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Regulation of the mitosis/meiosis decision in the *Caenorhabditis elegans* germline

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During the development of multicellular organisms, the processes of growth and differentiation are kept in balance to generate and maintain tissues and organs of the correct size, shape and cellular composition. We have investigated the molecular controls of growth and differentiation in the *Caenorhabditis elegans* germline. A single somatic cell, called the distal tip cell, promotes mitotic proliferation in the adjacent germline by GLP-1/Notch signalling. Within the germline, the decisions between mitosis and meiosis and between spermatogenesis and oogenesis are controlled by a group of conserved RNA regulators. FBF, a member of the PUF (for Pumilio and FBF) family of RNA-binding proteins, promotes mitosis by repressing *gld-1* mRNA activity; the GLD-1, GLD-2, GLD-3 and NOS-3 proteins promote entry into meiosis by regulating mRNAs that remain unknown. The regulatory balance between opposing FBF and GLD activities is crucial for controlling the extent of germline proliferation. PUF proteins regulate germline stem cells in both *Drosophila* and *C. elegans* and are localized to germline stem cells of the mammalian testis. Therefore, this post-transcriptional regulatory switch may be an ancient mechanism for controlling maintenance of stem cells versus differentiation.

Keywords: *Caenorhabditis elegans*; germline stem cells; PUF; RNA regulation; poly(A) polymerase; Notch

1. THE *CAENORHABDITIS ELEGANS* GERMLINE AND ITS BALANCE BETWEEN GROWTH AND DIFFERENTIATION

The adult *Caenorhabditis elegans* germline contains a continuously proliferating stem cell population as well as differentiated gametes. We discuss the regulatory mechanisms required for the decision between growth (mitosis) and differentiation (meiosis) in germline development. We focus on the adult hermaphrodite germline. The male germline is organized in a similar fashion and relies on many of the same molecular regulators.

During early larval development, the *C. elegans* germline is proliferative. In later larval development, some germ cells leave mitosis and enter meiosis. As the animal matures, gametes are made, and in the adult, the pattern of growth and differentiation is maintained. The adult hermaphrodite germline has an elongated U-shape with cells arranged in a gradient of maturation along its long axis (figure 1*a*). Mitotically dividing cells occupy the distal end, early stages of meiotic prophase occupy the transition zone, and gametogenesis occurs proximally. The germline is a specialized syncytium: each germline 'cell' is connected by a cytoplasmic bridge to the 'rachis', a core of cytoplasm that runs the length of the distal arm; primary spermatocytes are cellular, while oocytes remain connected to each other and the rachis by a thin bridge.

The mitotic region of the *C. elegans* germline generates additional mitotic cells as well as supplying meiotic cells for gametogenesis. This mitotic region must therefore include stem cells, although the location and number of these stem cells are not known. In other organisms, stem cells are controlled by one of two strategies (Watt & Hogan 2000). In some cases, stem cells divide asymmetrically to generate one stem cell daughter and one differentiating daughter; in other cases, stem cells divide symmetrically to generate either more stem cells or differentiated cells by a stochastic process that is not well understood (Watt & Hogan 2000; Marshman *et al.* 2002). Preliminary results have not identified cells within the *C. elegans* germline mitotic region that divide asymmetrically or in a reproducible orientation (S. Crittenden, unpublished data), suggesting that *C. elegans* germline stem cells may use the stochastic strategy.

Stem cells in many animal tissues rely on their surrounding microenvironment or stem cell 'niche' (Watt & Hogan 2000; Lin 2002). The niche for *C. elegans* germline stem cells is a single somatic cell called the DTC. This cell normally caps the distal germline where it controls the adjacent mitotic region: DTC removal eliminates germline stem cells, and DTC relocation results in the corresponding relocation of germline stem cells (Kimble & White 1981). Moreover, DTC duplication induces duplicated germline arms (Kipreos *et al.* 2000). Therefore, the DTC is both necessary and sufficient for the establishment and

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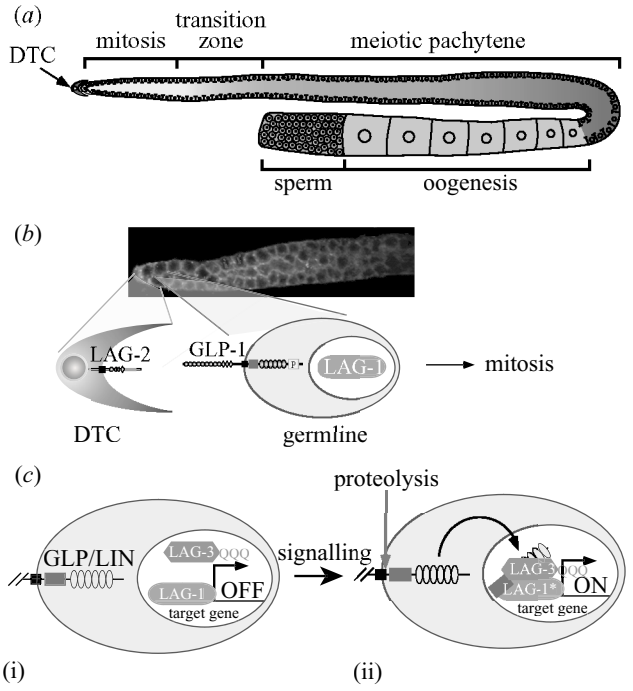

Figure 1. GLP-1/Notch signalling controls *C. elegans* germline proliferation. See text for more details. (*a*) Organization of adult hermaphrodite germline. DTC, somatic distal tip cell. Regions of the germline referred to in the text are indicated. (*b*) The LAG-2 ligand, localized to the DTC, signals to GLP-1 in the germline. Photograph shows *lag-2*::GFP in the DTC, and anti-GLP-1 staining in the germline. (*c*) Upon GLP-1 activation, the GLP-1 intracellular domain, LAG-1 and LAG-3 form a ternary complex in the nucleus and activate transcription of target genes.

maintenance of the germline stem cells (Kimble & White 1981).

2. CONTROL OF GERMLINE PROLIFERATION BY GLP-1/NOTCH SIGNALLING

The *C. elegans* DTC niche promotes germline proliferation by GLP-1/Notch signalling (Kimble & Simpson 1997, and references therein; figure 1*b*). In the absence of either the DTC or GLP-1 signalling, all germ cells enter meiosis and differentiate as functional gametes. The *C. elegans* genome encodes two Notch receptors, LIN-12 and GLP-1, which function with common components, designated LAG (for LIN-12 and GLP-1). The LAG-2 ligand, a DSL (Delta, Serrate, LAG-2) class transmembrane protein, is expressed by the DTC (figure 1*b*), which may restrict GLP-1 signalling to the distal end of the germline (Henderson *et al.* 1994). The germline expresses the GLP-1 receptor, which is localized to the mitotic region, probably by a post-transcriptional control (Crittenden *et al.* 1994; figure 1*b*). LAG-1 is a CSL (CBF-1, Su(H), LAG-1) family DNA-binding protein (Christensen *et al.* 1996). LAG-3 is a transcriptional coactivator (Petcherski & Kimble 2000*a*,*b*). In the absence of signalling, LAG-1 is thought to repress target genes (figure 1*c*(i)), by analogy with its vertebrate and fly homologues (for review, see Lai 2002). By contrast, signalling induces the formation of a complex that includes LAG-1, LAG-3, the intracellular domain of the GLP-1 receptor and the DNA target (figure 1*c*(ii); Petcherski & Kimble 2000*a*). GLP-1/Notch signalling therefore appears to act as a localized transcriptional switch in which LAG-1 is transformed from a transcriptional repressor to a transcriptional activator.

Our current model is that GLP-1/Notch signalling specifies the undifferentiated mitotic fate of germ cells. GLP-1/Notch signalling is likely to regulate the transcription of genes involved in promoting stem cell proliferation or inhibiting differentiation; however, those target genes remain unknown. As germ cells divide mitotically, they leave the influence of the DTC and its signal, which results in entry into the meiotic cell cycle. Excess GLP-1 activity, resulting from either a mutant ligand-independent receptor (Berry *et al.* 1997) or ectopic expression of the ligand (Henderson *et al.* 1997), leads to a tumorous germline, full of mitotic germ cells. By contrast, insufficient signalling, owing to loss-of-function mutations in *glp-1* or any of the *lag* genes, leads to the entry of all germ cells into meiosis (Kimble & Simpson 1997). Therefore, GLP-1 signalling must itself be controlled to achieve the proper balance between mitotically dividing and differentiated germ cells. Controls of GLP-1 activity include a transcriptional control that limits LAG-2 to the DTC (Henderson *et al.* 1994) and a post-transcriptional control that limits GLP-1 to the mitotic region (Crittenden *et al.* 1994). Recent evidence suggests that the post-transcriptional regulator is the GLD-1 RNA-binding protein. GLD-1 restricts GLP-1 protein to the mitotic region (Marin & Evans 2003). In addition, GLP-1 activity probably feeds back on itself (Kodoyianni *et al.* 1992; Christensen *et al.* 1996; Berry *et al.* 1997).

3. RNA REGULATION AND THE BALANCE OF MITOSIS AND MEIOSIS

Post-transcriptional regulation plays a major part within the *C. elegans* germline to control the mitosis/meiosis decision (figure 2). The FBF RNA-binding protein promotes mitosis and maintains germline stem cells (Crittenden *et al.* 2002). FBF belongs to the PUF (for Pumilio and FBF) family of RNA-binding proteins; it is encoded by two almost identical genes, *fbf-1* and *fbf-2*, and was originally identified for its role in germline sex determination (Zhang *et al.* 1997). FBF acts, at least in part, by repressing the activity of *gld-1* mRNA via two FBF binding sites in the *gld-1* 3′ UTR (Crittenden *et al.* 2002; figure 2*a*). The *gld-1* gene promotes commitment to the meiotic cell cycle (Francis *et al.* 1995; Kadyk & Kimble 1998). Animals lacking FBF activity have no germline stem cells; however, if even one copy of *gld-1* is removed in such animals, proliferation is restored (Crittenden *et al.* 2002). Therefore, *fbf* regulation of *gld-1* is critical for the maintenance of germline stem cells.

Three GLD proteins promote entry into the meiotic cell cycle as well as controlling a variety of other events in subsequent germline development, gametogenesis and embryogenesis (Jones & Schedl 1995; Kadyk & Kimble 1998; C. Eckmann and S. Crittenden, unpublished data). GLD-1 is a conserved translational repressor in the STAR/Quaking family (Jones & Schedl 1995; Jan *et al.* 1999; Lee & Schedl 2001; figure 2*a*). GLD-2 and GLD-3 act together as a cytoplasmic heterodimeric poly(A)

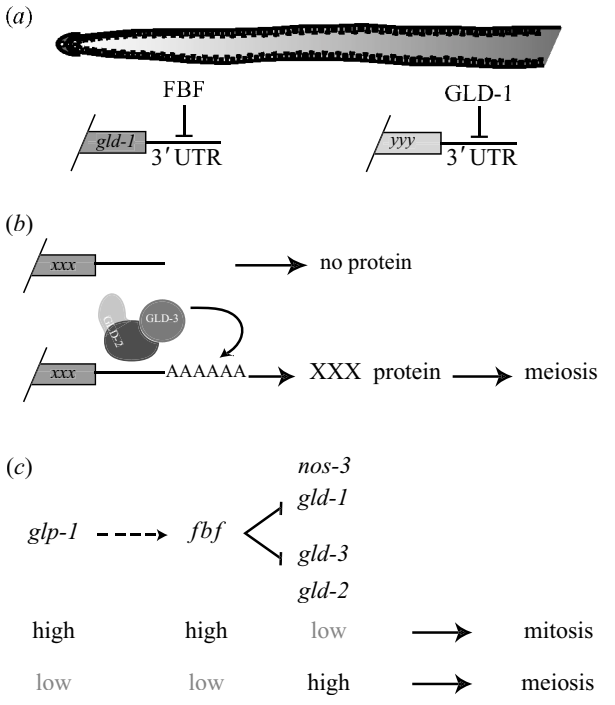

Figure 2. RNA regulators control the mitosis/meiosis decision within the germline. See text for more details. (*a*) FBF repression of *gld-1* mRNA is crucial for maintaining mitosis. GLD-1 probably represses mRNAs that inhibit meiosis. (*b*) Increased poly(A) tail length correlates with increased translation and stability of mRNAs. GLD-2 and GLD-3 together have poly(A) polymerase activity and are likely to activate targets that promote meiosis. (*c*) Genetic pathway for entry into meiosis.

polymerase (Wang *et al*. 2002; figure 2*b*). GLD-2 belongs to the DNA-β nucleotidyl transferase superfamily and encodes the catalytic subunit of the poly(A) polymerase (Wang *et al.* 2002). GLD-3 belongs to the Bicaudal-C family of KH-domain RNA-binding proteins (Eckmann *et al.* 2002) and may target poly(A) polymerase activity to specific targets. Thus, all three GLD proteins have roles in regulating mRNAs and all three are conserved throughout the animal kingdom.

The GLD genes fall into two apparently parallel pathways to promote entry into meiosis (figure 2*c*). Our first indication of these two pathways derived from studies with *gld-1* and *gld-2*: thus, *gld-1* and *gld-2* single mutants each leave the mitotic cell cycle and enter meiosis, but *gld-1 gld-2* double mutants fail to make this transition (Kadyk & Kimble 1998). Therefore, *gld-1* and *gld-2* are functionally redundant and these two genes act in parallel to drive germ cells towards the meiotic fate. Preliminary genetic experiments place GLD-3 in the GLD-2 branch of the pathway, consistent with its known molecular role as a GLD-2 partner (C. Eckmann, unpublished data). The targets that are regulated by each branch of the pathway to promote meiosis remain unknown. However, based on its poly(A) polymerase activity, we predict that the GLD-2/GLD-3 poly(A) polymerase will activate mRNAs that promote meiosis (figure 2*b*), whereas GLD-1 is likely to repress mRNAs that inhibit meiosis (Jan *et al*. 1999; Lee & Schedl 2001; figure 2*a*).

GLD-3 physically binds to FBF in addition to its binding to GLD-2 (Eckmann *et al.* 2002). We therefore explored the role of another FBF-interactor, called NOS-3, in the mitosis/meiosis decision. NOS-3 encodes a member of the Nanos RNA-binding protein family (Kraemer *et al.* 1999). In the mitosis/meiosis decision, NOS-3 promotes entry into meiosis, a role that appears to oppose that of FBF (C. Eckmann and S. Crittenden, unpublished data), whereas in sex determination, NOS-3 works together with FBF to promote oogenesis (Kraemer *et al.* 1999). Using double mutant analyses, we find that NOS-3 appears to act in the GLD-1 branch of the meiotic commitment pathway (figure 2*c*). Furthermore, both GLD-1/NOS-3 and GLD-2/GLD-3 branches act downstream of FBF, suggesting that FBF coordinately represses both pathways to inhibit entry into meiosis (figure 2*c*).

4. CONCLUSIONS AND PROSPECTS

This review describes the mitosis/meiosis decision in the germline of *C. elegans* and identification of two key regulatory pathways that govern that decision. One pathway is the GLP-1/Notch pathway, which mediates signalling between the DTC 'niche' and the germline. The second is the FBF/GLD/NOS RNA regulatory circuit within the germline. The balance between mitosis and meiosis appears to be controlled by GLP-1/Notch enhancement of FBF mitosis-promoting activity near the DTC, and a switch to GLD/NOS meiosis-promoting activities as germ cells leave the DTC influence (figure 2*c*). The actual mechanism of the switch from the FBF mode to the GLD/NOS mode is likely to involve feedback loops that strengthen the transition from FBF-dependent to GLD/NOS-dependent fates.

Do these two regulatory pathways control the mitosis/meiosis decision in germlines of other metazoans? A general role for Notch signalling in control of germline fates remains unknown, but Notch signalling does control proliferation of the *Drosophila* wing disc (Baonza & Garcia-Bellido 2000) and of myogenic progenitor cells and haemopoietic stem cells in vertebrates (Varnum-Finney *et al.* 2000; Conboy & Rando 2002). The PUF proteins control germline stem cells in *C. elegans* (Crittenden *et al.* 2002) and *Drosophila* (Lin & Spradling 1997; Forbes & Lehmann 1998), and are present in mammalian germline stem cells (Moore *et al.* 2003), suggesting that their role in germline development may be conserved. Indeed, PUF proteins may be involved even more broadly in stem cell regulation, since they promote mitosis in yeast (Kennedy *et al.* 1997) and *Dictyostelium* (Souza *et al.* 1999). Finally, the GLD proteins and NOS-3 are conserved RNA regulators (Jones & Schedl 1995; Kraemer *et al.* 1999; Eckmann *et al.* 2002; Wang *et al.* 2002); whether they control the mitosis/meiosis decision in other metazoan germlines is unknown.

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GLOSSARY

DTC: distal tip cell