gene *gld-1* result in a tumorous germline phenotype. These mutants are completely penetrant and fully expressed, allowing Ross Francis in my lab (unpublished work) to observe cytologically the tumorous phenotype as it unfolds. Our analysis demonstrates that germ cells in the mutant enter the meiotic pathway at the normal time and position and then progress to pachytene. Interestingly, germ cells fail to progress past pachytene and instead revert to mitotic proliferation, ultimately resulting in a germline tumour. Thus, *gld-1* is necessary for progression through or maintenance of meiotic prophase. The tumorous phenotype is observed in hermaphrodites, while *gld-1* null mutant males are unaffected. We have analysed the dependence of the tumorous phenotype on sexual fate by constructing double mutants with the sex determination genes: the tumorous phenotype is only observed when germ cells are following the female pathway. However, the *gld-1* tumorous germline never exhibits the cytological characteristics of oogenesis. The absence of oogenesis could result from either a failure to specify oocyte fate, or a failure in a very early step in oocyte differentiation. In either case, pachytene germ cells would exit meiotic prophase and begin proliferation.

gld-1 mutations also affect sex determination. Loss-of-function mutations display feminization of the hermaphrodite germline. Gain-of-function alleles exist which either feminize or masculinize the hermaphrodite germline, but which have no effect in entry or progression through meiotic prophase. Could the *gld-1* gene link the decisions of germline sex determination and entry into meiotic prophase? Possibly. However, a major problem with this hypothesis is that *gld-1* null mutants are not defective in entry into the meiotic pathway, but are instead defective in progression through meiotic prophase. One interpretation of our results is that *gld-1* acts in two distinct steps in germ cell development. First, it functions early for the specification of the male fate in the hermaphrodite germline and, second, it functions later for progression through female meiotic prophase and oocyte differentiation.

Fritz: Microsporocytes of *Lilium* (the lily) become irreversibly committed to meiosis near the end of the premeiotic S phase (Stern 1986). In this last S phase before the beginning of meiosis in *Lilium*, less than 100% of the DNA is replicated. Instead, the synthesis of approximately 0.3% of the DNA molecules is delayed until the zygotene and early pachytene stages of meiotic prophase. This delayed synthesis may be primarily associated with repair–replication of DNA associated with processes of genetic recombination; however, specific processes and regions of chromosomal DNA synthesis have also been implicated (Hotta & Stern 1971, Stern & Hotta 1973, Stern 1986). Meiotic cells can be cultured *in vitro*. At what stage does it no longer become possible for the germ cell to switch from meiosis to mitosis without aborting? I have the impression that this occurs in *Lilium* during the leptotene to zygotene transition.

McLaren: Irving [Fritz], you say that the previous S phase is unique in that not all of the DNA is replicated at that time, but replication is completed in zygotene. If that is so, it would seem to me reasonable that if, as in Tim Schedl's system, the cells have got past zygotene into pachytene, they would be able to revert to mitosis, because at least they would have all their DNA replicated. But if in *Lilium* you can revert from leptotene, which is before the zygotene DNA replication, surely there is a problem.

Fritz: I see what you mean: perhaps cells in meiosis from *Lilium* do not behave identically to those from *C. elegans*.

Carpenter: There is only one general statement known to be true about the premeiotic S phase for all meiotic organisms; it takes longer than does S phase for any other cell division in that organism. For most organisms, we simply do not know whether that DNA synthesis is complete or not. There is evidence from a few other organisms that it is complete. In the present context, however, this is irrelevant. Reverting meiosis to mitosis is a standard procedure in *Saccharomyces cerevisiae* (Baker et al 1976). You can take cells partly into meiosis and change the medium; at some point, when you change the medium the cells are committed to the meiotic pathway and they finish meiosis. Before that point, when the medium is changed the cells revert to mitosis. Yeast cells are committed to meiotic levels of recombination before they become committed to the meiotic pathway by this assay; but it is by no means clear that meiotic recombination has in fact occurred by that time, all one knows is that the potential has been induced. The actual physical events could occur after the return to growth medium. Exactly the same is true for completing DNA synthesis. It's not a problem, because cells that have started meiosis would be expected to complete DNA synthesis, should it be incomplete when they go back into the mitotic pathway.

References

Baker BS, Carpenter ATC, Esposito MS, Esposito RE, Sandler S 1976 The genic control of meiosis. Annu Rev Genet 10:53–134

Fehon RG, Kooh PJ, Rebay I et al 1990 Molecular interaction between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in Drosophila. Cell 61:523–534

Hotta Y, Stern H 1971 Analysis of DNA synthesis during meiotic prophase in *Lilium*. J Mol Biol 55:337–355

Stern H 1986 Meiosis: some considerations. J Cell Sci Suppl 4:29–43

Stern H, Hotta Y 1973 Biochemical controls of meiosis. Annu Rev Genet 7:37–66