Controls of Germline Stem Cells, Entry into Meiosis, and the Sperm/Oocyte Decision in *Caenorhabditis elegans*

Judith Kimble and Sarah L. Crittenden

Department of Biochemistry and Howard Hughes Medical Institute, University of Wisconsin, Madison, Wisconsin 53706-1544; email: jekimble@wisc.edu, slcritte@wisc.edu

Annu. Rev. Cell Dev. Biol. 2007. 23:405–33

First published online as a Review in Advance on June 22, 2007

The *Annual Review of Cell and Developmental Biology* is online at http://cellbio.annualreviews.org

This article's doi: 10.1146/annurev.cellbio.23.090506.123326

1081-0706/07/1110-0405$20.00

Key Words

stem cell niche, mitosis/meiosis decision, Notch signaling, PUF proteins, CPEB, regulatory network, cell number, hermaphrodite evolution

Abstract

The *Caenorhabditis elegans* germ line provides an exceptional model for analysis of the molecular controls governing stem cell maintenance, the cell cycle transition from mitosis to meiosis, and the choice of sexual identity—sperm or oocyte. Germline stem cells are maintained in an undifferentiated state within a well-defined niche formed by a single somatic cell, the distal tip cell (DTC). In both sexes, the DTC employs GLP-1/Notch signaling and FBF/PUF RNA-binding proteins to maintain stem cells and promote mitotic divisions, three additional RNA regulators (GLD-1/quaking, GLD-2/poly(A) polymerase, and GLD-3/Bicaudal-C) control entry into meiosis, and FOG-1/CPEB and FOG-3/Tob proteins govern sperm specification. These key regulators are part of a robust regulatory network that controls germ cell proliferation, stem cell maintenance, and sex determination. Parallels with controls in other organisms include the use of PUF proteins for stem cell maintenance and the prominence of mRNA regulation for the control of germline development.

Contents

INTRODUCTION

Germ cells are specialized to produce sperm and eggs and ultimately to create an entirely new organism. Throughout most of their life, germ cells reside in the gonad, an organ dedicated to their organization, development, and sustenance. In many adult gonads, germline stem cells (GSCs) are maintained to replenish stocks of germ cells as their number is depleted by gamete production. Gametogenesis is an ancient process with several common features. Gametes are generated by a modified cell cycle, called meiosis, that is characterized by two consecutive cell divisions to produce haploid nuclei. Gametes also possess sexual identity. Indeed, sperm and oocytes are differentiated cells with sex-specific morphologies, sex-specific behaviors, and sex-specific profiles of gene expression. One unusual aspect of gamete differentiation is its transience—upon fertilization, sperm and egg unite and become transformed into a zygote, revealing the underlying capacity of their genomes to support differentiation into all cell types, a quality called totipotency. Gametogenesis therefore faces the unusual regulatory challenge of needing to generate specific cell types while maintaining the

GSC: germline stem cell

Totipotency: having the potential to generate all differentiated cell types

signature capacity of the germ line for totipotency.

This review focuses on three fundamental aspects of germline development in the nematode *Caenorhabditis elegans*—GSC maintenance, the cell cycle transition between mitosis and meiosis, and the specification of a germ cell as sperm or oocyte. Our focus on these three seemingly disparate developmental processes reflects the emerging realization that their molecular controls are closely linked. Indeed, nearly all the same regulators influence all three processes, albeit in different ways. An unexpected molecular theme is the prominent use of posttranscriptional regulation to control germ cell fates. An understanding of the regulatory network controlling these events in the *C. elegans* germ line impacts several general issues of developmental regulation, which include balancing proliferation and differentiation, cell number determination, and network evolution.

GERMLINE STEM CELLS AND THEIR NICHE

GSCs are defined by their dual capacity for both self-renewal and the generation of a continuous supply of gametes. This broad definition encompasses GSCs that actually self-renew and GSCs in a reservoir that retain potential for self-renewal. In this section, we introduce *C. elegans* GSCs and their stem cell niche; later sections focus on molecular mechanisms that control GSC self-renewal or entry into a program of differentiation.

Organization of the Adult Germ Line

The adult germ line is organized with immature germ cells at the distal end and maturing gametes at the proximal end (**Figure 1*a***). Between those two ends, germ cells mature along a distal-proximal axis. The "mitotic region" resides at the distal end, where virtually all mitotically dividing germ cells are housed (**Figure 1*b***). Just proximal to the mitotic region is the "transition zone," which includes germ cells in early meiotic prophase (**Figure 1*b***) and in premeiotic S-phase as well as the rare straggling mitotic cell at the distal border. Further proximally, germ cells proceed through later stages of meiotic prophase (pachytene, diplotene, diakinesis) and undergo gametogenesis. This basic organization is typical of both sexes.

The Distal Tip Cell Forms the Germline Stem Cell Niche

The somatic distal tip cell (DTC) is essential for germline mitotic divisions (Kimble & White 1981). After laser ablation of the DTC, germ cells leave the mitotic cell cycle, enter meiosis, and undergo gametogenesis. Moreover, DTC relocation leads to a corresponding change in position of the mitotic region, and DTC duplication creates two pools of mitotically dividing germ cells (Kimble & White 1981, Kipreos et al. 2000, Kidd et al. 2005, Lam et al. 2006). The DTC employs GLP-1/Notch signaling to promote continued mitotic divisions: In mutants lacking any of the core elements of the GLP-1/Notch pathway (see below), germ cells leave the mitotic cell cycle, enter meiosis, and undergo gametogenesis (reviewed in Kimble & Simpson 1997). Indeed, the ligand for GLP-1/Notch signaling, called LAG-2, is expressed by the DTC (Henderson et al. 1994, Tax et al. 1994). A simple genetic experiment indicates that the strength of the LAG-2 signal drives the strength of the germ cell response: *lag-2*/+ heterozygotes possess a shorter mitotic region, containing fewer germ cells than does the wild type; by contrast, heterozygotes for other components of the GLP-1 signaling pathway do not similarly affect the size of the mitotic region (M.-H. Lee, S.L. Crittenden & J. Kimble, unpublished data). Therefore, the DTC and LAG-2 are essential components of the microenvironment that supports GSCs, the so-called GSC niche.

The architecture of the DTC provides clues about which germ cells may receive a strong LAG-2 signal. The DTC body resides

Germ line: cells dedicated to the production of gametes

Stem cell: a cell that is capable of generating both additional stem cells and differentiated cells

Niche: a microenvironment that is essential for stem cell maintenance

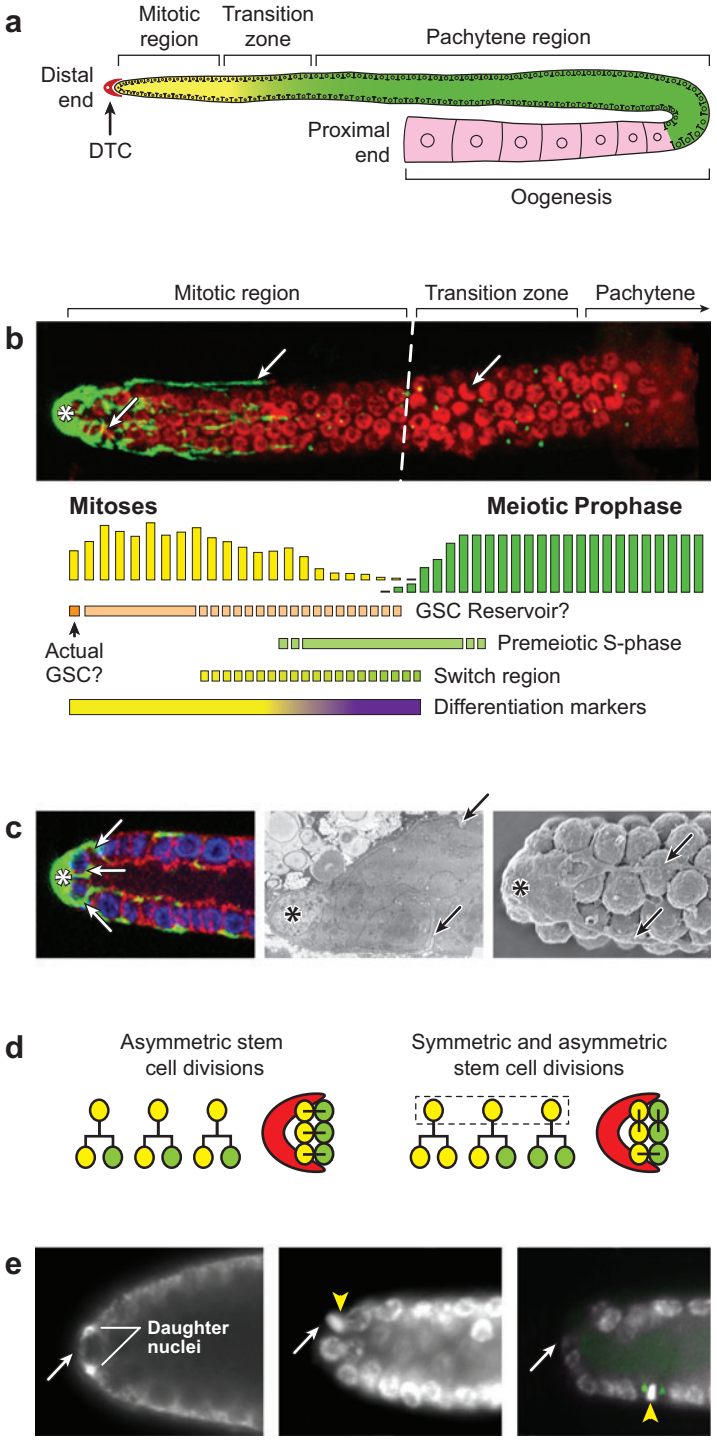
Self-renewal: the process by which a stem cell makes more stem cells

DTC: distal tip cell

GLP-1: one of two *C. elegans* Notch receptors

Notch signaling: a signal transduction pathway that controls proliferation and differentiation throughout the animal kingdom. Core components of this pathway (e.g., GLP-1, LIN-12) have been conserved from worms to humans

LAG-2: Delta homolog and ligand for the GLP-1/Notch receptor



at the distal end of the gonad (**Figure 1*b*,*c***), short DTC processes embrace the distal-most germ cells (**Figure 1*c***, left and middle), and longer DTC processes extend proximally along the surface of the germ line (**Figure1*b*,*c***). A LAG-2::MYC fusion protein, which rescues a *lag-2* null mutant and therefore reports a functional signal, is present throughout the DTC, including both its short and long processes (Crittenden et al. 2006). Germ cells in contact with the DTC body and nearly surrounded by its shorter processes are therefore most likely to receive a stronger LAG-2 signal than are more proximal germ cells. Germ cells in contact with the longer, more superficial DTC processes may also receive a signal, but there is no correlation between the length of these longer processes and the extent of the mitotic region, suggesting that signaling from the longer processes may

Figure 1

The germline stem cells (GSCs) and their niche. (*a*) The adult germ line. Mitotically dividing germ cells (*yellow*) are restricted to the mitotic region at the distal end of the elongate U-shaped gonadal arm. The transition zone is defined by a predominance of germ cells in the early stages of meiotic prophase I (leptotene, zygotene). From the transition zone, germ cells progress into pachytene and gametogenesis most proximally. Adult hermaphrodites make oocytes, as shown here; adult males make sperm (not shown). DTC, distal tip cell. Most germ "cells" are connected by intracellular bridges, even at the distal-most end. However, mitotic germ cell cycles are not synchronized, and neighboring germ cells can have distinct patterns of expression, suggesting at least partial cell autonomy (Jones et al. 1996, Thompson et al. 2005, Crittenden et al. 2006, Maciejowski et al. 2006). By convention, each germ nucleus with its surrounding cytoplasm and membranes is referred to as a cell. (*b*) The mitotic region and transition zone. (*Above*) Micrograph of the distal germ line. Green fluorescent protein (GFP) expression (*green*) from a DTC reporter highlights the DTC body (*asterisk*) as well as long DTC processes that extend along the germline tissue (*arrow*). DNA staining (*red*) highlights germline nuclei. The dashed line denotes the boundary between the mitotic region and transition zone that is defined by the presence of multiple nuclei with crescent-shaped DNA staining typical of early meiotic prophase (*arrow*). (*Below*) Yellow bars indicate the mitotic index, and the green bars indicate the percentage of nuclei in meiotic prophase at individual positions along the distal-proximal axis (see Crittenden et al. 2006 for details). The mitotic region–transition zone boundary is not sharp; rare mitotic nuclei are found at its distal border (overlap between *yellow* and *green bars*). The dark orange bar indicates the proposed position of actual GSCs; the light orange bars, the proposed reservoir of potential GSCs; the green bars, the extent of germ cells in premeiotic S-phase; and the dashed bar graded yellow to green, the extent of the switch region. The bar graded yellow to purple indicates that markers of differentiation (e.g., GLD-1) are expressed in the proximal mitotic region. (*c*) The adult hermaphrodite DTC and its processes. In all images, the DTC cell body is marked by an asterisk, and the processes are indicated by arrows. No other somatic cells are found within the mitotic region. (*Left*) Confocal image of an extruded germline with DTC and its processes highlighted by a transgene driving GFP under a DTC-specific promoter. Blue, germline nuclei; red, germline membranes. The green denotes DTC body and processes. Membranes are visualized through the use of an antibody to the GLP-1 receptor. Short processes surround the distal-most germ cells. (*Middle*) Transmission electron micrograph of the DTC body and its short processes extending around the distal-most germ cells. (*Right*) Scanning electron micrograph of distal gonad; image courtesy of David Greenstein (Hall et al. 1999). The basement membrane was removed to visualize the DTC body and its processes. All other cells in this image are germ cells. (*d*) Stem cell strategies. Stem cells are defined by their capacity to generate both additional stem cells (*yellow*) and differentiated progeny (*green*). The cell forming the stem cell niche is shown in red. (*Left*) individual stem cells can complete their dual task by asymmetric cell divisions. (*Right*) Small groups of stem cells (enclosed in *dashed line*) can complete their dual task by symmetrical and asymmetrical divisions. (*e*) Variable divisions within the GSC niche. In all three images, the distal end is to the left, and the DTC is indicated by an arrow. (*Left*) Symmetrical GSC division within the niche: Daughter nuclei are indicated by lines. (*Middle*) Oblique GSC division within the niche (the metaphase plate is marked by a *yellow arrowhead*). (*Right*) Axial germ cell division outside the niche (the metaphase plate is marked by a *yellow arrowhead*, and *green dots* denote centrosomes).

not be crucial (Hall et al. 1999, Crittenden et al. 2006). Instead, we surmise that the distal-most germ cells are signaled most strongly and that the short processes surrounding these germ cells may anchor them within the niche, because electron microscopic methods have not identified any adhesive junctions between the DTC and germ cells (Hall et al. 1999).

Germline Stem Cells Are Probably Located Next to the Distal Tip Cell Body

Where might GSCs be located within the mitotic region? Although GSCs have not been unambiguously identified in *C. elegans* by the gold standard of lineage tracing, we suggest that the actual GSCs, those that both self-renew and produce differentiated progeny, are located at the distal-most end next to the DTC cell body and surrounded by short DTC processes (**Figure 1*b***). This suggestion is based on two lines of reasoning. First, the distal-most germ cells reside nearest the source of GLP-1/Notch signaling. *Drosophila* GSCs lie immediately adjacent to signaling somatic cells that provide their niche (reviewed in Li & Xie 2005), and a similar scenario seems reasonable for *C. elegans* GSCs. Second, in other systems, stem cells have been defined as cells that proliferate and generate output cells but that have no input from cells other than themselves (Aherne et al. 1977, pp. 45–47 and 65). In the *C. elegans* germ line, this definition fits the distal-most germ cells. As germ cells mature, they move proximally from the mitotic region into meiotic zones (Crittenden et al. 2006; J. Snow & J. Kimble, unpublished data). As germ cells move proximally, they are replaced by more distal germ cells. At the distal-most end, germ cells cannot be replaced by more distal germ cells, and therefore they must replace themselves by self-renewing cell divisions. The actual GSCs—the germ cells located at the distal-most end of the germ line—are probably five or so in number.

The cell cycles of the proposed actual GSCs are similar to those of more proximal mitotic germ cells: The five or so germ cells at the distal-most end incorporate BrdU as actively as do more proximal germ cells, and their cell cycle lengths are indistinguishable from more proximal germ cells (Crittenden et al. 2006). In addition, no label-retaining cells are present anywhere in the mitotic region (Crittenden et al. 2006). Therefore, all germ cells in the mitotic cell cycle are largely equivalent with respect to cell cycle timing. The only detected difference is that germ cells in the distal-most rows have an approximately twofold-lower mitotic index than do those more proximal (Crittenden et al. 2006, Maciejowski et al. 2006). However, because the overall cell cycle times are similar in distal and proximal germ cells within the mitotic region, the lower mitotic index is likely to indicate a shorter M-phase in the distal-most germ cells. The significance of that difference is unclear.

Are all mitotically dividing germ cells equivalent? Preliminary experiments suggest that the mitotic region can be split into a distal half in which germ cells are maintained in a uniform undifferentiated state and a proximal half in which germ cells begin to transition into a differentiated state (O. Cinquin & J. Kimble, unpublished data). Consistent with this idea, molecular markers of germ cell differentiation [e.g., GLD-1 (Jones et al. 1996), HIM-3 (Zetka et al. 1999), FOG-1 (Thompson et al. 2005)] first appear in the proximal half of the mitotic region (**Figure 1*b***). An important question for the future is whether germ cells in these two regions have distinct potential.

Symmetrical and Asymmetrical Germline Stem Cell Divisions

A prevailing idea in recent years has been that stem cells divide asymmetrically to generate one daughter that retains stem cell potential and one daughter that has lost stem cell potential and is destined for differentiation (**Figure 1*d***, left). This idea may be true for some stem cells (e.g., *Drosophila* neuroblasts),

Hermaphrodite: in *C. elegans*, a modified female that produces ~70 primary spermatocytes and then switches to make oocytes continuously. Oocytes can be fertilized by the hermaphrodite's own sperm (self-fertilization) or by sperm from a male (cross-fertilization)

GLD-1: STAR/quaking RNA-binding protein and translational repressor

FOG-1: CPEB-related RNA-binding protein critical for germline proliferation and sperm specification

Actual germline stem cell: a cell that actually accomplishes both self-renewal and the generation of differentiated cells

but it is not true for all stem cells. One alternative possibility is that stem cells normally divide to generate one stem cell and one differentiating daughter but that the differentiating daughter retains stem cell potential (e.g., *Drosophila* GSCs; see below). A second alternative relies on symmetrical stem cell divisions, which are probably a common phenomenon (Morrison & Kimble 2006). By this reasoning, a cluster of stem cells can remain capable of both self-renewal and the generation of differentiated progeny, even if individual stem cells produce two daughters with a stem cell fate or two daughters with differentiated fates (**Figure 1*d***, right). This more plastic stem cell strategy provides an important mechanism for expansion and contraction of the stem cell pool in response to developmental or environmental cues.

In the *C. elegans* germ line, the orientation of germ cell divisions can be perpendicular, parallel, or oblique with respect to the distal-proximal axis (Kimble & White 1981, Crittenden et al. 2006). This is true during larval development, throughout the adult mitotic region, and, in particular, for the proposed GSCs residing adjacent to the DTC (**Figure 1*e***). Some germ cell divisions place both daughters in the same plane next to the main body of the DTC, and others leave one daughter next to the DTC body and locate the other daughter away from the DTC. Indeed, when the niche is depleted of one GSC, the remaining *Drosophila* GSC can divide symmetrically to generate two GSC daughters (Xie & Spradling 2000), and perhaps more importantly, differentiating cystoblast daughters can revert to the GSC fate (Brawley & Matunis 2004, Kai & Spradling 2004). This situation is reminiscent of the *C. elegans* larval germ line, in which both daughters of germ cell divisions retain potential for self-renewal and differentiation (Kimble & White 1981). A unifying idea is that germline stem cells generally produce daughters with equivalent potential and that the daughters' location relative to signals emanating within the niche determines their subsequent fate.

A Germline Stem Cell Reservoir Within the Mitotic Region?

GSC reservoir: cells that are capable of self-renewal but that instead normally differentiate

The discovery that *Drosophila* cystoblasts retain GSC potential suggested the existence of a GSC reservoir—a group of germ cells that retain GSC potential even though they normally have started to differentiate. A similar situation may well exist in the adult *C. elegans* germ line. In *C. elegans*, ablation of the two to three germ cells lying next to the DTC in the larval germ lines did not affect the capacity of the remaining germ cells to generate a functional germ line with both GSCs and differentiating gametes (Kimble & White 1981). This simple experiment showed that germ cells residing next to the DTC were not solely responsible for self-renewing divisions. In the adult germ line, similar laser ablations have not been attempted, owing to the collateral damage expected from ablation of the larger number of GSCs. The idea of a reservoir of germ cells with GSC potential within the mitotic region must therefore await verification. However, given the recent findings in *Drosophila* and the older work in *C. elegans* larvae, it seems likely that adult germ cells retain GSC potential even after they have left the niche. The mitotic region includes more than 200 germ cells, and we speculate that some or perhaps even all of them may provide a GSC reservoir (**Figure 1*b***).

GERMLINE PROLIFERATION, ENTRY INTO MEIOSIS, AND THE SPERM/OOCYTE DECISION

Germline development varies from species to species in details of timing and gamete morphology, but several processes stand out as fundamental to all germline programs: (*a*) commitment of a germ cell lineage during embryogenesis, (*b*) proliferation to expand the germ cell pool, (*c*) a cell cycle transition from mitosis to meiosis, and (*d*) sexual differentiation as sperm or oocyte. It remains a question whether all germ lines maintain a population

of adult stem cells as in the mammalian ovary, but in *C. elegans*, both sexes maintain GSCs in adults. This review focuses on controls of the mitosis/meiosis and sperm/oocyte decisions, which are intertwined with controls of larval proliferation and GSC maintenance. Here, we provide background on the development of the *C. elegans* germ line; later sections address molecular controls of both GSCs and the mitosis/meiosis and sperm/oocyte decisions.

Asymmetric division: the generation of daughters with distinct fates

The *C. elegans* life cycle includes a brief interval of embryogenesis (~12 h), four larval stages (L1–L4) that take a total of ~3 days, and adulthood, which lasts ~10 days. The embryo can develop as either of two sexes: XX hermaphrodites are essentially females that make sperm during larval development and then switch to oogenesis; XO males make sperm continuously. Closely related nematodes exist as male/female strains, and hermaphroditism is considered to be a recent modulation of an essentially female program (discussed below). Two primordial germ cells are generated in both XX and XO embryos, and these cells become incorporated into a four-celled gonadal primordium that appears morphologically similar in the two sexes (Sulston et al. 1983). Early larval gonads become sexually dimorphic after the first division of the two somatic gonadal progenitor cells in mid-L1; subsequently, male gonads develop with a single elongated gonadal arm, whereas hermaphrodite gonads develop with two gonadal arms (Kimble & Hirsh 1979). **Figure 2** diagrams germline proliferation, entry into meiosis, and gametogenesis

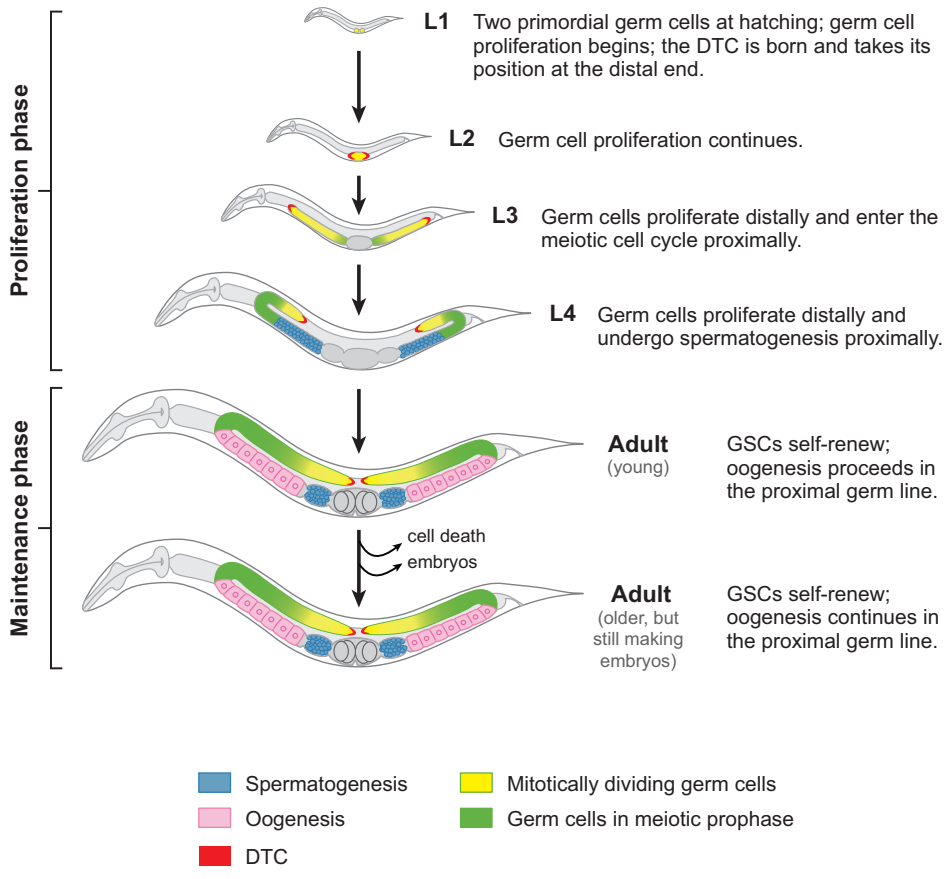


Figure 2

Germline proliferation, entry into meiosis, and gametogenesis. Hermaphrodite germline development takes place in two gonadal arms, which are shown here within the animal as it develops through the larval stages (L1–L4) and into adulthood. Male germline development follows a similar course but occurs only in a single arm and produces only sperm (not shown). Each of the two hermaphrodite gonadal arms possesses a single distal tip cell (DTC); the single male gonadal arm has two DTCs at its distal end (not shown).

in hermaphrodites; a similar sequence occurs in males.

During L1 and L2, primordial germ cells proliferate, expanding the germ cell pool from 2 to ~60. During L3 and L4, the total germ cell number continues to increase until it reaches ~2000 in adult hermaphrodites (~1000 per arm) and ~1000 in adult males. Larval proliferation depends on the DTCs in both sexes but also receives input from other somatic gonadal cells in hermaphrodites. In particular, two AC/VU (anchor cell/ventral uterine) precursor cells express LAG-2, the GLP-1/Notch ligand, during L2 and contribute to robust larval germline proliferation (Wilkinson et al. 1994, Pepper et al. 2003). In addition, the distal sheath cells support the normal extent of germline proliferation (McCarter et al. 1997, Killian & Hubbard 2005).

The adult hermaphrodite germ line is maintained with a steady-state average of ~2000 germ cells (Crittenden et al. 2006). The average length of a germ cell cycle is ~4 h during larval proliferation (Kipreos et al. 1996) and ~16–24 h in the adult maintenance phase (Crittenden et al. 2006). In larvae, germ cell divisions are symmetrical with respect to the capacity of their daughters to self-renew or differentiate, do not follow a fixed lineage, and are not reproducibly oriented (Kimble & Hirsh 1979, Kimble & White 1981). In adults, the orientation of germ cell divisions is also not fixed, and symmetrical divisions have been postulated (Crittenden et al. 2006). The single DTC is the only somatic cell in the adult hermaphrodite mitotic region (two DTCs are present in males). In hermaphrodites, two epithelial sheath cells incompletely enclose germ cells in early meiotic prophase (Hirsh et al. 1976, Hall et al. 1999), whereas in males no other somatic cells are found until the region of gametogenesis. There currently is no evidence that adult sheath cells play any role in controlling the adult mitotic region.

Entry into meiosis first occurs in L3 larvae, when proximal germ cells enter the meiotic cell cycle. As the gonad elongates during L4, germ cells continue to enter meiosis, and in adults, germ cells transition from mitosis into meiosis as they progress from the mitotic region into the transition zone. Indeed, germ cells in premeiotic S-phase extend over a broad switch region that includes the proximal mitotic region and distal transition zone (**Figure 1*b***) (Crittenden et al. 2006).

The timing of entry into meiosis is sex specific (Kimble & White 1981, Austin & Kimble 1987). In males (XO), germ cells enter the pachytene stage of meiotic prophase in mid-L3 and begin overt spermatogenesis in mid-L4; male germ cells continue to enter meiosis and differentiate as sperm throughout adulthood. In hermaphrodites (XX), germ cells are first seen in the pachytene stage during the late-L3 stage, a few hours later than in males; those first germ cells to enter meiosis become sperm during the L4 stage, whereas germ cells entering meiosis during mid-L4 or later differentiate into oocytes in adults. The temporal program of hermaphrodite germ cell development can be uncoupled from specific larval stages. Laser ablation of one of the two primordial germ cells in L1 larvae reduced germ cell number, which in turn delayed meiotic entry, presumably because germ cells must attain a certain distance from the DTC before entering meiosis (Kimble & White 1981). Importantly, sperm maturation and the switch into oogenesis were similarly delayed, even though somatic lineages and larval progression were normal. Hermaphrodite sperm number was essentially normal in these experiments. Therefore, the initial entry into meiosis, determination of sperm number, and sperm/oocyte switch do not appear to be rigidly coupled to any stage-specific process.

Specification of a germ cell as sperm or oocyte is a continuous process: XO male germ lines making sperm can be induced to make oocytes instead by RNA interference (RNAi) to *fog-3*, one of the key sperm/oocyte regulators (Chen et al. 2000). Moreover, XX

FOG-3: a Tob/Btg-related protein critical for germline proliferation and sperm specification

animals that have switched into oogenesis can be switched back into spermatogenesis, either by shifting a *fem-3* gain-of-function (gf) mutant to restrictive temperature or by RNAi directed against *daz-1* into a *fem-3*(*gf*) mutant maintained at permissive temperature (Barton et al. 1987, Otori et al. 2006). Therefore, germ cells must be continuously instructed to differentiate as sperm or oocyte. Where within the germ line does specification as sperm or oocyte occur? The answer is not known, but several lines of evidence suggest that germline sex determination may occur in the distal gonad. To wit, sex-specific gene expression [e.g., of GLD-1 (Jones et al. 1996), TRA-1 (Segal et al. 2001), and FOG-1 (Thompson et al. 2005)] begins in the proximal mitotic region and extends into the transition zone (**Figure 1*b***). The sexual dimorphism within the mitotic region may be coupled to the specification of germ cells as sperm or oocyte, consistent with the idea that regulation of entry into meiosis is linked to regulation of the sperm/oocyte decision (see below). Indeed, the switch from production of one gamete to the other in the proximal germ line takes 36–48 h after the manipulation of sperm/oocyte regulators (shifting a temperature-sensitive mutant or RNAi; see above). That rather lengthy interval corresponds roughly to the time it takes germ cells to move proximally from the mitotic region to the region where they begin overt differentiation (Crittenden et al. 2006).

CONTROL OF DISTAL TIP CELL FATE

The DTC is a major regulator of germ cell fate, and the molecular regulators of the DTC fate must therefore have profound consequences for germ cell development. This section summarizes the cellular origins of the DTC and the molecular regulators that control its fate.

The DTC arises during early larval development from an asymmetric cell division of the somatic gonadal progenitor cell (**Figure 3*a***) (Kimble & Hirsh 1979). After this division, one daughter acquires DTC potential. In males, that daughter differentiates as a DTC, but in hermaphrodites, it divides asymmetrically once more to generate the DTC.

A divergent Wnt signaling pathway, the Wnt/MAPK pathway (**Figure 3*b*,*c***), is critical for specification of the DTC fate. DTCs are not made in animals depleted of frizzled receptors, dishevelled proteins, or either of two terminal transcription factors, POP-1/TCF and SYS-1/β-catenin (**Figure 3*b***) (Siegfried & Kimble 2002, Siegfried et al. 2004, Kidd et al. 2005, Phillips et al. 2007). Furthermore, overexpression of SYS-1/β-catenin produces extra DTCs (Kidd et al. 2005). Wnt signaling has been implicated in stem cell control in other systems (e.g., Reya et al. 2003, Reya & Clevers 2005), and a key question is whether Wnt signaling controls the stem cells themselves or the regulatory cells that define the niche.

A nuclear hormone receptor, called NHR-25, also controls the DTC fate (Asahina et al. 2006). The asymmetric cell division that normally generates one DTC is rendered symmetrical in *nhr-25* mutants, such that both daughters acquire DTC potential, and extra DTCs are made. Importantly, depletion of *nhr-25* can suppress both *pop-1* and *sys-1* mutants, suggesting that NHR-25 acts antagonistically to POP-1/TCF and SYS-1/β-catenin. The current model is that the DTC fate is controlled by a balance between transcriptional controls by POP-1 and SYS-1 on the one hand and by NHR-25 on the other hand.

A direct target of Wnt/MAPK signaling controls DTC potential. Specifically, *ceh-22b* is transcriptionally activated by POP-1/TCF and SYS-1/β-catenin in cells destined to become DTCs (Lam et al. 2006). The *ceh-22b* gene encodes a conserved homeodomain transcription factor, CEH-22/Nkx2.5/tinman, that was originally identified as a regulator of embryonic cell fates (Okkema et al. 1997). CEH-22b depletion leads to DTC loss

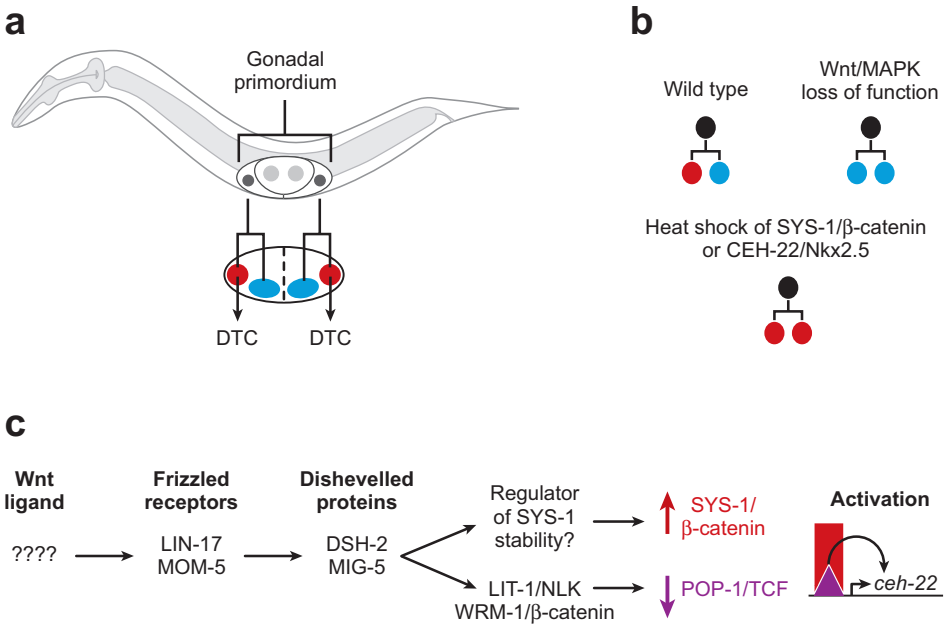


Figure 3

Specification of distal tip cell (DTC) fate. (*a*) DTCs arise by asymmetric cell division of somatic gonadal progenitor cells. The gonadal primordium is composed of four cells: two somatic gonadal progenitor cells (SGPs) (*dark gray circles*) and two primordial germ cells (*light gray circles*). Both SGPs divide to generate one daughter with DTC potential (*red*) and one daughter without DTC potential (*blue*). (*b*) The Wnt/mitogen-activated protein kinase (MAPK) pathway controls DTC potential. Wild-type SGPs divide asymmetrically, mutant SGPs lacking components of the Wnt/MAPK pathway divide symmetrically to generate two daughters without DTC potential, and transgenic SGPs overexpressing either SYS-1/β-catenin or CEH-22/Nkx2.5 generate multiple DTCs. (*c*) The Wnt/MAPK pathway controls transcription of *ceh-22* to specify DTC fate. No Wnt ligand is known for DTC control; two frizzled receptors, LIN-17 and MOM-5, and two dishevelled proteins, DSH-2 and MIG-5, control a forked pathway in which SYS-1/β-catenin abundance is increased, probably by control of stability (Phillips et al. 2007), and LIT/Nemo-like kinase (NLK) and WRM-1/β-catenin decrease POP-1/TCF abundance (Siegfried et al. 2004, Eisenmann 2005). POP-1 (*purple triangle*) and SYS-1 (*red rectangle*) work together to activate transcription of the *ceh-22b* promoter, which drives expression of the CEH-22 homeodomain transcription factor, a key regulator of the DTC fate (Lam et al. 2006).

and a consequent loss of GSCs; conversely, CEH-22b overexpression generates extra DTCs and extra GSC pools (**Figure 3*b***) (Lam et al. 2006). An additional player in DTC fate specification is the E/daughterless ortholog HLH-2 (Karp & Greenwald 2004). The *hlh-2* promoter possesses potential binding sites for both CEH-22 and POP-1/TCF (M. Chesney & J. Kimble, unpublished data). Therefore, the Wnt/MAPK pathway may control two transcription factors that contribute to the specification of DTC fate.

REGULATORS OF GERMLINE STEM CELL MAINTENANCE AND THE MITOSIS/MEIOSIS DECISION

This review focuses on regulators of germline mitotic divisions that promote the mitotic cell cycle at the expense of entry into meiosis. Germ cell divisions controlled in this manner include proliferative divisions that expand the germ cell population during larval development and stem cell divisions more broadly. Germ cell divisions exempt from this control

include the first few primordial germ cell divisions, including that of P4 in embryos and the first one or two divisions of Z2 and Z3 in L1s. In addition, continued mitotic divisions in the germ line rely on controls of germ cell survival, which are beyond the scope of this review.

GLP-1/Notch Signaling

Proliferative germ cell divisions during larval development and GSC maintenance divisions in adults are controlled by the GLP-1/Notch signaling pathway (reviewed in Kimble & Simpson 1997). The *glp-1* gene encodes a Notch-related receptor that is expressed in the germ line and that transduces the DTC signal to promote mitotic divisions at the expense of entry into meiosis (Austin & Kimble 1989, Yochem & Greenwald 1989, Crittenden et al. 1994). In *glp-1* null mutants, the two primordial germ cells present at hatching divide once or twice before entry into meiosis and differentiation. At virtually any stage of the life cycle, when *glp-1* temperature-sensitive mutants are shifted to a restrictive temperature, germ cells leave the mitotic cell cycle and enter meiosis. In contrast to *glp-1* loss-of-function mutants, a *glp-1* gain-of-function mutant is unregulated for GLP-1 signaling, which results in a germline tumor with all germ cells in the mitotic cell cycle (Berry et al. 1997). Therefore, GLP-1/Notch signaling is both necessary and sufficient for germline mitotic divisions at the expense of differentiation.

The restriction of active GLP-1/Notch signaling to the distal germ line is achieved by localized expression of both the signaling ligand, LAG-2/Delta, and the GLP-1/Notch receptor. Specifically, LAG-2 expression is restricted to DTCs in the adult gonad (Henderson et al. 1994), and the GLP-1 protein is most abundant in the mitotic region (**Figure 1*b***) (Crittenden et al. 1994). How LAG-2 expression is governed in DTCs is not understood, but progress has been made with the mechanism confining GLP-1 to the mitotic region. *glp-1* mRNA is uniformly distributed throughout the germ line, but expression of the GLP-1 protein tapers off quickly in the transition zone, suggesting translational control (Crittenden et al. 1994). Indeed, the GLD-1 translational repressor (see below) represses *glp-1* mRNA translation and restricts GLP-1 protein expression to the mitotic region (Marin & Evans 2003).

What about target genes that act downstream of GLP-1/Notch signaling to promote mitotic germline divisions? Bioinformatics has revealed many candidates (Yoo et al. 2004; A. Kershner & J. Kimble, unpublished data), but only two direct target genes have been validated to date: *fbf-2* (Lamont et al. 2004) and *lip-1* (Lee et al. 2006). The *lip-1* gene had been previously identified as a target of LIN-12/Notch signaling in the vulva (Berset et al. 2001). Both *fbf-2* and *lip-1* promoters contain LAG-1-binding sites. The *fbf-2* mRNA is restricted to the mitotic region, and normal levels of FBF-2 protein expression are dependent on GLP-1 signaling. The *lip-1* mRNA is also found in the mitotic region but only in *fbf* mutants: *lip-1* is a target of FBF repression as well as a target of GLP-1 activation. This unexpected dual control eliminates *lip-1* expression in the distalmost germ cells in wild-type germ lines but ensures *lip-1* expression in the proximal mitotic region. The coupling of GLP-1/Notch signaling to FBF repression therefore provides a mechanism for patterning gene expression at a distance from the source of signaling. Other GLP-1/Notch targets must also exist (see below), but the identification and analysis of their roles in GSC maintenance or later steps of germline development are just starting.

RNA Regulators

Within the germ line, a battery of RNA regulators controls GSC maintenance and the mitosis/meiosis decision. The FBF and GLD proteins constitute the heart of a regulatory switch that governs the cell cycle transition

from mitosis to meiosis (**Figure 4*a*,*b***). In addition to providing insights into control of the mitosis/meiosis decision, the analysis of these key regulators has begun to distinguish between controls of GSC maintenance and larval germline proliferation.

FBF-1 and FBF-2. FBF-1 and FBF-2 are PUF (for Pumilio and FBF) RNA-binding proteins that are essential for GSC maintenance (Zhang et al. 1997, Crittenden et al. 2002). These two nearly identical FBF proteins are largely redundant: *fbf-1* and *fbf-2* single mutants are both self-fertile with germ lines organized as in wild type (Crittenden et al. 2002, Lamont et al. 2004). By contrast, in *fbf-1 fbf-2* double mutants, germ cells divide normally until the L4 stage but then leave the mitotic cell cycle, enter meiosis, and undergo spermatogenesis. Therefore, FBF, a collective term for FBF-1 and FBF-2, is not essential for germline mitoses per se but instead governs GSC maintenance. Fly Pumilio has a similar role in GSC maintenance (Lin & Spradling 1997, Forbes & Lehmann 1998), and murine Pumilio 2 (Pum2) has been implicated in GSC maintenance (Moore et al. 2003, Xu et al. 2007). Therefore, understanding how FBF controls GSC maintenance in *C. elegans* is likely to have important implications for other stem cells.

FBF controls germline fates by the posttranscriptional repression of numerous target mRNAs (**Figure 4*d*,*g***) (Wickens et al. 2002). These target mRNAs possess FBF-binding elements (FBEs) in their 3′UTRS (3′ untranslated regions) and generate more protein than normal in germ lines with no or reduced FBF (Zhang et al. 1997, Crittenden et al. 2002, Eckmann et al. 2004, Lamont et al. 2004, Thompson et al. 2005, Lee et al. 2006). To promote mitosis, FBF represses the expression of two critical regulators of entry into the meiotic cell cycle, *gld-1* and *gld-3* (see below) (**Figure 4*b***). Yeast and human PUF proteins repress target mRNAs, at least in part, by recruitment of the deadenylation machinery (Goldstrohm et al. 2006), and preliminary evidence suggests a similar mechanism for FBF (A. Goldstrohm & M. Wickens, unpublished data).

FBF-1 and FBF-2 themselves are controlled by at least four different mechanisms (**Figure 4*g***). First, *fbf-1* and *fbf-2* mRNAs are subject to FBF repression, an autoregulation that keeps FBF abundance in check (Lamont et al. 2004). Second, *fbf-1* and *fbf-2* mRNAs are activated by DAZ-1, a positive regulation that promotes FBF activity (Otori et al. 2006). Third, *fbf-2* is a direct target of GLP-1/Notch signaling (Lamont et al. 2004). [One might have thought that both *fbf* genes would be GLP-1/Notch targets, but *fbf-1* lacks consensus LAG-1-binding sites, and its expression is not sensitive to signaling changes. Upon close analysis, Lamont et al. (2004) found that *fbf-1* and *fbf-2* have diverged in their specific biological roles within the mitotic region, an exemplary case of subfunctionalization.] Fourth, FBF protein activity is antagonized by GLD-3, a homolog of Bicaudal-C that binds FBF (Eckmann et al. 2002). In addition, GLD-1 represses expression of both FBF-1 and FBF-2 proteins (S.L. Crittenden, L.B. Lamont & J. Kimble, unpublished data), although it is not known whether that repression is direct (Lee & Schedl 2001, Ryder et al. 2004).

GLD-1, GLD-2, GLD-3, and NOS-3. Three GLD proteins and NOS-3 are key regulators of entry into meiosis (Kadyk & Kimble 1998, Eckmann et al. 2004, Hansen et al. 2004b). All four proteins reside in the cytoplasm and have been implicated in posttranscriptional regulation. They control meiotic entry via a two-pronged regulatory pathway, with GLD-1 and NOS-3 in one branch and GLD-2 and GLD-3 in the other (**Figure 4*b*,*g***). Entry into meiosis is blocked nearly completely in double mutants that delete one gene in each of these branches, and that block is found in both hermaphrodite and male germ lines. A third, more minor branch has been predicted but remains uncharacterized (Hansen et al. 2004a, Hansen & Schedl 2006).

FBF-1 and FBF-2 (collectively called FBF): PUF RNA-binding proteins that control GSC maintenance and germline sex determination

PUF proteins: conserved family of RNA-binding proteins that control gene expression by regulating mRNA translation or stability

GLD-3: Bicaudal-C homolog that antagonizes FBF and enhances GLD-2

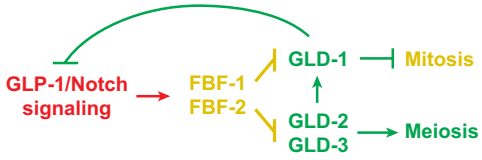
GLD-2: cytoplasmic poly(A) polymerase and translational activator

NOS-3: RNA regulator related to *Drosophila* Nanos that can act with PUF proteins

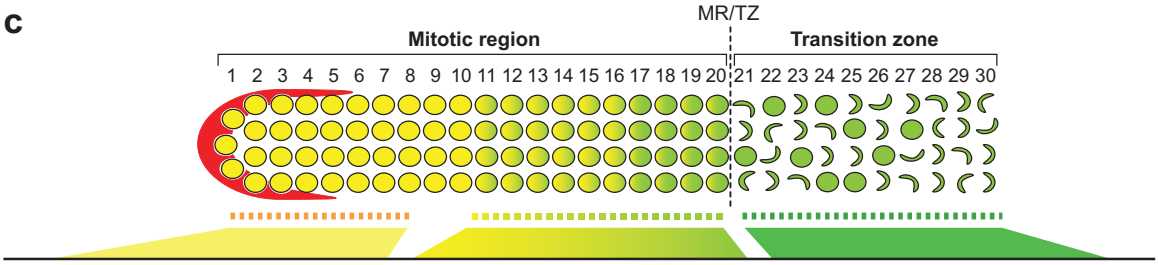
a

Primary regulators	Vertebrate homolog	Molecular identity
FBF-1	Pumilio	PUF RNA-binding protein
FBF-2	Pumilio	PUF RNA-binding protein
GLD-1	Quaking	STAR/SGS RNA-binding protein
GLD-2	X-GLD-2	Poly(A) polymerase

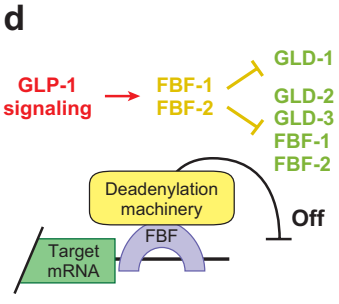
b



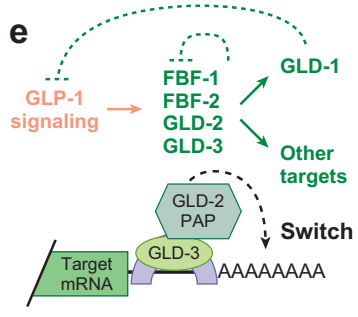
c



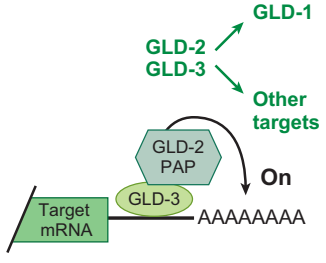
d



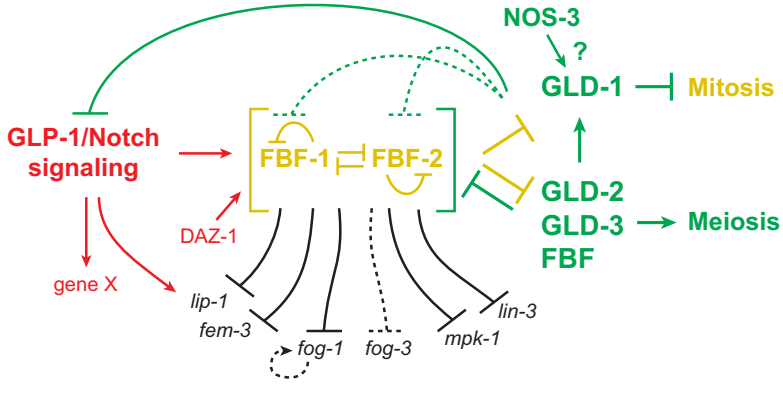
e



f



g



GLD-1 and GLD-2 stand out among the GLD/NOS proteins as the primary regulators of meiotic entry (**Figure 4*b***). GLD-1, a sequence-specific RNA-binding protein of the STAR/quaking family, functions as a translational repressor (Jones & Schedl 1995, Jan et al. 1999, Lee & Schedl 2001, Marin & Evans 2003, Ryder et al. 2004). GLD-2, by contrast, is a cytoplasmic poly(A) polymerase and translational activator (Wang et al. 2002, Suh et al. 2006). Therefore, GLD-1 and GLD-2 are hypothesized to drive germ cells from mitosis into meiosis by simultaneously repressing mitosis-promoting mRNAs and activating meiosis-promoting mRNAs, respectively. Consistent with this idea, GLD-1 represses *glp-1* mRNA directly (Marin & Evans 2003) as well as expression of both FBF-1 and FBF-2 (S.L. Crittenden, L.B. Lamont & J. Kimble, unpublished data). GLD-1 therefore appears to promote meiosis at least in part by negative feedback on regulators that promote mitosis. The only known target of activation by the GLD-2 poly(A) polymerase is *gld-1* mRNA (Suh et al. 2006), which provides a positive feed-forward step to drive germ cells robustly into meiosis. However, GLD-2 must control other target mRNAs as well because in *gld-1* null mutants it can drive germ cells into the meiotic cell cycle.

GLD-3 and NOS-3 appear to control meiotic entry by modulating GLD-2 and GLD-1, respectively. GLD-3, a member of the Bicaudal-C family of RNA-binding proteins, enhances GLD-2 poly(A) polymerase activity (Wang et al. 2002, Eckmann et al. 2004) [and also antagonizes FBF repression

Figure 4

Regulation of germline stem cell (GSC) maintenance and the mitosis/meiosis decision. (*a*) Primary regulators. FBF-1 and FBF-2 are essential for GSC maintenance in both hermaphrodite and male germ lines (Crittenden et al. 2002); GLD-1 and GLD-2 drive germ cells out of the mitotic cell cycle and into meiosis (Kadyk & Kimble 1998). (*b*) Backbone of the network controlling the mitosis/meiosis decision. GLP-1/Notch signaling promotes mitosis, in part by transcriptional activation of the *fbf-2* gene (Lamont et al. 2004). FBF-1 and FBF-2 maintain GSCs, largely by repressing the activity of the *gld-1* and *gld-3* mRNAs (Crittenden et al. 2002, Eckmann et al. 2004). GLD-1 is a translational repressor (Jan et al. 1999, Lee & Schedl 2001), and GLD-2 is a translational activator (Suh et al. 2006). GLD-1 translational repression of mitosis-promoting mRNAs and GLD-2 translational activation of meiosis-promoting mRNAs are proposed to drive germ cells robustly into the meiotic cell cycle. GLD-1 provides negative feedback within the pathway by repressing translation of the *glp-1* mRNA (Marin & Evans 2003), and GLD-2 provides positive feed-forward by activating *gld-1* mRNA (Suh et al. 2006). (*c*) The switch from mitosis to meiosis in the distal germ line. Yellow, germ cells in mitotic cell cycle; green, germ cells in meiotic cell cycle; gradient from yellow to green, germ cells switching from mitosis to meiosis; red, DTC; dotted line, mitotic region (MR)/transition zone (TZ) boundary. (*d*) Control of mitotic divisions in the distal-most germ line. GLP-1/Notch signaling from the distal tip cell to the germ line promotes FBF repression of regulators of meiotic entry. FBF binds specifically to regulatory elements in the 3′UTR (3′ untranslated region) of target mRNAs (reviewed in Wickens et al. 2002). In yeast and humans, PUF proteins recruit the deadenylation machinery to repress mRNAs (Goldstrohm et al. 2006), and FBF appears to work by a similar mechanism (A. Goldstrohm, unpublished data). (*e*) The switch to meiosis. Germ cells start to switch into meiosis as they acquire distance from the source of GLP-1/Notch signaling. Several lines of evidence suggest that FBF facilitates the switch together with GLD-2 and GLD-3, perhaps by recruiting the GLD-2 poly(A) polymerase (PAP) to its target mRNAs. GLD-1 expression begins in the switch region and is proposed to initiate negative feedback on both GLP-1 signaling and FBF (*dashed lines*). (*f*) Control of entry into meiosis and meiotic progression. Germ cells in the transition zone possess little FBF (Crittenden et al. 2002, Lamont et al. 2004), and GLD proteins dominate to drive germ cells through the meiotic cell cycle. (*g*) A more complete view of the network controlling the mitosis/meiosis decision in the adult germ line (see text for a description of most individual elements). Gene X is postulated as a target of GLP-1/Notch signaling because *glp-1(gf); fbf-1 fbf-2* triple-mutant germ lines are tumorous. This means that unregulated GLP-1 can promote mitoses in the absence of FBF and suggests the existence of some other regulator.

CPEB: cytoplasmic polyadenylation element binding protein

(Eckmann et al. 2002) (see below)]. NOS-3, a member of the Nanos family of RNA-binding proteins, affects GLD-1 accumulation by an unknown mechanism (Hansen et al. 2004b); NOS-3 binds specifically to FBF and is likely a translational corepressor of at least some target mRNAs (Kraemer et al. 1999).

Other RNA regulators. Numerous other RNA regulators have been implicated in the control of germline proliferation and the mitosis/meiosis decision. These include FOG-1 (Thompson et al. 2005), ATX-2 (Ciosk et al. 2004, Maine et al. 2004), EGO-1 (Smardon et al. 2000, Vought et al. 2005), PRG-1 and PRG-2 (Cox et al. 1998, Yigit et al. 2006), and six MOG proteins (Graham & Kimble 1993, Graham et al. 1993, Puoti & Kimble 1999, Puoti & Kimble 2000, Belfiore et al. 2004) as well as three more general translation factors: a poly(A) binding protein (PAB-1), an elongation factor 1-α homolog (GLP-3/EFT-3), and the L11 protein of the large ribosomal subunit (RPL-11.1) (Maciejowski et al. 2005). ATX-2 binds PAB-1 and may provide a link between mRNA-specific regulators (e.g., GLD-1) and the general translation machinery (Ciosk et al. 2004). Furthermore, ATX-2 may be involved in the third pathway for entry into meiosis (Maine et al. 2004).

The *fog-1* gene acts redundantly with FBF to direct larval germline proliferation (Thompson et al. 2005). Thus, FOG-1 and FBF can each promote germline proliferation in the absence of the other. The mechanism by which FBF and FOG-1 fulfill this same biological function remains unknown. FOG-1 is a CPEB (cytoplasmic polyadenylation element binding protein) homolog that is localized in the cytoplasm and thought to act post-transcriptionally (Luitjens et al. 2000, Jin et al. 2001, Thompson et al. 2005). One possibility is that FBF and FOG-1 redundantly maintain low levels of *gld-1* mRNA during larval development but that FBF alone fulfills that role in adults. This idea, however, is too simple because FBF is essential for GSC maintenance in male germ lines, in which FOG-1 expression continues. That paradox can be explained by an additional role for FBF in the maintenance of low FOG-1 levels (Thompson et al. 2005).

EGO-1 belongs to a conserved family of RNA-directed RNA polymerases (RdRPs) and affects both germline development and RNAi (Smardon et al. 2000). Other central RNAi proteins, including the *C. elegans* Dicer homolog, DCR-1, affect germline development (Knight & Bass 2001). EGO-1 stimulates germline proliferation in parallel with GLP-1/Notch signaling and functions in the organization of nuclear pores and P granules (Vought et al. 2005). The identification of EGO-1 and RNAi pathway regulators as critical for germline proliferation suggests that small noncoding mRNAs are also involved. Consistent with this idea, the Argonaute/Piwi/Zwille homologs PRG-1 and PRG-2 also affect germline proliferation (Cox et al. 1998, Yigit et al. 2006).

Six MOG proteins are required for robust germline proliferation (Graham & Kimble 1993, Graham et al. 1993). MOG-1, MOG-4, and MOG-5 belong to a subclass of nuclear DEAH-box RNA helicases (Puoti & Kimble 1999, 2000), and MOG-6 is related to cyclophilin (Belfiore et al. 2004). Several *mog; gld-3* double mutants (*mog-1*, *-4*, *-5*, and *-6*) develop germline tumors, but the role of the MOG proteins in the mitosis/meiosis decision remains largely uncharacterized.

LIP-1 and MPK-1

LIP-1 belongs to a family of dual-specificity phosphatases that inhibit MAPK activity in vertebrates, the so-called MKP (MAPK phosphatase) proteins (Camps et al. 1998, Berset et al. 2001); MPK-1 is the major MAPK in *C. elegans* (Lackner & Kim 1998). The *lip-1* gene is a direct target of GLP-1/Notch signaling in the distal-most germ line and is required for robust germline proliferation (**Figure 4*g***) (Lee et al. 2006). In addition to its activation by GLP-1/Notch signaling, the *lip-1* mRNA is repressed by FBF in the distal-most germ line (Lee et al. 2006). LIP-1 protein becomes

detectable in the switch region, consistent with its role in extending germline mitotic divisions. Lowering MPK-1 by RNAi ameliorates the *lip-1* proliferation defect, suggesting that LIP-1, like its vertebrate homologs, promotes proliferation by MAPK inhibition. In vertebrates, MKPs have been implicated in stem cell self-renewal (Burdon et al. 2002) and can be upregulated in tumor cell lines (Vogt et al. 2005). The vertebrate MKPs appear to have a role in proliferation that is remarkably similar to that of LIP-1 in the distal *C. elegans* germ line.

FOG-3: Another RNA Regulator?

Like FOG-1, the FOG-3 protein acts redundantly with FBF to promote larval proliferation (Thompson et al. 2005). Indeed, *fog-3* behaves like *fog-1* in both its biological functions and genetic position as a terminal regulator of germline sexual identity (Barton & Kimble 1990, Ellis & Kimble 1995, Thompson et al. 2005). Unlike FOG-1, which is homologous to the well-characterized RNA regulator CPEB (see above), FOG-3 belongs to a family of proteins with uncertain molecular function, the Tob (Transducer of ErbB-2) family (Chen et al. 2000). Vertebrate Tob proteins interact with Caf1 (CCR4-associated factor 1) (Rouault et al. 1998, Ikematsu et al. 1999), a central component of the deadenylase complex (Tucker et al. 2001), and they interact with poly(A) binding protein, a general regulator of translation (Okochi et al. 2005). These physical interactions with RNA regulatory proteins suggest that FOG-3 may also be an RNA regulator. However, vertebrate Tob proteins also interact with SMAD, a DNA-binding protein (Yoshida et al. 2000, Tzachanis et al. 2001), and β-catenin, a transcriptional coactivator (Xiong et al. 2006). Therefore, Tob family proteins have been implicated in both transcriptional and posttranscriptional controls. Vertebrate Tob family proteins have antiproliferative activity (e.g., Yoshida et al. 2003) and are MAPK substrates (Maekawa et al. 2002). Intriguingly, FOG-3 contains potential MAPK-docking sites (Maekawa et al. 2002), which may tie together MPK-1 effects with FOG-3 regulation.

CONTROLLING THE SWITCH BETWEEN MITOSIS AND MEIOSIS

Once regulators of the mitosis/meiosis decision are identified, one can begin to ask how they control the cell cycle transition from mitosis to meiosis. Germ cells leave the mitotic cell cycle and enter meiosis as they move proximally from the niche into the transition zone (**Figure 4*c***). Adjacent to the DTC, germ cells receive a strong GLP-1/Notch signal and are maintained in the mitotic cell cycle and in an undifferentiated state, at least in part by FBF repression of meiosis-promoting mRNAs (e.g., *gld-1*) (**Figure 4*d***). Once germ cells have left the niche, GLP-1/Notch signaling is postulated to decrease, which in turn leads to the expression of GLD proteins and the transition into meiosis. Importantly, not only is the *gld-1* mRNA a target of FBF repression (Crittenden et al. 2002), but it is also a target of GLD-2 activation (**Figure 4*f***) (Suh et al. 2006). One aspect of the switch therefore involves a shift from FBF repression to GLD-2 activation. However, that change is certainly not the only mechanism: Germ cells enter meiosis in mutants lacking GLD-2, albeit in a delayed fashion. Therefore, a full understanding of the switch mechanism must consider controls exerted by the complete regulatory network.

How might the transformation from FBF repression to GLD-2 activation occur? Details of the mechanism remain unknown, but two familiar regulators appear to be involved. GLD-3 binds physically to both FBF and GLD-2 and is enriched in the switch region; moreover, GLD-3 antagonizes FBF RNA binding and enhances GLD-2 poly(A) polymerase activity (Eckmann et al. 2002, 2004). Therefore, GLD-3 is a superb candidate for driving the transition from

FBF repression to GLD-2 activation. By this model, GLD-3 would incorporate into the FBF–*gld-1* mRNA complex in the switch region, where it could both antagonize FBF repression and enhance GLD-2 polyadenylation (**Figure 4*e***). Another candidate is FBF itself: In addition to its role in GSC maintenance, FBF also acts genetically in the GLD-2/GLD-3 regulatory branch to stimulate entry into meiosis (Crittenden et al. 2002). We envision two possible mechanisms by which FBF may promote meiosis. FBF may initially repress mRNAs but at the same time mark them for later recruitment of GLD-2 and GLD-3. Given the physical association among FBF, GLD-3, and GLD-2, this direct model is attractive. Alternatively or in addition, FBF may stimulate entry into meiosis indirectly by repressing an inhibitor of GLD-2/GLD-3, a model that retains the FBF molecular function as a repressor.

A REGULATORY NETWORK CONTROLS THE BALANCE BETWEEN PROLIFERATION AND DIFFERENTIATION

The bare bones pathway presented in **Figure 4*b*** is clearly oversimplified. A more complete picture brings in additional regulators, more functional interactions, added feedback loops, and other levels of redundancy (**Figure 4*g***). This section focuses on the properties of the more extended network rather than on its details. In particular, we focus on two features found in many biological networks: robustness and plasticity.

The mitosis/meiosis transition is highly reproducible in wild-type animals. Among the numerous regulators of this switch (see above section), only the GLP-1/Notch signaling pathway is essential. Any of the other individual regulators can be deleted without eliminating the change in cell cycle. The robustness of the mitosis/meiosis decision relies on a pervasive redundancy within the regulatory circuitry (**Figure 4*g***). The best-understood examples are FBF-1 and FBF-2 on the one hand and the GLD-1 and GLD-2 branches on the other hand. Other examples include gene X downstream of GLP-1/Notch signaling (Lamont et al. 2004), a third minor branch driving germ cells into meiosis (Hansen et al. 2004a), and a parallel branch to GLP-1/Notch signaling (Vought et al. 2005). Deletions of single components of the network do not eliminate the switch but instead shift its position in the gonad. For example, in *fbf-1* single mutants, the switch occurs closer to the DTC than in wild type because fewer germ cells than normal are maintained in mitosis (Crittenden et al. 2002, Lamont & Kimble 2007). In *gld-2* and *gld-3* single mutants, more germ cells than normal occupy the mitotic region, and the switch occurs further from the DTC, suggesting that removal of the GLD-2/GLD-3 branch makes the switch less efficient (Eckmann et al. 2004). The network is therefore buffered to make it robust and is designed with an inherent flexibility or plasticity. Indeed, the regulation can occur at different individual nodes to modulate the circuitry. Plasticity is likely crucial for balancing proliferation and differentiation in response to changes in reproduction, aging, nutrition, or other environmental cues.

THE SPERM/OOCYTE DECISION

The sperm/oocyte decision is controlled by many of the same regulators that are used for the mitosis/meiosis decision. This section summarizes major points about how germ cells are specified to differentiate as sperm or oocytes. The next section compares regulation of the mitosis/meiosis and sperm/oocyte decisions.

Regulators of the Sperm/Oocyte Decision

The sperm/oocyte decision is controlled by regulators that act globally to specify male or female development in all tissues (reviewed in Zarkower 2006) as well as by

germline-specific regulators (**Figure 5*a***) (reviewed in Ellis & Schedl 2006). Sex determination is initiated by the ratio of X chromosomes to autosomes (reviewed in Meyer 2005), but hermaphrodite germ cells circumvent that X:A control to make both male and female gametes—sperm and oocytes. For the sake of brevity, we focus here on terminal regulators of the sperm/oocyte decision and on controls that permit the transient burst of spermatogenesis in hermaphrodites.

The terminal regulators of the sperm/oocyte regulatory pathway appear to be FOG-1/CPEB and FOG-3/Tob (**Figure 5*a***) (Barton & Kimble 1990, Ellis & Kimble 1995). No genes downstream of FOG-1 and FOG-3 have been identified. FOG-1 cannot specify sperm fate on its own because FOG-1 is expressed in the oocytes of either *fog-3* null mutants or feminized *tra-1* null mutants (Lamont & Kimble 2007); a similar experiment has not yet been done for FOG-3. Recent experiments reveal that FOG-1 is not essential for sperm specification in compound mutants that lack multiple regulators (Cho et al. 2007; M.-H. Lee & J. Kimble, unpublished data), but again, similar experiments have not been done with *fog-3* null mutants. The mechanism by which FOG-1 and FOG-3 specify sperm fate remains an important unanswered question.

Controls of *fog-1* and *fog-3* expression are crucial for specification as sperm or oocyte. Their most direct regulators are the FBF RNA-binding protein (Thompson et al. 2005) and TRA-1/GLI, a conserved transcription factor and regulator of nuclear export (Zarkower & Hodgkin 1992, Chen & Ellis 2000, Jin et al. 2001, Segal et al. 2001, Lamont & Kimble 2007). FBF promotes oogenesis by repressing *fog-1*, *fog-3*, and *fem-3* mRNAs (**Figure 5*a***) (Zhang et al. 1997, Thompson et al. 2005). TRA-1 has a more complex effect. It promotes oogenesis (Hodgkin & Brenner 1977, Hodgkin 1980), probably by transcriptional repression of *fog-1* and *fog-3* (Chen & Ellis 2000, Jin et al. 2001, Lamont & Kimble 2007), but it also sustains continued spermatogenesis in males (Hodgkin & Brenner 1977, Hodgkin 1986, Schedl & Kimble 1988), perhaps by positively regulating *fog-3* under special circumstances (Chen & Ellis 2000).

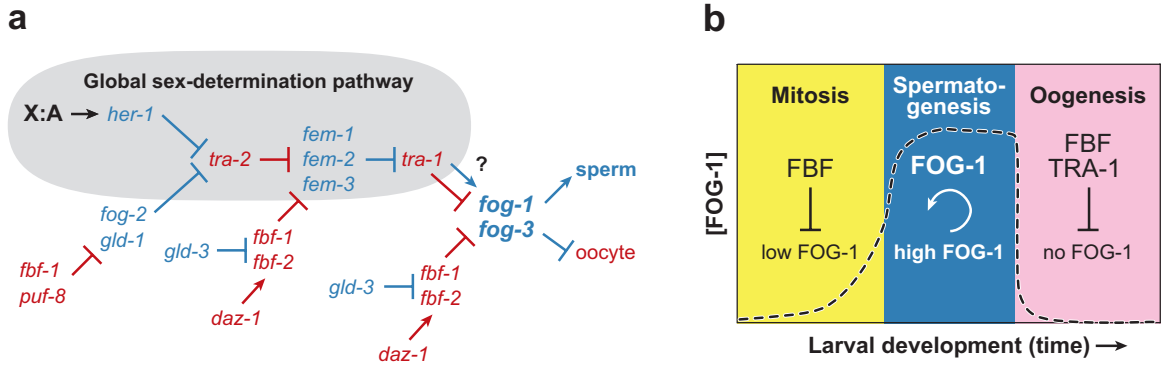


Figure 5

Regulation of the sperm/oocyte decision. (*a*) Global sex-determination regulators (contained in the *gray circle*) and germline-specific regulators converge on control of *fog-1* and *fog-3*, the terminal regulators of the sperm/oocyte decision. Regulators promoting female development (*red*) and regulators promoting male development (*blue*) are shown. (*b*) A FOG-1 gradient controls the larval mitosis/meiosis decision as well as the sperm/oocyte decision. In young larvae, FBF represses *fog-1* and probably maintains FOG-1 at a low level appropriate for mitotic divisions. In mid-stage larvae, a predicted positive autoregulation may generate abundant FOG-1, which is critical for sperm specification. In late-stage larvae, TRA-1 and FBF are likely to act together to turn FOG-1 off and permit oogenesis.

Therefore, *fog-1* and *fog-3* are directly controlled by both the global sex-determination pathway and germline-specific regulators.

Regulation of Hermaphrodite Spermatogenesis and Sperm Number

Hermaphrodite spermatogenesis relies on regulatory machinery that first activates *fog-1* and *fog-3* for sperm specification and then represses them for the switch into oogenesis. The first step is accomplished largely by translational repression of *tra-2*, a global sex-determination regulator that promotes female development upstream of the *fog* genes (**Figure 5*a***) (reviewed in Zarkower 2006). Intriguingly, GLD-1, introduced above as a mitosis/meiosis regulator, represses *tra-2* expression together with FOG-2, a *C. elegans*–specific F-box protein (Doniach 1986, Schedl & Kimble 1988, Goodwin et al. 1993, Jan et al. 1999, Clifford et al. 2000). In addition, a protein interaction between TRA-2 and TRA-1 facilitates hermaphrodite spermatogenesis, perhaps by freeing FEM-3 or sequestering TRA-1 (Lum et al. 2000, Wang & Kimble 2001). As spermatocytes are specified during later L3 and early L4, FOG-1 expression increases sharply but transiently just prior to the appearance of an early marker of sperm differentiation (Lamont & Kimble 2007). The hermaphrodite switch from spermatogenesis to oogenesis is controlled by FBF repression of *fem-3*, *fog-1*, and probably *fog-3* mRNAs (Barton et al. 1987, Zhang et al. 1997, Thompson et al. 2005). The FOG-1 protein disappears from the hermaphrodite germ line as it transitions into oogenesis during L4 (Lamont & Kimble 2007); antibodies are not yet available for either FEM-3 or FOG-3. TRA-1/GLI also represses *fog-1* and *fog-3*, which may facilitate the switch into oogenesis (Chen & Ellis 2000, Jin et al. 2001, Lamont & Kimble 2007).

Normally, the hermaphrodite germ line makes ~280 sperm, and that number appears to be a selected optimum: Mutant hermaphrodites with either fewer or more sperm cannot compete with those making the wild-type number (Hodgkin & Barnes 1991). The modulation of hermaphrodite sperm number relies on the RNA regulatory network, including FBF and GLD-1: *fbf-1* single mutants make nearly twice as many sperm as normal (~500–600) (Crittenden et al. 2002), and *gld-1/+* heterozygotes make approximately half the normal number (~120) in otherwise normal germ lines (Francis et al. 1995). The germ lines with altered sperm number display a corresponding change in the extent of FOG-1 expression (Lamont & Kimble 2007). In *fbf-1* mutants, FOG-1 is expressed earlier than normal and stays on later, and in *gld-1/+* mutants, FOG-1 comes up late and goes off early. Expression of other regulators (e.g., FBF-2, GLD-1) was not affected during larval development in these same mutants. Therefore, hermaphrodite sperm number is controlled at least in part by regulating the extent of FOG-1 expression.

Evolution of Hermaphroditism

Strains that reproduce as self-fertile hermaphrodites and males have evolved independently at least three times from female/male strains, which are more typical of caenorhabditid species (Kiontke et al. 2004). Nematode hermaphroditism therefore provides an excellent model for analysis of the capacity of a regulatory network to evolve, a property termed evolvability (Kirschner & Gerhart 1998). Although molecular and genetic studies addressing hermaphrodite evolution remain in their infancy, some answers have started to emerge. Importantly, in other nematode species FOG-3 has been conserved as a key regulator of sperm specification (Chen et al. 2001). Therefore, evolutionary studies can focus on how the sex-determination pathway has been modulated to express FOG-3 in XX animals.

We mention two recent findings to demonstrate that the nematode sperm/oocyte regulatory network has evolved within a time frame that makes it an accessible model

for in-depth analyses of network evolution. The first centers on the *fog-2* gene, which is required for the onset of hermaphrodite spermatogenesis but not for spermatogenesis per se (Schedl & Kimble 1988). The FOG-2 protein turns out to be a recently evolved F-box protein that does not exist in closely related nematodes (Clifford et al. 2000, Nayak et al. 2005). Because other genes controlling the sperm/oocyte decision have been conserved (Haag 2005), FOG-2 stands out as a species-specific regulator (Nayak et al. 2005). A second exciting discovery is that *fem-3* null mutants remain hermaphroditic in *Caenorhabditis briggsae*, a phenotype that differs dramatically from that in *C. elegans* (Hill et al. 2006). The machinery controlling hermaphroditism is therefore likely to reside downstream of the *fem* genes in *C. briggsae*, whereas in *C. elegans*, it acts largely on *tra-2* and *fem-3*. What regulates *C. briggsae* hermaphroditism remains unknown, but candidates include controls of *fog-1* and *fog-3* by TRA-1 or FBF as well as the TRA-1–TRA-2 interaction.

COUPLING THE MITOSIS/MEIOSIS AND SPERM/OOCYTE DECISIONS

The mitosis/meiosis and sperm/oocyte decisions are affected by many of the same regulators, most notably FOG-1, FOG-3, FBF, NOS-3, GLD-1, and GLD-3. In this section, we compare the roles of these key regulators in the two decisions and begin to ask how the two networks might be integrated.

FOG-1 and FOG-3 Link the Mitosis/Meiosis and Sperm/Oocyte Decisions

FOG-1/CPEB and FOG-3/Tob are molecularly distinct proteins but have remarkably similar biological roles in both the mitosis/meiosis and sperm/oocyte decisions. Whether these proteins act together in a functional complex or separately is not yet known. FOG-1 controls germ cell fates in a dose-dependent manner: High levels of FOG-1 promote sperm specification, low levels of FOG-1 maintain germ cells in mitosis (in the absence of FBF), and no FOG-1 results in oogenesis (Barton & Kimble 1990, Thompson et al. 2005). This FOG-1 dose dependency is likely to be critical for the dynamic fate changes typical of hermaphrodite germline development (**Figure 5*b***). FOG-3 similarly affects both decisions, but its dose dependency has not yet been carefully examined. How might FOG-1 and FOG-3 direct both mitotic divisions and sperm specification? The answer to this critical question awaits the identification of target genes and a better understanding of how the two proteins work. The FOG-1 dose dependence is reminiscent of a similar finding with its homolog, *Xenopus* CPEB, which drives mitosis at low abundance and stimulates progression through meiosis at higher levels (Mendez et al. 2002). CPEB may therefore provide a conserved link between these two cell cycles.

Additional Links Between the Two Decisions

The regulatory circuits in **Figures 4*b*** and **5*a*** include many of the same regulators, but the roles and regulatory relationships of these regulators are intriguingly different in the mitotis/meiosis and sperm/oocyte decisions. For example, GLD-3 acts upstream of FBF to antagonize the activity of the latter and promote continued spermatogenesis (Eckmann et al. 2002), but it acts downstream of FBF to enhance GLD-2 activity in the mitosis/meiosis circuit (Eckmann et al. 2004). FBF and NOS-3 work together to promote oogenesis (Kraemer et al. 1999). In contrast, they probably work independently in the mitosis/meiosis circuit: NOS-3 functions in the GLD-1 branch (Eckmann et al. 2004, Hansen et al. 2004b), whereas FBF acts upstream of both GLD branches to promote mitosis and in the GLD-2/GLD-3 branch to promote

meiosis (Crittenden et al. 2002, Eckmann et al. 2004). Therefore, the regulatory circuits controlling these two decisions have independent features despite the use of the same regulators.

FBF and GLD-1 provide two additional links between the mitosis/meiosis and sperm/oocyte decisions. However, the roles of these molecules in the sperm/oocyte decision are both linked to hermaphroditism: GLD-1 represses *tra-2* for the onset of hermaphrodite spermatogenesis, and FBF represses *fem-3* for the hermaphrodite switch from spermatogenesis to oogenesis. These two regulators may therefore have been recruited into the sex-determination pathway while still retaining more ancient roles in GSC maintenance (FBF) and entry into meiosis (GLD-1).

WHY SO MUCH RNA REGULATION?

FBF-1, FBF-2, GLD-1, GLD-2, GLD-3, NOS-3, and FOG-1 are all cytoplasmic proteins that control gene expression at the level of mRNA translation or stability, and even FOG-3 is implicated by homology in RNA regulation. By contrast, in-depth studies over many years have failed to identify posttranscriptional regulators as critical for cell fates in somatic tissues. What is the difference? Why does RNA regulation play such a crucial role in the control of germ cell fates? Spermatogenesis and oogenesis impose transient states of differentiation upon germ cells, which must be reversible in the early embryo. Perhaps posttranscriptional regulation represents a molecular strategy to facilitate that reversibility. We suggest that gene-specific regulators of translation (e.g., GLD-1) partner with global regulators of transcription (e.g., polycomb repressors) to maintain germline totipotency. In support of this basic idea, two translational regulators, GLD-1 and MEX-3, were recently identified as critical for germ cell totipotency: Germ cells depleted for both differentiate as one of several somatic cell types (Ciosk et al. 2006). Therefore, the differentiated fates adopted by germ cells more generally are affected by RNA regulators.

SUMMARY POINTS

1. The somatic distal tip cell (DTC) controls germline stem cell (GSC) maintenance by Notch signaling.
2. The actual GSCs are likely to be located immediately adjacent to the DTC.
3. Wnt/MAPK signaling specifies the DTC, at least in part by the activation of CEH-22/tinman/Nkx2.5.
4. RNA regulation plays a prominent role in governing germ cell fates.
5. A robust regulatory network acts downstream of Notch signaling to control the decision between continued mitotic divisions and entry into meiosis.
6. PUF RNA-binding proteins control GSC maintenance in *C. elegans*, *Drosophila*, and probably mammals.
7. FOG-1/CPEB and FOG-3/Tob provide two key molecular links between the mitosis/meiosis and sperm/oocyte decisions.
8. The regulatory circuitry controlling hermaphroditism in caenorhabditids provides an accessible model for the analysis of network evolution.

DISCLOSURE STATEMENT

The authors are not aware of any potential biases that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

Aherne WA, Camplejohn RS, Wright NA. 1977. *An Introduction to Cell Population Kinetics*. London: Edward Arnold

Asahina M, Valenta T, Silhankova M, Korinek V, Jindra M. 2006. Crosstalk between a nuclear receptor and β-catenin signaling decides cell fates in the *C. elegans* somatic gonad. *Dev. Cell* 11:203–11

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* 51:589–99

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* 58:565–71

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* 115:107–19

Belfiore M, Pugnale P, Saudan Z, Puoti A. 2004. Roles of the *C. elegans* cyclophilin-like protein MOG-6 in MEP-1 binding and germline fates. *Development* 131:2935–45

Berry LW, Westlund B, Schedl T. 1997. Germ-line tumor formation caused by activation of *glp-1*, a *Caenorhabditis elegans* member of the *Notch* family of receptors. *Development* 124:925–36

Berset T, Fröhli Hoier E, Battu G, Canevascini S, Hajnal A. 2001. Notch inhibition of RAS signaling through MAP kinase phosphatase LIP-1 during *C. elegans* vulval development. *Science* 291:1055–58

Brawley C, Matunis E. 2004. Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. *Science* 304:1331–34

Burdon T, Smith A, Savatier P. 2002. Signalling, cell cycle and pluripotency in embryonic stem cells. *Trends Cell Biol.* 12:432–38

Camps M, Nichols A, Gillieron C, Antonsson B, Muda M, et al. 1998. Catalytic activation of the phosphatase MKP-3 by ERK2 mitogen-activated protein kinase. *Science* 280:1262–65

Chen PJ, Cho S, Jin SW, Ellis RE. 2001. Specification of germ cell fates by FOG-3 has been conserved during nematode evolution. *Genetics* 158:1513–25

Chen PJ, Ellis RE. 2000. TRA-1A regulates transcription of *fog-3*, which controls germ cell fate in *C. elegans*. *Development* 127:3119–29

Chen PJ, Singal A, Kimble J, Ellis RE. 2000. A novel member of the Tob family of proteins controls sexual fate in *Caenorhabditis elegans* germ cells. *Dev. Biol.* 217:77–90

Cho S, Rogers KW, Fay DS. 2007. The *C. elegans* glycopeptide hormone receptor ortholog, FSHR-1, regulates germline differentiation and survival. *Curr. Biol.* 17:203–12

Ciosk R, DePalma M, Priess JR. 2004. ATX-2, the *C. elegans* ortholog of ataxin 2, functions in translational regulation in the germline. *Development* 131:4831–41

Ciosk R, DePalma M, Priess JR. 2006. Translational regulators maintain totipotency in the *Caenorhabditis elegans* germline. *Science* 311:851–53

Clifford R, Lee MH, Nayak S, Ohmachi M, Giorgini F, Schedl T. 2000. FOG-2, a novel F-box containing protein, associates with the GLD-1 RNA binding protein and directs male sex determination in the *C. elegans* hermaphrodite germline. *Development* 127:5265–76

This paper and Ellis & Kimble (1995) reported the discovery of terminal regulators of germline sex determination.

Showed that the *C. elegans* FSH receptor homolog acts in the soma to affect the sperm/oocyte decision.

Extended the role of RNA regulators to include control of totipotency.

Cox DN, Chao A, Baker J, Chang L, Qiao D, Lin H. 1998. A novel class of evolutionarily conserved genes defined by *piwi* are essential for stem cell self-renewal. *Genes Dev.* 12:3715–27

Crittenden SL, Bernstein DS, Bachorik JL, Thompson BE, Gallegos M, et al. 2002. A conserved RNA-binding protein controls germline stem cells in *Caenorhabditis elegans*. *Nature* 417:660–63

Reported that FBF controls GSC maintenance in *C. elegans* and identifies *gld-1* mRNA as a key FBF target.

Crittenden SL, Leonhard KA, Byrd DT, Kimble J. 2006. Cellular analyses of the mitotic region in the *Caenorhabditis elegans* adult germ line. *Mol. Biol. Cell* 17:3051–61

Crittenden SL, Troemel ER, Evans TC, Kimble J. 1994. GLP-1 is localized to the mitotic region of the *C. elegans* germ line. *Development* 120:2901–11

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

Eckmann CR, Crittenden SL, Suh N, Kimble J. 2004. GLD-3 and control of the mitosis/meiosis decision in the germline of *Caenorhabditis elegans*. *Genetics* 168:147–60

Eckmann CR, Kraemer B, Wickens M, Kimble J. 2002. GLD-3, a Bicaudal-C homolog that inhibits FBF to control germline sex determination in *C. elegans*. *Dev. Cell* 3:697–710

Eisenmann D. 2005. Wnt signaling. In *WormBook*, ed. The *C. elegans* Research Community, 10.1895/wormbook.1.7.1. **http://www.wormbook.org**

Ellis R, Schedl T. 2006. Sex determination in the germ line. In *WormBook*, ed. The *C. elegans* Research Community, 10.1895/wormbook.1.82.1. **http://www.wormbook.org**

Ellis RE, Kimble J. 1995. The *fog-3* gene and regulation of cell fate in the germ line of *Caenorhabditis elegans*. *Genetics* 139:561–77

Forbes A, Lehmann R. 1998. Nanos and Pumilio have critical roles in the development and function of *Drosophila* germline stem cells. *Development* 125:679–90

Francis R, Barton MK, Kimble J, Schedl T. 1995. *gld-1*, a tumor suppressor gene required for oocyte development in *Caenorhabditis elegans*. *Genetics* 139:579–606

Goldstrohm AC, Hook BA, Seay DJ, Wickens M. 2006. PUF proteins bind POP2p to regulate mRNAs. *Nat. Struct. Mol. Biol.* 13:533–39

Goodwin EB, Okkema PG, Evans TC, Kimble J. 1993. Translational regulation of *tra-2* by its 3′ untranslated region controls sexual identity in C. elegans. *Cell* 75:329–39

Graham PL, Kimble J. 1993. The *mog-1* gene is required for the switch from spermatogenesis to oogenesis in *Caenorhabditis elegans*. *Genetics* 133:919–31

Graham PL, Schedl T, Kimble J. 1993. More *mog* genes that influence the switch from spermatogenesis to oogenesis in the hermaphrodite germ line of *Caenorhabditis elegans*. *Dev. Genet.* 14:471–84

Haag ES. 2005. The evolution of nematode sex determination: *C. elegans* as a reference point for comparative biology. In *WormBook*, ed. The *C. elegans* Research Community, 10.1895/wormbook.1.120.1. **http://www.wormbook.org**

Hall DH, Winfrey VP, Blaeuer G, Hoffman LH, Furuta T, et al. 1999. Ultrastructural features of the adult hermaphrodite gonad of *Caenorhabditis elegans*: relations between the germ line and soma. *Dev. Biol.* 212:101–23

Hansen D, Hubbard EJA, Schedl T. 2004a. Multi-pathway control of the proliferation versus meiotic development decision in the *Caenorhabditis elegans* germline. *Dev. Biol.* 268:342–57

Hansen D, Schedl T. 2006. The regulatory network controlling the proliferation-meiotic entry decision in the *Caenorhabditis elegans* germ line. *Curr. Top. Dev. Biol.* 76:185–215

Hansen D, Wilson-Berry L, Dang T, Schedl T. 2004b. Control of the proliferation versus meiotic development decision in the *C. elegans* germline through regulation of GLD-1 protein accumulation. *Development* 131:93–104

Henderson ST, Gao D, Lambie EJ, Kimble J. 1994. *lag-2* may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans*. *Development* 120:2913–24

Hill RC, de Carvalho CE, Salogiannis J, Schlager B, Pilgrim D, Haag ES. 2006. Genetic flexibility in the convergent evolution of hermaphroditism in *Caenorhabditis* nematodes. *Dev. Cell* 10:531–38

Hirsh D, Oppenheim D, Klass M. 1976. Development of the reproductive system of *Caenorhabditis elegans*. *Dev. Biol.* 49:200–19

Hodgkin J. 1980. More sex-determination mutants of *Caenorhabditis elegans*. *Genetics* 96:649–64

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, Barnes TM. 1991. More is not better: brood size and population growth in a self-fertilizing nematode. *Proc. R. Soc. London Ser. B* 246:19–24

Hodgkin JA, Brenner S. 1977. Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. *Genetics* 86:275–87

Ikematsu N, Yoshida Y, Kawamura-Tsuzuku J, Ohsugi M, Onda M, et al. 1999. Tob2, a novel antiproliferative Tob/BTG1 family member, associates with a component of the CCR4 transcriptional regulatory complex capable of binding cyclin-dependent kinases. *Oncogene* 18:7432–41

Jan E, Motzny CK, Graves LE, Goodwin EB. 1999. The STAR protein, GLD-1, is a translational regulator of sexual identity in *Caenorhabditis elegans*. *EMBO J.* 18:258–69

Jin SW, Kimble J, Ellis RE. 2001. Regulation of cell fate in *Caenorhabditis elegans* by a novel cytoplasmic polyadenylation element binding protein. *Dev. Biol.* 229:537–53

Jones AR, Francis R, Schedl T. 1996. GLD-1, a cytoplasmic protein essential for oocyte differentiation, shows stage- and sex-specific expression during *Caenorhabditis elegans* germline development. *Dev. Biol.* 180:165–83

Jones AR, Schedl T. 1995. Mutations in *gld-1*, a female germ cell-specific tumor suppressor gene in *Caenorhabditis elegans*, affect a conserved domain also found in Src-associated protein Sam68. *Genes Dev.* 9:1491–504

Kadyk LC, Kimble J. 1998. Genetic regulation of entry into meiosis in *Caenorhabditis elegans*. *Development* 125:1803–13

Kai T, Spradling A. 2004. Differentiating germ cells can revert into functional stem cells in *Drosophila melanogaster* ovaries. *Nature* 428:564–69

Karp X, Greenwald I. 2004. Multiple roles for the E/Daughterless ortholog HLH-2 during *C. elegans* gonadogenesis. *Dev. Biol.* 272:460–69

Kidd AR III, Miskowski JA, Siegfried KR, Sawa H, Kimble J. 2005. A β-catenin identified by functional rather than sequence criteria and its role in Wnt/MAPK signaling. *Cell* 121:761–72

Killian DJ, Hubbard EJA. 2005. *Caenorhabditis elegans* germline patterning requires coordinated development of the somatic gonadal sheath and the germ line. *Dev. Biol.* 279:322–35

Kimble J, Hirsh D. 1979. The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* 70:396–417

Kimble J, Simpson P. 1997. The LIN-12/Notch signaling pathway and its regulation. *Annu. Rev. Cell Dev. Biol.* 13:333–61

Kimble JE, White JG. 1981. On the control of germ cell development in *Caenorhabditis elegans*. *Dev. Biol.* 81:208–19

Kiontke K, Gavin NP, Raynes Y, Roehrig C, Piano F, Fitch DH. 2004. *Caenorhabditis* phylogeny predicts convergence of hermaphroditism and extensive intron loss. *Proc. Natl. Acad. Sci. USA* 101:9003–8

Reported that the same key genes control both somatic and germline sexual identity in *C. elegans*.

Along with Jan et al. (1999) and Lee & Schedl (2001), this is part of a series of papers showing that GLD-1 is a translational repressor and identifying potential target mRNAs.

Identified *gld-1* and *gld-2* as key regulators of entry into meiosis.

Showed that the proximal somatic gonad affects germline proliferation and patterning.

Identified the somatic DTC as a critical component of the GSC niche.

Kipreos ET, Gohel SP, Hedgecock EM. 2000. The *C. elegans* F-box/WD-repeat protein LIN-23 functions to limit cell division during development. *Development* 127:5071–82
Kipreos ET, Lander LE, Wing JP, He WW, Hedgecock EM. 1996. *cul-1* is required for cell cycle exit in *C. elegans* and identifies a novel gene family. *Cell* 85:829–39
Kirschner M, Gerhart J. 1998. Evolvability. *Proc. Natl. Acad. Sci. USA* 95:8420–27
Knight SW, Bass BL. 2001. A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. *Science* 293:2269–71
Kraemer B, Crittenden S, Gallegos M, Moulder G, Barstead R, et al. 1999. NANOS-3 and FBF proteins physically interact to control the sperm-oocyte switch in *Caenorhabditis elegans*. *Curr. Biol.* 9:1009–18
Lackner MR, Kim SK. 1998. Genetic analysis of the *Caenorhabditis elegans* MAP kinase gene *mpk-1*. *Genetics* 150:103–17
Lam N, Chesney MA, Kimble J. 2006. Wnt signaling and CEH-22/tinman/Nkx2.5 specify a stem cell niche in *C. elegans*. *Curr. Biol.* 16:287–95
Lamont LB, Crittenden SL, Bernstein D, Wickens M, Kimble J. 2004. FBF-1 and FBF-2 regulate the size of the mitotic region in the *C. elegans* germline. *Dev. Cell* 7:697–707
Lamont LB, Kimble J. 2007. Developmental expression of FOG-1/CPEB protein and its control in the *Caenorhabditis elegans* hermaphrodite germ line. *Dev. Dyn.* 36:871–79
Lee MH, Hook B, Lamont LB, Wickens M, Kimble J. 2006. LIP-1 phosphatase controls the extent of germline proliferation in *Caenorhabditis elegans*. *EMBO J.* 25:88–96
Lee MH, Schedl T. 2001. Identification of in vivo mRNA targets of GLD-1, a maxi-KH motif containing protein required for *C. elegans* germ cell development. *Genes Dev.* 15:2408–20
Li L, Xie T. 2005. Stem cell niche: structure and function. *Annu. Rev. Cell Dev. Biol.* 21:605–31
Lin H, Spradling AC. 1997. A novel group of *pumilio* mutations affects the asymmetric division of germline stem cells in the *Drosophila* ovary. *Development* 124:2463–76
Luitjens C, Gallegos M, Kraemer B, Kimble J, Wickens M. 2000. CPEB proteins control two key steps in spermatogenesis in *C. elegans*. *Genes Dev.* 14:2596–609
Lum DH, Kuwabara PE, Zarkower D, Spence AM. 2000. Direct protein-protein interaction between the intracellular domain of TRA-2 and the transcription factor TRA-1A modulates feminizing activity in *Caenorhabditis elegans*. *Genes Dev.* 14:3153–65
Maciejowski J, Ahn JH, Cipriani PG, Killian DJ, Chaudhary AL, et al. 2005. Autosomal genes of autosomal/X-linked duplicated gene pairs and germ-line proliferation in *Caenorhabditis elegans*. *Genetics* 169:1997–2011
Maciejowski J, Ugel N, Mishra B, Isopi M, Hubbard EJA. 2006. Quantitative analysis of germline mitosis in adult *C. elegans*. *Dev. Biol.* 292:142–51
Maekawa M, Nishida E, Tanoue T. 2002. Identification of the anti-proliferative protein Tob as a MAPK substrate. *J. Biol. Chem.* 277:37783–87
Maine EM, Hansen D, Springer D, Vought VE. 2004. *Caenorhabditis elegans atx-2* promotes germline proliferation and the oocyte fate. *Genetics* 168:817–30
Marin VA, Evans TC. 2003. Translational repression of a *C. elegans* Notch mRNA by the STAR/KH domain protein GLD-1. *Development* 130:2623–32
McCarter J, Bartlett B, Dang T, Schedl T. 1997. Soma-germ cell interactions in *Caenorhabditis elegans*: multiple events of hermaphrodite germline development require the somatic sheath and spermathecal lineages. *Dev. Biol.* 181:121–43
Mendez R, Barnard D, Richter JD. 2002. Differential mRNA translation and meiotic progression require Cdc2-mediated CPEB destruction. *EMBO J.* 21:1833–44
Meyer BJ. 2005. X-chromosome dosage compensation. In *WormBook*, ed. The *C. elegans* Research Community, 10.1895/wormbook.1.8.1. **http://www.wormbook.org**

Moore FL, Jaruzelska J, Fox MS, Urano J, Firpo MT, et al. 2003. Human Pumilio-2 is expressed in embryonic stem cells and germ cells and interacts with DAZ (Deleted in AZoospermia) and DAZ-like proteins. *Proc. Natl. Acad. Sci. USA* 100:538–43

Morrison SJ, Kimble J. 2006. Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* 441:1068–74

Nayak S, Goree J, Schedl T. 2005. *fog-2* and the evolution of self-fertile hermaphroditism in *Caenorhabditis*. *PLoS Biol.* 3:e6

Reported that FOG-2 is not conserved in closely related hermaphroditic species, and proposed convergent evolution of nematode hermaphroditism.

Okkema PG, Ha E, Haun C, Chen W, Fire A. 1997. The *Caenorhabditis elegans* NK-2 homeobox gene *ceh-22* activates pharyngeal muscle gene expression in combination with *pha-1* and is required for normal pharyngeal development. *Development* 124:3965–73

Okochi K, Suzuki T, Inoue J, Matsuda S, Yamamoto T. 2005. Interaction of antiproliferative protein Tob with poly(A)-binding protein and inducible poly(A)-binding protein: implication of Tob in translational control. *Genes Cells* 10:151–63

Otori M, Karashima T, Yamamoto M. 2006. The *Caenorhabditis elegans* homologue of *Deleted in Azoospermia* is involved in the sperm/oocyte switch. *Mol. Biol. Cell* 17:3147–55

Pepper ASR, Lo TW, Killian DJ, Hall DH, Hubbard EJA. 2003. The establishment of *Caenorhabditis elegans* germline pattern is controlled by overlapping proximal and distal somatic gonad signals. *Dev. Biol.* 259:336–50

Phillips BT, Kidd III AR, King R, Hardin J, Kimble J. 2007. Reciprocal asymmetry of SYS-1/β-catenin and POP-1/TCF controls asymmetric divisions in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA.* 104:3231–36

Puoti A, Kimble J. 1999. The *Caenorhabditis elegans* sex determination gene *mog-1* encodes a member of the DEAH-box protein family. *Mol. Cell. Biol.* 19:2189–97

Puoti A, Kimble J. 2000. The hermaphrodite sperm/oocyte switch requires the *Caenorhabditis elegans* homologs of PRP2 and PRP22. *Proc. Natl. Acad. Sci. USA* 97:3276–81

Reya T, Clevers H. 2005. Wnt signaling in stem cells and cancer. *Nature* 434:843–50

Reya T, Duncan AW, Ailles L, Domen J, Scherer DC, et al. 2003. A role for Wnt signaling in self-renewal of hematopoietic stem cells. *Nature* 423:409–14

Rouault JP, Prévôt D, Berthet C, Birot AM, Billaud M, et al. 1998. Interaction of *BTG1* and p53-regulated *BTG2* gene products with mCaf1, the murine homolog of a component of the yeast CCR4 transcriptional regulatory complex. *J. Biol. Chem.* 273:22563–69

Ryder SP, Frater LA, Abramovitz DL, Goodwin EB, Williamson JR. 2004. RNA target specificity of the STAR/GSG domain post-transcriptional regulatory protein GLD-1. *Nat. Struct. Mol. Biol.* 11:20–28

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

Segal SP, Graves LE, Verheyden J, Goodwin EB. 2001. RNA-regulated TRA-1 nuclear export controls sexual fate. *Dev. Cell* 1:539–51

Siegfried K, Kimble J. 2002. POP-1 controls axis formation during early gonadogenesis in *C. elegans*. *Development* 129:443–53

Siegfried KR, Kidd III AR, Chesney MA, Kimble J. 2004. The *sys-1* and *sys-3* genes cooperate with Wnt signaling to establish the proximal-distal axis of the *C. elegans* gonad. *Genetics* 166:171–86

Smardon A, Spoerke JM, Stacey SC, Klein ME, Mackin N, Maine EM. 2000. EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in *C. elegans*. *Curr. Biol.* 10:169–78

Suh N, Jedamzik B, Eckmann CR, Wickens M, Kimble J. 2006. The GLD-2 poly(A) polymerase activates *gld-1* mRNA in the *C. elegans* germ line. *Proc. Natl. Acad. Sci. USA* 103:15108–12

Sulston JE, Schierenberg E, White JG, Thomson JN. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* 100:64–119

Tax FE, Yeargers JJ, Thomas JH. 1994. Sequence of *C. elegans lag-2* reveals a cell-signaling domain shared with *Delta* and *Serrate* of *Drosophila*. *Nature* 368:150–54

Showed that FOG-1 controls both germline proliferation and sperm fate in a dose-dependent fashion.

Thompson BE, Bernstein DS, Bachorik JL, Petcherski AG, Wickens M, Kimble J. 2005. Dose-dependent control of proliferation and sperm specification by FOG-1/CPEB. *Development* 132:3471–81

Tucker M, Valencia-Sanchez MA, Staples RR, Chen J, Denis CL, Parker R. 2001. The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in *Saccharomyces cerevisiae*. *Cell* 104:377–86

Tzachanis D, Freeman GJ, Hirano N, van Puijenbroek AA, Delfs MW, et al. 2001. Tob is a negative regulator of activation that is expressed in anergic and quiescent T cells. *Nat. Immunol.* 2:1174–82

Vogt A, Tamewitz A, Skoko J, Sikorski RP, Giuliano KA, Lazo JS. 2005. The benzo[*c*]phenanthridine alkaloid, sanguinarine, is a selective, cell-active inhibitor of mitogen-activated protein kinase phosphatase-1. *J. Biol. Chem.* 280:19078–86

Found that EGO-1 is an RNA-directed RNA polymerase that acts in the mitosis/meiosis decision.

Vought VE, Ohmachi M, Lee MH, Maine EM. 2005. EGO-1, a putative RNA-directed RNA polymerase, promotes germline proliferation in parallel with GLP-1/Notch signaling and regulates the spatial organization of nuclear pore complexes and germline P granules in *Caenorhabditis elegans*. *Genetics* 170:1121–32

Wang L, Eckmann CR, Kadyk LC, Wickens M, Kimble J. 2002. A regulatory cytoplasmic poly(A) polymerase in *Caenorhabditis elegans*. *Nature* 419:312–16

Wang S, Kimble J. 2001. The TRA-1 transcription factor binds TRA-2 to regulate sexual fates in *Caenorhabditis elegans*. *EMBO J.* 20:1363–72

Wickens M, Bernstein DS, Kimble J, Parker R. 2002. A PUF family portrait: 3′UTR regulation as a way of life. *Trends Genet.* 18:150–57

Wilkinson HA, Fitzgerald K, Greenwald I. 1994. Reciprocal changes in expression of the receptor *lin-12* and its ligand *lag-2* prior to commitment in a *C. elegans* cell fate decision. *Cell* 79:1187–98

Xie T, Spradling AC. 2000. A niche maintaining germ line stem cells in the *Drosophila* ovary. *Science* 290:328–30

Xiong B, Rui Y, Zhang M, Shi K, Jia S, et al. 2006. Tob1 controls dorsal development of zebrafish embryos by antagonizing maternal β-catenin transcriptional activity. *Dev. Cell* 11:225–38

Xu EY, Chang R, Salmon NA, Reijo Pera RA. 2007. A gene trap mutation of a murine homolog of the *Drosophila* stem cell factor *Pumilio* results in smaller testes but does not affect litter size or fertility. *Mol. Reprod. Dev.* 74:912–21

Yigit E, Batista PJ, Bei Y, Pang KM, Chen CC, et al. 2006. Analysis of the *C. elegans* Argonaute family reveals that distinct Argonautes act sequentially during RNAi. *Cell* 127:747–57

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* 58:553–63

Yoo AS, Bais C, Greenwald I. 2004. Crosstalk between the EGFR and LIN-12/Notch pathways in *C. elegans* vulval development. *Science* 303:663–66

Yoshida Y, Nakamura T, Komoda M, Satoh H, Suzuki T, et al. 2003. Mice lacking a transcriptional corepressor Tob are predisposed to cancer. *Genes Dev.* 17:1201–6

Yoshida Y, Tanaka S, Umemori H, Minowa O, Usui M, et al. 2000. Negative regulation of BMP/Smad signaling by Tob in osteoblasts. *Cell* 103:1085–97

Zarkower D. 2006. Somatic sex determination. In *Wormbook*, ed. The *C. elegans* Research Community, doi/10.1895/wormbook1.84.1. **http://www.wormbook.org**

Discovered that GLD-2 is a cytoplasmic poly(A) polymerase that is likely to promote meiosis by activating target mRNAs.

Zarkower D, Hodgkin J. 1992. Molecular analysis of the C. elegans sex-determining gene *tra-1*: a gene encoding two zinc finger proteins. *Cell* 70:237–49

Zetka MC, Kawasaki I, Strome S, Müller F. 1999. Synapsis and chiasma formation in *Caenorhabditis elegans* require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. *Genes Dev.* 13:2258–70

Zhang B, Gallegos M, Puoti A, Durkin E, Fields S, et al. 1997. A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. *Nature* 390:477–84

Reported the identity of FBF as a 3′UTR regulator of germline sexual identity.