Cyclin E and Cdk2 Control GLD-1, the Mitosis/Meiosis Decision, and Germline Stem Cells in Caenorhabditis elegans

Johan Jeong1, Jamie M. Verheyden2, Judith Kimble1,2,3

1 Program in Cellular and Molecular Biology, University of Wisconsin–Madison, Madison, Wisconsin, United States of America, 2 Howard Hughes Medical Institute, University of Wisconsin–Madison, Madison, Wisconsin, United States of America, 3 Department of Biochemistry, University of Wisconsin–Madison, Madison, Wisconsin, United States of America

Abstract

Coordination of the cell cycle with developmental events is crucial for generation of tissues during development and their maintenance in adults. Defects in that coordination can shift the balance of cell fates with devastating clinical effects. Yet our understanding of the molecular mechanisms integrating core cell cycle regulators with developmental regulators remains in its infancy. This work focuses on the interplay between cell cycle and developmental regulators in the Caenorhabditis elegans germline. Key developmental regulators control germline stem cells (GSCs) to self-renew or begin differentiation: FBF RNA–binding proteins promote self-renewal, while GLD RNA regulatory proteins promote meiotic entry. We first discovered that many but not all germ cells switch from the mitotic into the meiotic cell cycle after RNAi depletion of CYE-1 (C. elegans cyclin E) or CDK-2 (C. elegans Cdk2) in wild-type adults. Therefore, CYE-1/CDK-2 influences the mitosis/meiosis balance. We next found that GLD-1 is expressed ectopically in GSCs after CYE-1 or CDK-2 depletion and that GLD-1 removal can rescue cye-1/cdk-2 defects. Therefore, GLD-1 is crucial for the CYE-1/CDK-2 mitosis/meiosis control. Indeed, GLD-1 appears to be a direct substrate of CYE-1/CDK-2: GLD-1 is a phosphoprotein; CYE-1/CDK-2 regulates its phosphorylation in vivo; and human cyclin E/Cdk2 phosphorylates GLD-1 in vitro. Transgenic GLD-1(AAA) harbors alanine substitutions at three consensus CDK phosphorylation sites. GLD-1(AAA) is expressed ectopically in GSCs, and GLD-1(AAA) transgenic germlines have a smaller than normal mitotic zone. Together these findings forge a regulatory link between CYE-1/CDK-2 and GLD-1. Finally, we find that CYE-1/CDK-2 works with FBF-1 to maintain GSCs and prevent their meiotic entry, at least in part, by lowering GLD-1 abundance. Therefore, CYE-1/CDK-2 emerges as a critical regulator of stem cell maintenance. We suggest that cyclin E and Cdk-2 may be used broadly to control developmental regulators.

Introduction

Metazoan development coordinates progression through the cell cycle with key developmental events, such as stem cell maintenance, cell fate specification and patterning. An emerging theme is that core cell cycle regulators can influence development in addition to their more traditional role. For example, cyclin E affects the Drosophila endocycle [1,2], CDK1 influences Drosophila neuroblast asymmetric divisions [3] and cyclin D controls the Caenorhabditis elegans asymmetric division of somatic gonadal precursors [4]. Yet our understanding of the molecular interplay between cell cycle and developmental regulators remains in its infancy with only a few exceptions (e.g. MyoD and CKI [5–7]).

The C. elegans adult germline provides a superb model to investigate cell cycle controls in a developmental context [8]. Germ cells progress from mitotic divisions at the distal end of the adult gonad through meiotic prophase I in the middle to overt differentiation as sperm or oocyte at the proximal end. Here we focus on the distal germline, including the “mitotic zone” and adjacent “transition zone” (Figure 1A). The mitotic zone houses a pool of >200 mitotically dividing germ cells, whereas the transition zone contains germ cells that have entered the meiotic cell cycle. The transition zone is recognized by the presence of crescent-shaped DAPI-staining of nuclei, typical of the first step in pairing homologous chromosomes [9]. As germ cells move proximally through the mitotic zone, most enter meiotic S-phase prior to their entry into the transition zone [10–12]. The distal germline therefore presents an orderly maturation from stem cell through transit-amplifying cell to meiotic entry and differentiation.

Major developmental regulators controlling the choice between germline self-renewal and differentiation are well established (Figure 1B) [8]. A single somatic cell, the distal tip cell (DTC), provides the stem cell niche, and Notch signaling from the DTC drives self-renewal at the expense of meiotic entry and differentiation. Germ cells within the niche express FBF-1 and FBF-2, two nearly identical and largely redundant PUF (Pamilio and FBF) RNA-binding proteins [13,14]; the FBF proteins promote germline self-renewal and repress differentiation [15–17]. When the...
DTC is removed by laser ablation or when GLP-1/Notch signaling or both FBFs are removed by either mutation or RNAi, germ cells leave the mitotic cell cycle, enter the meiotic cell cycle, and progress through meiotic prophase and gametogenesis. Previous work has identified conserved developmental regulators that control the choice between self-renewal and differentiation in this tissue. In this work, we focus on cyclin E/Cdk-2, which is a core cell cycle kinase, and GLD-1, a key regulator of stem cell differentiation. Our work shows that cyclin E/Cdk-2 phosphorylates GLD-1 and lowers its abundance in stem cells via a post-translational mechanism. We also find that a post-transcriptional GLD-1 regulator, called FBF-1, works synergistically with cyclin E/Cdk-2 to ensure that GLD-1 is off in germline stem cells. When both FBF-1 and cyclin E/Cdk-2 are removed, the stem cells are no longer maintained and instead differentiate. Our findings reveal that cyclin E/Cdk-2 kinase is a critical stem cell regulator and provide a paradigm for how cell cycle regulators interface with developmental regulators.

**Figure 1. The mitosis/meiosis decision and its regulation.** (A) Schematic of adult distal germline. The distal tip cell (DTC) niche (red) resides at the distal end. The mitotic zone contains mostly germ cells in the mitotic cell cycle (yellow) but also includes some germ cells in meiotic S-phase (green) at its proximal edge; the germline stem cell (GSC) pool is located at the distal end and consists of 30–70 germ cells. The transition zone contains germ cells in meiotic S-phase (green circles) and meiotic prophase (green crescents). The dashed line marks the boundary between mitotic and transition zones. (B) Skeleton of regulatory network controlling the decision between germline self-renewal and differentiation, a decision that also controls the choice between mitotic and meiotic cell cycles. See Introduction for details. doi:10.1371/journal.pgen.1001348.g001

**Author Summary**

How are cell cycle regulators coordinated with cell fate and patterning regulators during development? Several studies suggest that core cell cycle regulators can influence development, but molecular mechanisms remain unknown for the most part. We have tackled this question in the nematode Caenorhabditis elegans. Specifically, we have investigated how cell cycle regulators affect germline stem cells. Previous work had identified conserved developmental regulators that control the choice between self-renewal and differentiation in this tissue. In this work, we focus on cyclin E/Cdk-2, which is a core cell cycle kinase, and GLD-1, a key regulator of stem cell differentiation. Our work shows that cyclin E/Cdk-2 phosphorylates GLD-1 and lowers its abundance in stem cells via a post-translational mechanism. We also find that a post-transcriptional GLD-1 regulator, called FBF-1, works synergistically with cyclin E/Cdk-2 to ensure that GLD-1 is off in germline stem cells. When both FBF-1 and cyclin E/Cdk-2 are removed, the stem cells are no longer maintained and instead differentiate. Our findings reveal that cyclin E/Cdk-2 kinase is a critical stem cell regulator and provide a paradigm for how cell cycle regulators interface with developmental regulators.

FBF is a broad-spectrum regulator of >1000 mRNAs, which include the three gld mRNAs [15,17,20,27]. FBF also controls many core components of the meiotic cellular machinery, including HIM-3 [17,20,29]. Most relevant here is the FBF repression of gld-1 mRNA, which is exerted directly through a cis-acting regulatory element in the gld-1 3’ untranslated region and which is responsible for lowering gld-1 expression in the distal mitotic zone [15,27]. In addition, the roles of FBF-1 and FBF-2 are subtly different even though they are redundant for maintenance of germline self-renewal [15,30]. For example, in fbf-1 single mutants (but not in fbf-2 mutants), GLD-1 is expressed ectopically in the distal-most germ cells within the niche, even though those distal germ cells remain in the mitotic cell cycle and germline self-renewal is maintained in both single mutants. Therefore, simply the presence of GLD-1 protein does not drive germ cells from the mitotic into the meiotic cell cycle [15,30].

Cyclin-dependent-kinases (CDKs) govern the cell cycle in virtually all eukaryotes, and cyclins are universal regulators of CDK activity and substrate specificity [31–34]. Phosphorylation by an active cyclin/CDK complex often affects the stability or activity of its substrates [31–33]. The C. elegans CDKs control cell cycle progression in both somatic and germline tissues [36–38]. Here we focus on cyclin E, which is best known as a regulator of the G1/S transition [39,40]. In C. elegans, the cyclin E homolog, known as CYE-1, acts with Cdk-2, the Cdk2 homolog, to facilitate the G1/S transition in somatic cells and to promote proliferation of germ cells [36,41–45]. In the adult germline, CYE-1 is easily detectable throughout the mitotic zone but becomes undetectable in the transition zone and pachytene region [43]. Its presence in germ cells throughout the mitotic zone suggests that CYE-1 may have a broader role than control of the G1/S transition, because half of the mitotic zone germ cells are in S-phase [11]. In the transition zone, the GLD-1 translational repressor acts directly on cye-1 mRNA to reduce its activity [23]. This GLD-1 control of cye-1 mRNA translation provided the first link between developmental and cell cycle regulators in this system.

In addition to its role in cell cycle regulation, cyclin E can affect developmental events. In C. elegans, non-vulval cells are transformed into vulval cells upon CYE-1 removal [42], the asymmetric division of somatic gonadal precursor cells is rendered symmetrical [4] and quiescent somatic precursor cells are spurred to differentiate [45]. In Drosophila, cyclin E affects neuroblasts [46,47] and ovarian follicle stem cells [48]. Most recently, cyclin E has been implicated in the control of human embryonic stem cells [49]. Therefore, the coordination of cell cycle with development appears to be achieved, at least in part, by a dual role of key cell cycle regulators [39,50].

Here we report that CYE-1/CDK-2 controls the balance between proliferation and differentiation in the C. elegans germ-line, at least in part, by lowering GLD-1 abundance in dividing cells. We provide multiple lines of evidence to suggest that GLD-1 is a direct substrate of CYE-1/CDK-2 phosphorylation. These results forge a molecular and functional link between the CYE-1/CDK-2 cell cycle regulator and the GLD-1 developmental regulator. Because GLD-1 represses cye-1 mRNA translation [23], these two regulators engage in a double negative feedback loop. Finally, we demonstrate that CYE-1/CDK-2 and FBF-1 normally function together to repress GLD-1 and prevent meiotic entry of germ cells in the stem cell pool. Therefore, CYE-1/CDK-2 emerges as a critical regulator of germline stem cell maintenance.
**Results**

**CYE-1/CDK-2 controls the mitosis/meiosis balance in adult germlines**

To assess CYE-1 function in the adult hermaphrodite germline, we placed mid to late L4 larvae on bacteria expressing double-stranded RNA (dsRNA) complementary to *cye-1* mRNA and scored germlines after 48 hours, well into adulthood. This regimen effectively reduced *cye-1* expression (Figure 2A), and is referred to henceforth as adult RNAi (aRNAi).

*aRNAi* had a dramatic effect on the mitotic zone, which extends from the distal end of the adult germline along the gonadal axis to the first set of crescent-shaped nuclei in early meiotic prophase. Normally, the mitotic zone harbors actively cycling germ cells, it extends 18-20 germ cell diameters (gcd) along the gonadal axis from the distal end, and its total germ cell number exceeds 200 [51]. However, after *cye-1* aRNAi, this region was abnormal: germ cell nuclei were grossly enlarged (Figure 2A–2C), and enlarged distal germ cells were still observed. The possibility that the enlarged distal germinal nuclei might reflect partial rather than full depletion of CYE-1/CDK-2 activity. A *cye-1* null mutant or *rrf-1* mutants [54,55]; however, GLD-1 was abundant throughout the mitotic zone in both wild-type and *cye-1* (aRNAi) distal germlines: cells were not abnormally enlarged and HIM-3 distribution suggested that the enlarged distal germ cells may originate from some other part or parts of the mitotic zone and that the remaining germ cells are squeezed proximally as the ~75 enlarge. Regardless, we conclude that *cye-1* depletion causes ~75 of the >200 germ cells in the mitotic zone to enlarge abnormally and that it also causes the remaining >100 germ cells in the mitotic zone to enter meiotic prophase prematurely (Figure 2H, dashed lines between wild-type and *cye-1* (aRNAi) distal germlines). An alternative explanation might have been that >100 germ cells die, but no germ cell corpses or degenerating germ cells were seen during the transition from the wild-type >200 germ cells mitotic zone to the *cye-1* (aRNAi) ~75 germ cells mitotic zone. A third possibility is that the 75 enlarged germ cells come from some other part or parts of the mitotic zone and that the remaining germ cells are squeezed proximally as the ~75 enlarge. Regardless, we conclude that *cye-1* depletion causes ~75 of the >200 germ cells in the mitotic zone to enlarge abnormally and that it also causes the remaining >100 germ cells in the mitotic zone to enter meiotic prophase. Therefore, CYE-1/CDK-2 is required to achieve and maintain the normal balance between numbers of germ cells in the mitotic and meiotic cell cycles.

**CYE-1/CDK-2 acts through GLD-1 to control distal germ cells**

We postulated that CYE-1/CDK-2 might exert its influence in the mitotic zone through GLD-1, a key repressor of germ line proliferation (see Introduction). We first tested for a change in GLD-1 abundance after *cye-1* (aRNAi) depletion. In wild-type and control germlines, GLD-1 was barely detectable in the proximal mitotic zone and became abundant in the transition zone (Figure 3A, top), as seen previously [26,57]. By contrast, GLD-1 was abundant throughout the mitotic zone in both *cye-1* (aRNAi) and *cdk-2* (aRNAi) germlines (Figure 3A, middle and bottom). A similar GLD-1 increase was also observed in *gld-1* (q361) homozygotes (Figure S3A, S3B), which harbor a G227D point mutation in the KH domain resulting in loss of GLD-1 function [58]. Therefore, the GLD-1 increase upon *cye-1* (aRNAi)/CDK-2 depletion is not dependent on GLD-1 function. We conclude that CYE-1 and CDK-2 lower the abundance of GLD-1 in the wild-type mitotic zone.

To ask genetically if CYE-1/CDK-2 might function through GLD-1 in the mitotic zone, we depleted CYE-1 or *cdk-2* in *gld-1* null mutant germlines. Remarkably, the *cye-1* (aRNAi) and *cdk-2* (aRNAi) distal germlines: cells were not abnormally enlarged and HIM-3 failed to extend distally (Figure 3B, upper panels). Instead germ cells in the mitotic zone appeared similar to those in the wild-type. GLD-1 function in the wild-type mitotic zone.

We next attempted to ask if the enlarged distal germ cells were in the mitotic or meiotic cell cycle. To this end, we first stained for cytoplasmic REC-8, a marker of mitotic germ cells [10], and chromosomal HIM-3, a marker of meiotic germ cells [28]. In most *cye-1* (aRNAi) germlines (97.1%, n = 33), REC-8 was lower in the mitotic zone relative to controls (Figure 2B) and HIM-3 expanded distally (82.5%, n = 40; Figure 2C). This shift in marker distribution suggested that the enlarged distal germ cells may have entered the meiotic cell cycle. However, the finite mitotic index, albeit low, suggested that at least some germ cells remained in the mitotic cell cycle. To explore this question further, we stained DNA content of DAPI-stained nuclei, comparing values for the abnormally enlarged germ line nuclei to those of nuclei in the somatic DTC (2n) and pachytene germ cells (4n). This analysis revealed a range of DNA content from 2n to 4n in the abnormally enlarged germ line nuclei that was essentially the same as seen in wild-type mitotic zone nuclei (Figure 2G). DNA content therefore does not distinguish between germ cells being in mitotic or meiotic S-phase.
Figure 2. CYE-1/CDK-2 controls the mitosis/meiosis balance. (A–C) Dissected gonads stained with antibodies (left panels) and DAPI (right) after arNAi treatment for 48 hrs from L4. Arrowhead, distal end of the gonad; dashed line, boundary between mitotic and transition zones; arrows, nuclei with crescent-shape typical of meiotic prophase. Right/left images are the same gonad; germlines in each panel were treated identically and...
protein prepared from *E. coli* with purified human cyclin E/CDK2 complex (Cell Signaling) and radiolabelled ATP. As expected, cyclin E/CDK2 phosphorylated the positive control histone H1 but not the negative control BSA (Figure 4F, lanes 1–4). More importantly, the complex phosphorylated wild-type GLD-1(wt) (Figure 4F, lane 5), but not a mutant, GLD-1(AAA), which had its three putative CDK phosphorylation site residues substituted to alanines (S22A, S39A, T348A) (Figure 4F, lane 7). We conclude that human cyclin E/CDK2 directly phosphorylates GLD-1 *in vivo*.

**GLD-1 CDK phosphorylation sites affect its abundance and mitotic zone size**

To ask if CYE-1/CDK2 controls GLD-1 abundance via its predicted phosphorylation sites, we generated transgenes encoding a GFP- and FLAG-tagged GLD-1 that was either wild-type or mutated at its putative CDK phosphorylation sites [dubbed transgenic (tr) GLD-1(wt) and trGLD-1(AAA), respectively] (Figure 5A). Both trGLD-1(wt) and trGLD-1(AAA) made functional protein, as assayed by *gld-1(0)* mutant rescue to fertility. The distribution of the transgenic proteins was analyzed in five independent lines for each protein with equivalent results. Expression of the trGLD-1(wt) protein was very low or undetectable in the distal mitotic zone (Figure 5B, top), but trGLD-1(AAA) was easily seen (Figure 5B, bottom). Indeed, the abundance of trGLD-1(AAA) in the distal germline was greater than that of trGLD-1(wt), when quantified using ImageJ (Figure 5C). The mitotic zone length in trGLD-1(AAA) germlines was also shorter: 13.9 gcd on average in trGLD-1(AAA) germlines (n = 40) compared to 17.8 gcd on average in trGLD-1(wt) control germlines (n = 36) (Figure 5B, 5D). A similar effect was observed in a *gld-1(0)* mutant background, both with respect to higher protein abundance at the distal end and shortening of the mitotic zone (Figure 5E). We note however that *cye-1* depletion increased trGLD-1(AAA) in the distal-most germ cells even more (Figure S9), suggesting that CYE-1/CDK2 represses GLD-1 abundance both directly and indirectly. We also measured total transgenic GLD-1 protein with anti-GLD-1 antibodies in Western blots, and total transgenic *gld-1* mRNA with primers to the *gfp* portion of the mRNA. The total trGLD-1(AAA) protein was twice as abundant as total trGLD-1(wt) protein (Figure 5E), but mRNA levels were comparable (Figure 5F). We conclude that the CDK phosphorylation sites in GLD-1 contribute to the control of its abundance in the distal germline and also contribute to control of mitotic zone length.

**CYE-1/CDK2 works with FBF-1 to maintain germline stem cells**

Earlier work showed that FBF-1 and FBF-2 repress *gld-1* expression in the distal germline [15], and this work shows that CYE-1/CDK2 lowers GLD-1 abundance in the same region. To ask if these two controls might function synergistically to control cell fate in the distal germline, we treated wild-type, *fbf-1(0)* and *fbf-2(0)* single mutants with *cye-1* aRNAi and examined their mitotic zones as described earlier. The distal-most germ cells did
GLD-1 abundance increases (left) and germ cells enlarge (right) in the cdk-2 aRNAi, showing that RNAi was effective. (C) Increases in the distal germline after cye-1 null mutant. Distal germline (top) or loop region, where germ cells normally dependent. (A–C) Dissected gonads stained after aRNAi treatment for the same settings. Scale bar, 20 μm. (A) Wild-type. GLD-1 abundance increases in the distal germline after cye-1 or cdk-2 aRNAi, each treated with control, cye-1, or cdk-2 aRNAi (Figure 6C). Two equivalent positions were scored: the distal-most region corresponding to what normally is the germline stem cell pool and a more proximal region corresponding to what normally is the transition zone (”distal” and ”proximal” squares in Figure 6C). GLD-1 was significantly more abundant in fbf-1(0); cye-1(aRNAi) and fbf-1(0); cdk-2(aRNAi) than in the equivalent position of controls (Figure 6D, middle). Indeed, the GLD-1 was more abundant in fbf-1(0); cye-1(aRNAi) and fbf-1(0); cdk-2(aRNAi) distal germ cells than in any region of control germlines (Figure 6D and 6E). Comparison of GLD-1 abundance in more proximal regions revealed only minor differences (Figure 6E). In contrast to fbf-1(0), we found no dramatic increase of GLD-1 in fbf-2(0); cye-1(aRNAi) germlines (Figure 6B, 6D, 6E). We conclude that the combined action of the CYE-1/CDK-2 and FBF-1 prevents the distal germ cells from entering into meiotic prophase.

To ask whether FBF-1 and CYE-1 normally function together to lower GLD-1 in the distal germline, we quantitated GLD-1 protein in confocal images of wild-type, fbf-1, and fbf-2 germlines, each treated with control, cye-1, or cdk-2 aRNAi (Figure 6C). GLD-1 was significantly more abundant in fbf-1(0); cye-1(aRNAi) and fbf-1(0); cdk-2(aRNAi) than in the equivalent position of controls (Figure 6D, middle). Indeed, the GLD-1 was more abundant in fbf-1(0); cye-1(aRNAi) and fbf-1(0); cdk-2(aRNAi) distal germ cells than in any region of control germlines (Figure 6D and 6E). Comparison of GLD-1 abundance in more proximal regions revealed only minor differences (Figure 6E). In contrast to fbf-1(0), we found no dramatic increase of GLD-1 in fbf-2(0); cye-1(aRNAi) germlines (Figure 6B, 6D, 6E). We conclude that the combined action of CYE-1/CDK-2 and FBF-1 maintains the extremely low GLD-1 abundance typical of wild-type distal-most germ cells. This combined action may work directly on GLD-1, indirectly through other proteins or perhaps most likely through action on both GLD-1 and other proteins in the network.

GLD-1 is normally repressed in self-renewing germ cells [15]. To test if entry into meiotic prophase in the distal-most fbf-1(0); cye-1(aRNAi) germ cells might rely on high GLD-1, we examined the effect of varying gld-1 dosage. We first examined germlines lacking GLD-1, and found that entry into meiotic prophase did not occur in the distal-most cells of gld-1(0); fbf-1(0) or gld-1(0); fbf-1(0); cye-1(aRNAi) germlines (Figure S6, upper and middle panels). This result is consistent with both CYE-1/CDK-2 and FBF-1 acting upstream of GLD-1 to prevent entry into meiotic prophase. Next we tested a germline with a single dose of wild-type gld-1: again entry into meiotic prophase did not occur in the distal-most cells in gld-1(0); fbf-1(0); cye-1(aRNAi) germlines (Figure S6, bottom). Therefore, the entry into meiotic prophase observed in the fbf-1(0); cye-1(aRNAi) distal-most germ cells (Figure 6A) is suppressed by lowering GLD-1 to a null or heterozygous state. We conclude that two copies of wild-type gld-1 are required to drive the distal-most germ cells into meiotic prophase when CYE-1 is depleted in fbf-1 mutants.

Distinct effects of CDK-1 and CDK-2 on the mitosis/meiosis decision

CDK-1 is the C. elegans homolog of mammalian CDK1 and is required for M-phase, in both somatic and germline tissues [44].
Like \textit{cdk-2} aRNAi, \textit{cdk-1} depletion shortened the mitotic zone (Figure S7A and S7B), as shown previously [44]. However, this \textit{cdk-1} effect was not accompanied by an expansion of GLD-1 to the distal end (95%, n = 21) (Figure S7A, top), and it was not synergistic with \textit{fbf-1(0)} or \textit{fbf-2(0)} mutants (Figure S7A and S7B). Most importantly, the distal-most germ cells in \textit{fbf-1(0); cdk-1(aRNAi)} did not acquire crescent-shaped nuclei typical of meiotic prophase, even though they were enlarged (Figure S7A, middle, and S7B). Similarly aRNAi depletion of CDK-4, CYA-2 and CYB-1 did not mimic \textit{cye-1/cdk-2} effects. We suggest that CDK-1 functions in the germline as a core cell cycle regulator of M-phase, but does not repress the developmental regulator GLD-1.

\section*{Discussion}

This work explores the role of CYE-1 and CDK-2 in the \textit{C. elegans} germline and comes to two major conclusions. First, CYE-1 and CDK-2 regulate the mitosis/meiosis balance, and do so, at least in part, by downregulating abundance of the GLD-1 translational repressor. Second, CYE-1 and CDK-2 work together
with FBF-1 to prevent cells in the germline stem cell pool from precocious meiotic entry and differentiation. Our discussion places these findings in context with previously known controls of the mitosis/meiosis decision and stem cell maintenance.

The GLD-1 developmental regulator is likely a direct CYE-1/CDK-2 substrate

GLD-1 normally facilitates the switch of germ cells from the mitotic cell cycle into the meiotic cell cycle (see Introduction). In wild type, the GLD-1 protein is either extremely low or absent in the germline stem cell pool, but after depletion of either CYE-1 or CDK-2, GLD-1 becomes abundant in the distal-most germ cells. That GLD-1 increase is not simply a reflection of a mitotic cell cycle defect since depletion of other cell cycle regulators (e.g. CDK-1, CDK-4, CYA-2, EMB-30) does not lead to a similar GLD-1 increase in the distal germine [this work and [63]].

Therefore, CYE-1/CDK-2 appears to govern GLD-1 independently of its canonical role in the cell cycle.

Several lines of evidence suggest that GLD-1 is a direct substrate of CYE-1/CDK-2. First, endogenous GLD-1 is a phosphoprotein, as shown here and corroborated by mass spectrometry [64]. Indeed, CDK-2 was predicted to be the best candidate kinase for the GLD-1 peptides identified in the *C. elegans* phosphoproteome [64]. Second, GLD-1 phosphorylation depends on CYE-1 and CDK-2 in vivo, and human cyclin E/CDK2 phosphorylates GLD-1 in *vitro*. Third, transgenic GLD-1 with serine/threonine to alanine substitutions at predicted CDK phosphorylation sites is ectopically expressed in the distal germline. Yet no obvious cell cycle perturbation was observed in the transgenic GLD-1(AAA) germlines, supporting the suggestion that GLD-1 phosphorylation represents a cell cycle independent role of CYE-1/CDK-2. Intriguingly, cyclin B/Cdk1 phosphorylates a mammalian GLD-
1 homolog, Sam68 (Src-associated in mitosis), during mitosis [65], implying a conserved link between proteins in the GLD-1 family and regulators of the cell cycle. We suggest that CYE-1/CDK-2 phosphorylation of GLD-1 affects GLD-1 stability. This mechanism is attractive both for its simplicity and the existence of precedents [66,67]. Consistent with that idea, abundance of the trGLD-1(AAA) protein increased substantially over trGLD-1(wt) protein. However, the trGLD-1(AAA) increase was not as great as the GLD-1 increase in cye-1(aRNAi) or cdk-2(aRNAi) distal germlines (compare Figure 3A and Figure 5B). Furthermore, the trGLD-1(AAA) was further increased after cye-1 depletion. Therefore, CYE-1/CDK-2 is likely to act on GLD-1 abundance by both direct and indirect mechanisms.

Mutual repression between GLD-1 and CYE-1/CDK-2

This work together with a previous study [23] reveals a double-negative feedback loop between CYE-1/CDK-2 and GLD-1 (Figure 7, blue shading). Mutual repression is a classical network motif and has been suggested to serve as a ‘toggle switch’ for controlling decisions between two states [reviewed in 68]. Here the two relevant states are the mitotic and meiotic cell cycles. CYE-1/CDK-2 represses GLD-1 to promote mitotic divisions [this work], and GLD-1 represses cye-1 mRNA to promote meiotic progression [23]. However, loss of CYE-1 or CDK-2 does not flip germ cells from the mitotic to meiotic cell cycle; instead it shifts the balance between the two cycles in favor of meiotic entry. Therefore, the mutual repression between GLD-1 and CYE-1/CDK-2 does not provide a toggle switch on its own. We suggest instead that this CYE-1/CDK-2 and GLD-1 double-negative feedback loop contributes to the bi-stable switch between the mitotic and meiotic cell cycles and helps to maintain the normal balance between them.

The C. elegans mitosis/meiosis decision is influenced not only by CYE-1/CDK-2 and GLD-1 but also by a distinct double-negative feedback loop between Notch signaling and GLD-1 (Figure 7, yellow shading). Notch signaling promotes mitotic divisions through GLD-1 repression, albeit indirectly via the FBFs [15,30], while GLD-1 directly represses translation of the glp-1/Notch receptor mRNA [69]. Therefore, two circuits of mutual repression converge on GLD-1, one involving core cell cycle regulators and the other involving differentiation regulators. We suggest that this two-fisted mutual repression renders the switch more robust and helps to ensure that the two cell cycle programs are incompatible.

Mutual repression between key regulators of the cell cycle and cell differentiation is emerging as a theme of developmental control. In addition to the example reported here, other examples exist. One example is in developing skeletal muscle of mammals.

Figure 6. CYE-1/CDK-2 and FBF-1 work together to lower GLD-1 and to prevent meiotic entry in the germ line stem cell pool. (A,B) Dissected gonads stained after aRNAi; conventions same as in Figure 2; germ lines were treated identically and images taken at the same settings. Scale bar, 20 μm. (A) CYE-1/CDK-2 depletion in fbf-1 single mutants. Whereas control distal germ lines appear wild-type (top), most fbf-1; cye-1(aRNAi) (middle) and fbf-1; cdk-2(aRNAi) (bottom) germ lines have abundant GLD-1 to the distal end and also have lost nuclei typical of the mitotic cell cycle and gained crescent-shaped nuclei typical of meiotic prophase all the way to the distal end (73%, n = 70). (B) CYE-1/CDK-2 depletion in fbf-2 single mutants. The effects of CYE-1/CDK-2 depletion in fbf-2 single mutants are similar to those seen in wild-type (compare to Figure 2). (C–E) GLD-1 quantitation in confocal images measured using ImageJ, averaged, and plotted. Error bars indicate 95% confidential limits; asterisks denote a statistically significant difference with p<0.002 using Student’s t-test. (C) Quantitation was done at two sites: the distal-most germ cells (Distal) and more proximal germ cells (Proximal). The proximal site was located just beyond the MZ/TZ boundary in those germ lines with a boundary; however in fbf-1; cye-1(aRNAi) and fbf-1; cdk-2(aRNAi), no MZ/TZ boundary exists so this proximal site was defined arbitrarily as 12 germ cell diameters from the distal end. (D) GLD-1 abundance at the distal-most site, quantitated in strains as noted. (E) GLD-1 abundance at the more proximal site, quantitated in strains as noted.

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Figure 7. Two double negative feedback loops control GLD-1 abundance. Blue shading, mutual repression of GLD-1 and CYE-1/CDK-2; yellow shading, mutual repression of GLD-1 and the Notch/FBF arm of GLD-1 control; green shading, GLD-1 promotes meiotic entry and differentiation and it represses mitotic divisions and germline self-renewal.

MyoD activates expression of CDK inhibitors and thereby inhibits cell cycle progression; conversely, CDK1 or CDK2 phosphorylates MyoD and promotes its turnover to prevent cell differentiation [reviewed in 6]. A second example occurs in Drosophila neuroblasts. Prospero represses transcription of cell cycle regulators, including cyclin E, to block cell cycle progression [70, 71], and conversely cyclin E inhibits Prospero by promoting its cortical localization [47]. Those two examples focus on mutual repression between cell cycle regulators and transcription factors. With this work, the mutual repression between GLD-1 and CYE-1/CDK-2 extends this important theme to translational regulators.

CYE-1/CDK-2 is a critical regulator of germline stem cell maintenance

A stem cell pool resides at the distal end of the adult germline [8,63]. The FBF-1 and FBF-2 proteins are well-established and largely redundant regulators of germline self-renewal; iffy-1 and iffy-2 single mutants maintain germline stem cells, but iffy-1 iffy-2 double mutants do not – instead, their germline stem cells enter meiotic prophase and differentiate [15,30]. We have found that CYE-1/CDK-2 depletion in iffy-1 single mutants similarly causes germline stem cells to enter meiotic prophase and differentiate. Therefore, CYE-1/CDK-2 emerges as a critical regulator of germline self-renewal.

The iffy-1 cdk-2 effect on germline self-renewal occurs in iffy-1, but not in iffy-2 mutants. Why might this be true? One interpretation is that FBF-1 and CYE-1/CDK-2 function in parallel pathways, while FBF-2 and CYE-1/CDK-2 function together in the same pathway. We cannot exclude this idea, but suggest that an alternate explanation, which is based on FBF-specific effects of FBF cross-regulation, may be simpler. In iffy-1 and iffy-2 single mutants, the remaining FBF protein increases in abundance, but effects on distribution are different. The increased FBF-1 expands proximally in iffy-2 mutants with the result that more germ cells than normal possess FBF; by contrast, the increased FBF-2 remains limited to a small region at the distal-most end in iffy-1 mutants with the result that fewer germ cells than normal have FBF [30]. We suspect that this difference may result from a differential stability of FBF-1 and FBF-2 proteins, although we have not been tested. Regardless of the underlying mechanism, the spatially expanded FBF-1 may compensate for the loss of FBF-2 better than the spatially restricted FBF-2 can compensate for loss of FBF-1. This spatial difference could explain why GLD-1 expands distally in iffy-1 but not iffy-2 mutants [15] and why iffy-1(aRNAi) has a synergistic effect with iffy-1 but not iffy-2 mutants (this work). Based on this reasoning, Figure 7 depicts FBF-1 and FBF-2 in one pathway and CYE-1/CDK-2 in a parallel pathway. However, we cannot exclude regulatory interactions between the FBF and CDK pathways.

Both FBF-1 and CYE-1/CDK-2 down-regulate GLD-1 in the germline stem cell pool (Figure 7) ([13,27] and this work). FBF-1 keeps GLD-1 low by its direct post-transcriptional repression of GLD-1 mRNA, while CYE-1/CDK-2 keeps GLD-1 low post-translationally. One simple idea is that GLD-1 must be increased to a critical threshold to switch from the mitotic cell cycle into meiotic prophase. Consistent with that idea, reduction of GLD-1 by one copy prevents entry into meiotic prophase in the distal germ cells of either an iffy-1 iffy-2 double mutant or an iffy-1; iffy-1(aRNAi) animal ([15] and this work). It is important to emphasize that other factors (e.g. GLD-2/GLD-3) also affect the decision [19,20]. Moreover, FBF-1 is a broad-spectrum regulator of >1000 mRNAs [17], with multiple targets that promote differentiation (e.g. ERK/MAP kinase [16]). We suspect that CYE-1/CDK-2 may similarly have many substrates. Therefore the regulatory network depicted in Figure 7 is highly simplified: it highlights the two double-negative feedback loops that converge on GLD-1 but does not illustrate the complete network.

A central role for cyclin E and CDK2 in stem cell maintenance may be conserved. In human embryonic stem cells (hESCs), CDK2 promotes the G1/S transition and prevents differentiation, perhaps by maintaining a short G1 that is not subject to differentiation signals [49]. In addition, Drosophila CycE maintains neuronal stem cells by inhibiting the Prospero transcription factor, either directly or indirectly [47]. Our results support and extend this idea in several ways. Most importantly, our analysis places the C. elegans cyclin E and CDK2 homologs into the developmental pathway controlling self-renewal or differentiation. We identify a double-negative feedback loop between CYE-1/CDK-2 and GLD-1 that operates in parallel to a second double-negative feedback loop between Notch signaling and GLD-1. Although details will surely be different among the various stem cell systems, this regulatory logic provides a paradigm for the control of stem cell identity.

Materials and Methods

Nematode strains and RNA interference

All strains were derived from Bristol strain N2 and maintained at 20°C as described [72]. Mutants used in this work were: iffy-1(ch10) [43], gld-1(g485) [58], gld-1(q361) [58], gld-3(q730) [73], him-3(e1147) [74], nos-3(q650) [75], iffy-1(ok91) [15], iffy-2(q738) [30], eri-1(mg366) [54], rrf-1(pk1477) and nfr-3(pk1426) [55]. Those mutants not viable as homozygotes were maintained in balanced strains: JK4057, iffy-1(ch10)/ht2(q485); JK3025, gld-1(ch10)/ht2(q485); JK4018, gld-1(ch10)/ht2(q485); JK3182, gld-3(q730) nos-3(q650)/mhn1[+]/mhn1[+]; msn1[+]/msn1[+]; dpy-10/e128].

RNA interference (RNAi) was performed essentially as described [76]. For adult RNAi, synchronized L4s were placed on NGM plates seeded with dsRNA-expressing or empty vector control bacteria, and incubated at 20°C.

Generation of transgenic lines

JK4117 (iffy-1/kht2; q485); q134): two DNAs (a PCR product harboring the wild-type iffy-1 genomic fragment with 1.2 kb 5’ and 854 bp 3’ flanking sequences and pTG96 carrying the Pm4::gfp co-transformation marker [77]) were co-injected into adult wild-type gonads to generate the qEs650 extrachromosomal array.
qEx660 was integrated into a chromosome using UV-TMP [78] to
generate qIs154, which was crossed to cye-1(eh10) to create JK4117.

trGLD-1(wt); pMM016 DNA (gift from T. Schedl) harbors (1) a
wild-type genomic gld-1 fragment that includes 1.1 kb 5’ and
1.5 kb 3’ flanking sequences and that was altered to include GFP-
and FLAG-encoding sequences fused to the GLD-1 C-terminus
and (2) unc-119 to select for transformants. Microparticle
bombardment was done using established protocols ([79] and T.
Schedl lab protocol), with modifications. Briefly, 4 μg of linear
plasmids were mixed with 40 μl of 0.1 M spermidine-treated 1 μm
gold-beads (Bio-Rad) and 100 μl of 2.5 M CaCl2; particles were
transferred to the macrorocket after ethanol washes and then
bombarded as normal. The bombarded worms were collected with
M9 buffer after 1 hr incubation at room temperature, placed on
rich media plates, and grown at 20°C. Bombardment of pMM016
generated five independent transgenic lines, qIs168 – qIs172.

The five had similar effects on GLD-1 abundance and
distribution; generated five independent transgenic lines,
qIs168 – qIs172. Bombardment generated five trGLD-1(AAA) lines,
confirmation of gld-1(q485). All five had similar effects on GLD-1 abundance and
distribution; (qIs173 – qIs176) was selected for further analyses, including confirmation of
gld-1(q485) rescue. trGLD-1(AAA); pJK1571 DNA modifies three sites in pMM016
to encode GLD-1(S22A, S39A, and T348A); GFP::FLAG. Bombardment
generated five trGLD-1(AAA) lines, qIs173 – qIs176 and
qIs182. All five had similar effects on GLD-1 abundance and
distribution; (qIs174) was selected for further analyses, including confirmation of
qIs174 rescue. The human cyclin E/CDK2 complex (Cell Signaling) was
diluted in [20 mM MOPS (pH 7.5), 1 mM EDTA, 0.03% Brij-35,
5% glycerol, 1 mg/ml BSA, 0.1% beta-mercaptoethanol]. About
27 pmole of purified recombinant GLD-1 was incubated in
[8 mM MOPS (pH 7.0), 1 mM EDTA with 0.92 pmole of cyclin
E/CDK2 and 10 μCi of [gamma-32P]ATP] for 1 hr at room
temperature, boiled in protein sample buffer, and separated on a
4–12% SDS-PAGE gel. Phosphorylation was visualized by
autoradiography.

**Supporting Information**

**Figure S1** CYE-1 effects on the mitotic zone, additional views.
(A) After 72 or 96 hours of cye-1 aRNAi treatment of wild-type hermaphrodites, germ cells degenerate in the region that had been the
discoted mitotic zone. Dissected gonads stained for HIM-3 (left) and
DAPI (right). Open triangles indicate distal tip cells, which are
somatic cells that do not express HIM-3. Other marks follow
conventions of Figure 2: closed arrowheads mark the boundary between mitotic and
transition zones and small arrows point to crescent-shaped germ
cells. (B) The mitotic zone shortens after aRNAi in wild-type, L1
RNAi in nfi-1(pk1417) mutants and aRNAi in ori-1(ng366) and rrf-
3(pk1426) mutants from L4 stage. Error bars indicate 95%
confidence limits; one asterisk denotes a statistically significant
difference (p<0.05. E−10 using Student’s t-test).

Preparation of recombinant GLD-1

A full-length wild-type gld-1 cDNA was cloned into pET-21b
using Sal I and Xho I restriction sites to make pJK1341. To create
pJK1347 (GLD-1 S22A, S39A, T348A), we changed the
three conserved CDK phosphorylation sites in GLD-1 by site-directed
mutagenesis (Stratagene). Primer sequences are available upon
request. Recombinant proteins were expressed in BL21(DE3)
(Novagen) cells by adding 0.1 mM IPTG and incubating 4 hrs at
30°C. Proteins were purified using Ni-NTA columns (Novagen)
under native conditions using the manufacturer’s protocol.

**In vitro GLD-1 phosphorylation**

The human cyclin E/CDK2 complex (Cell Signaling) was
diluted in [20 mM MOPS (pH 7.5), 1 mM EDTA, 0.03% Brij-35,
5% glycerol, 1 mg/ml BSA, 0.1% beta-mercaptoethanol]. About
27 pmole of purified recombinant GLD-1 was incubated in
[8 mM MOPS (pH 7.0), 1 mM EDTA with 0.92 pmole of cyclin
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difference (p<0.05. E−10 using Student’s t-test).

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**Figure S2** CYE-1 promotes germline cell divisions autonomously.
(A) Above, cye-1 gene structure. Below: black shows extent of
cye-1 deletion in the eh10 null allele [43]; red shows the region used
to make the q134 transgene. (B–D) Images include the somatically
rescued strain, JK4117 with genotype cye-1(eh10); qIs134. Specific
genotypes labeled in the figure. (B) The q134 transgene expresses
CYE-1 in somatic but not germ cells. Embryos stained with anti-
cye-1 (red), anti-Angel1 (green), and DAPI (blue); red shows the region used to make the q134 transgene. (C) The q134 transgene expresses
CYE-1 in somatic but not germ cells. Embryos stained with anti-
cye-1 (red), anti-Angel1 (green), and DAPI (blue); red shows the region used to make the q134 transgene. (D) Specific
concepts of Figure 2: closed arrowheads mark the boundary between mitotic and
transition zones and small arrows point to crescent-shaped germ
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RNAi in nfi-1(pk1417) mutants and aRNAi in ori-1(ng366) and rrf-
3(pk1426) mutants from L4 stage. Error bars indicate 95%
confidence limits; one asterisk denotes a statistically significant
difference (p<0.05. E−10 using Student’s t-test).

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**Figure S3** cye-1 aRNAi defects in the mitotic zone depend on
functional GLD-1. gld-1(q361) is a point mutation that inactivates
GLD-1 without affecting its stability [21,22,58]. (A) gld-1(q361)
homzygotes were treated with either control, cye-1, or cdk-2 aRNAi for 48 hrs and then dissected and stained for GLD-1 (left) or DAPI (right). Confocal images of GLD-1 staining were stacked. Asterisks indicate sites of GLD-1 increase in the mitotic zone; arrowheads, distal end of gonad; scale bar, 20 μm. (B) Quantitation of GLD-1 abundance at the distal end of the gonad in gld-1(q361) homozygotes after control, cye-1, or cdk-2 aRNAi. GLD-1 intensities were quantified and graphed. Error bars indicate 95% confidence limits; one asterisk denotes a statistically significant difference (p<0.001 using Student’s t-test).

Figure S4 CYE-1 is depleted after cye-1 aRNAi treatment of the gld-1 null mutant. Dissected gonads stained for CYE-1 (left) or DAPI (right) in gld-1(0) mutants (above) or gld-1(0) mutants depleted for CYE-1 (below).

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Figure S5 CYE-1 and GLD-1 distributions in wild-type and gld-3(0) nas-3(0) tumorous germline. (A, B) Dissected germlines were stained for CYE-1 (red), GLD-1 (green) and DAPI (blue). Arrowheads mark distal end and broken lines depict boundary between mitotic and transition zones. (A) Distal wild-type gonad. CYE-1 and GLD-1 distributions have little overlap in the mitotic zone. (B) gld-3 nas-3 tumorous germline. The distribution of CYE-1 expands while that of GLD-1 shrinks.

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Figure S6 GLD-1 removal in either one or two doses restores the mitotic zone to fbf-1; cye-1(aRNAi) germlines. Dissected germlines stained for CYE-1 (red) and DAPI (white). Arrowheads mark distal end and broken lines depict boundary between mitotic and transition zones. Top, CYE-1 is expressed normally in the distal gonad. (A) Dissected gonads with conventions and transition zones. Top, CYE-1 is expressed normally in the distal gonad. (B) Quantitation of GFP intensity in the distal-most germline. Error bars indicate 95% confidence limits; asterisk denotes a statistically significant difference (p<0.0001 using Student’s t-test).

Found at: doi:10.1371/journal.pgen.1001348.s005 (1.77 MB TIF)

Figure S7 CDK-1 does not lower GLD-1 abundance in the distal gonad. (A) Dissected gonads with conventions and treatments as described in Figure 2 legend. Germlines were treated identically and GLD-1 images taken at the same settings. Scale bar, 20 μm. (B) Miotic zone lengths. Error bars indicate 95% confidence limits. One asterisk denotes a statistically significant difference (p<6.5x10^-5 using Student’s t-test); two asterisks denote the lack of a statistically significant difference (p>0.6 using Student’s t-test).

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Figure S8 CDK phosphorylation influences both GLD-1 abundance in the distal germline and the mitotic zone length. (A) Distal end of dissected gonads. Left panels, stacked confocal images of GFP fluorescence to show abundance of transgenic GLD-1; right panels, DAPI staining to show distal end and mitosis/meiosis boundary. Solid triangle marks distal end; small v and larger V mark steps of comparable GLD-1 abundance; dashed line indicates boundary between mitotic and transition zones. Germlines were treated identically and confocal fluorescent images taken at the same settings and then stacked along Z-axis. Scale bar, 20 μm. (B) Quantitation of GFP intensity in the distal-most germline. Pixel intensity was measured using ImageJ and plotted using Microsoft Excel. Error bars indicate 95% confidence limits; asterisk denotes a statistically significant difference (p<1.0x10^-5 using Student’s t-test). (C) Mitotic zone length is reduced in gld-1(0); trGLD-1(AAA) compared to gld-1(0); trGLD-1(SET). Error bars indicate 95% confidence limits; asterisk denotes a statistically significant difference (p<3.65x10^-11 using Student’s t-test).

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Figure S9 CYE-1/CDK2 represses GLD-1 abundance both directly and indirectly. (A–C) Conventions are same as Figure S8. (A) Dissected gonads treated aRNAi for 48 hrs from L4. TrGLD-1(AAA) in the mitotic zone increases and germ cell nuclei become enlarged after cye-1 aRNAi. Left, stacked confocal images of GFP fluorescence; right panels, DAPI staining (right). (B) Quantitation of GFP intensity in the distal-most germline. Error bars indicate 95% confidence limits; asterisk denotes a statistically significant difference (p<0.0003 using Student’s t-test). (C) Mitotic zone length, measured in gcf from the distal end, is reduced after cye-1 aRNAi. Error bars indicate 95% confidence limits; asterisk denotes a statistically significant difference (p<2.19x10^-8 using Student’s t-test).

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Author Contributions

Conceived and designed the experiments: JJ JMV JK. Performed the experiments: JJ JMV. Analyzed the data: JJ JMV JK. Contributed reagents/materials/analysis tools: JJ. Wrote the paper: JJ JK.

References

1. Duronio RJ, O’Farrell PH (1995) Developmental control of the G1 to S transition in Drosophila: cyclin Ea is a limiting downstream target of E2F. Genes Dev 9: 1456–1468.
2. Lilly MA, Spradling AC (1996) The Drosophila cell cycle and asymmetric division machineries. Nature 409: 1063–1067.
3. Talamà C, Kimble J (2005) Cyclin D regulation of a sexually dimorphic mitotic region in the C. elegans hermaphrodite germ line. Dev Biol 286: 342–357.
4. Tilmann C, Kimble J (2005) Cyclin D regulation of a sexually dimorphic mitotic region in the C. elegans hermaphrodite germ line. Dev Biol 286: 342–357.
5. Budrahaardja Y, Gonczy P (2009) Coupling the cell cycle to development. Development 136: 2661–2672.
6. Kimble J, Crittenden SL (2007) Controls of germine stem cells, entry into meiosis, and the sperm/oocyte decision in Caenorhabditis elegans. Annu Rev Cell Dev Biol 23: 403–433.
7. Talamà C, Kimble J (2005) Cell cycle and transition zones. Top, CYE-1 is expressed normally in the distal gonad. (A) Distal end of dissected gonads. Left panels, stacked confocal images of GFP fluorescence to show abundance of transgenic GLD-1; right panels, DAPI staining to show distal end and mitosis/meiosis boundary. Solid triangle marks distal end; small v and larger V mark steps of comparable GLD-1 abundance; dashed line indicates boundary between mitotic and transition zones. Germlines were treated identically and confocal fluorescent images taken at the same settings and then stacked along Z-axis. Scale bar, 20 μm. (B) Quantitation of GFP intensity in the distal-most germline. Pixel intensity was measured using ImageJ and plotted using Microsoft Excel. Error bars indicate 95% confidence limits; asterisk denotes a statistically significant difference (p<1.0x10^-5 using Student’s t-test). (C) Mitotic zone length is reduced in gld-1(0); trGLD-1(AAA) compared to gld-1(0); trGLD-1(SET). Error bars indicate 95% confidence limits; asterisk denotes a statistically significant difference (p<3.65x10^-11 using Student’s t-test).

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21. Jan E, Motzny CK, Graves LE, Goodwin EB (1999) The STAR protein, GLD-21.
20. Eckmann CR, Crittenden SL, Suh N, Kimble J (2004) GLD-3 and control of the cell cycle regulation in the germline of Caenorhabditis elegans. Genetics 146: 147-160.
27. Merritt C, Rasoloson D, Ko D, Seydoux G (2008) Cyclin E/Cdk2 Controls Mitosis/Meiosis Decision.
23. Biedermann B, Wright J, Senften M, Kalchhauser I, Sarathy G, et al. (2009) Translational repression of cyclin E prevents precocious mitosis and embryonic lethality. Dev Biol 324: 682-693.
22. Jan E, Motzny CK, Graves LE, Goodwin EB (1999) The STAR protein, GLD-20.
21. Jan E, Motzny CK, Graves LE, Goodwin EB (1999) The STAR protein, GLD-21.
17. Kershner AM, Kimble J (2010) Genome-wide analysis of mRNA targets for GLP-1-related kinases. Curr Biol 20: 1181-1183.
16. Lee M-H, Hook B, Pan G, Kershner AM, Merritt C, et al. (2007) Conserved regulators of gene expression in the Drosophila melanogaster germline. Dev Biol 296: 427-439.
14. Wickens M, Bernstein DS, Kimble J, Parker R (2002) A PUF family portrait: conserved regulators of gene expression in the Caenorhabditis elegans germ line. Curr Opin Genet Dev 12: 43-47.
13. Brodigan TM, Liu J, Park M, Kipreos ET, Krause M (2003) Cyclin E expression requires SHC, a small guanine nucleotide exchange factor important for Cdk2 activity. Dev Biol 254: 102-115.
12. Fay DS, Han M (2000) Mutations in gld-1, a Caenorhabditis elegans homolog, reveal coordination between cell-cycle control and vulval development. Genes Dev 13: 1415-1425.
11. Neiman MS, Xu S, Montgomery MK, Fire A (1997) Divergent functions for the STAR/KH domain protein GLD-1 in the C. elegans germline. Genetics 146: 227-239.
10. Endicott JA, Noble ME, Tucker JA (1999) Cyclin-dependent kinases: inhibition and substrate recognition. Curr Opin Struct Biol 9: 738-744.
9. Levine EM (2004) Cell cycling through development. Curr Opin Cell Biol 16: 662-669.
8. Boxem M, Srinivasan DG, van den Heuvel S (1999) The Caenorhabditis elegans gene sex-1 encodes related kinase required for M phase in meiotic and mitotic cell divisions, but not for S phase. Development 126: 2227-2229.
7. Fujita M, Takeshita H, Sawa H (2007) Cyclin E and CDK2 repress the terminal differentiation of quiescent cells after asymmetric division in C. elegans. PLoS Genet 3: 4039-4049.
6. Berger C, Mallik SK, Prasad M, Shashidhara LS, Teichmann GM (2005) A critical role for Cyclin E in cell fate determination in the central nervous system of Drosophila melanogaster. Nat Cell Biol 7: 56-62.
5. Jones AR, Schedl T (1995) Mutations in gld-1, a Caenorhabditis elegans homolog, reveal coordination between cell-cycle control and vulval development. Genes Dev 13: 1415-1425.
4. Suzuki N, Jardan-Sanz M, Eckmann CR, Kimble J (2006) The GLD-2 poly(A) polymerase activates gld-1 mRNA in the Caenorhabditis elegans germline. Proc Natl Acad Sci USA 103: 15108-15112.
3. Kershner AM, Kimble J (2010) Genome-wide analysis of mRNA targets for GLP-1-related kinases. Curr Biol 20: 1181-1183.
2. Nagano H, Zhang X, Atkinson S, Lako M (2009) Expression and functional analyses reveal an important role for Cdk2 in cell cycle regulation in human embryonic stem cells. Oncogene 28: 20-30.
1. Fox DS, Han M (2000) Mutations in gld-1, a Caenorhabditis elegans homolog, reveal coordination between cell-cycle control and vulval development. Genes Dev 13: 1415-1425.
78. Gengyo-Ando K, Mitani S (2000) Characterization of mutations induced by ethyl methanesulfonate, UV, and trimethylpsoralen in the nematode Caenorhabditis elegans. Biochem Biophys Res Commun 269: 64–69.

79. Praitis V, Casey E, Collar D, Austin J (2001) Creation of low-copy integrated transgenic lines in Caenorhabditis elegans. Genetics 157: 1217–1226.

80. Pasierbek P, Jantsch M, Melcher M, Schleiffer A, Schweizer D, et al. (2001) A Caenorhabditis elegans cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes Dev 15: 1349–1360.