A sensitized genetic screen to identify regulators of Caenorhabditis elegans germline stem cells

Sarah Robinson-Thiewes,† Aaron M. Kershner,‡ Heaji Shin,§ Kimberly A. Haupt,¶ Peggy Kroll-Connor,* and Judith Kimble

1Department of Genetics, University of Wisconsin-Madison, Madison, WI 53706, USA
2Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA
3Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, USA
4Department of Chemical Biology and Therapeutics, St. Jude Children’s Research Hospital, Memphis, TN 38105, USA
†Present address: Department of Chemical Biology and Therapeutics, St. Jude Children’s Research Hospital, Memphis, TN 38105, USA
‡Present address: Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA
§Present address: Department of Genetics, University of Wisconsin-Madison, Madison, WI 53706, USA
¶Present address: The David H. Koch Institute for Integrative Cancer Research at MIT, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
*Corresponding author: Department of Genetics, University of Wisconsin-Madison, Madison, WI 53706, USA. Email: jekimble@wisc.edu

Abstract

GLP-1/Notch signaling and a downstream RNA regulatory network maintain germline stem cells in Caenorhabditis elegans. In mutants lacking the GLP-1 receptor, all germline stem cells enter the meiotic cell cycle precociously and differentiate into sperm. This dramatic germline stem cell defect is called the “Glp” phenotype. The lst-1 and sygl-1 genes are direct targets of Notch transcriptional activation and functionally redundant. Whereas single lst-1 and sygl-1 mutants are fertile, lst-1 sygl-1 double mutants are sterile with a Glp phenotype. We set out to identify genes that function redundantly with either lst-1 or sygl-1 to maintain germline stem cells. To this end, we conducted forward genetic screens for mutants with a Glp phenotype in genetic backgrounds lacking functional copies of either lst-1 or sygl-1. The screens generated 9 glp-1 alleles, 2 lst-1 alleles, and 1 allele of pole-1, which encodes the catalytic subunit of DNA polymerase ε. Three glp-1 alleles reside in Ankyrin repeats not previously mutated, pole-1 single mutants have a low penetrance Glp phenotype that is enhanced by loss of sygl-1. Thus, the screen uncovered 1 locus that interacts genetically with sygl-1 and generated useful mutations for further studies of germline stem cell regulation.

Keywords: Caenorhabditis elegans; stem cells; Notch; DNA polymerase; forward genetics screen

Introduction

Stem cells maintain a robust balance between self-renewal and differentiation to ensure tissue homeostasis despite physiological and environmental challenges. Failure to maintain that balance can lead to tissue dysfunction, disease, and death (Simons and Clevers 2011). Therefore, understanding the molecular circuitry governing stem cell regulation is critical. Yet biologically robust regulatory circuits are notoriously difficult to disentangle.

The C. elegans germline is a powerful system for the study of stem cell regulation (Hubbard and Schedl 2019). The adult hermaphrodite germline is contained in 2 U-shaped gonadal arms and produces oocytes; sperm are made during larval development and stored for later fertilization (Fig. 1a, top). Germline stem cells (GSCs) are maintained at the distal end of each gonadal arm by a single-celled somatic niche, while GSC daughters differentiate as they move proximally away from the niche and ultimately undergo oogenesis (Fig. 1a, middle) (Hubbard and Greenstein 2000).

GSC self-renewal depends on GLP-1/Notch signaling from the niche and on a downstream RNA regulatory network. In glp-1 null mutants, GSCs fail to self-renew and instead differentiate precociously into sperm—the “Glp” phenotype (Austin and Kimble 1987) (Fig. 1a, bottom). Downstream of GLP-1/Notch, a “PUF hub” is required for self-renewal (Fig. 1b). This regulatory hub comprises 4 genes encoding PUF RNA-binding proteins (FBF-1, FBF-2, PUF-3, and PUF-11) as well as 2 direct GLP-1/Notch target genes, lst-1 and sygl-1, that encode novel PUF interacting proteins (Crittenden et al. 2002; Kershner et al. 2014; Shin et al. 2017; Haupt et al. 2019; Haupt et al. 2020; Qiu et al. 2019).

The PUF hub is characterized by pervasive genetic redundancy. For example, mutants lacking 3 PUF homologs are able to sustain some GSC self-renewing divisions, but animals lacking all 4 homologs phenocopy glp-1 null mutants (Haupt et al. 2020). Moreover, single mutants lacking lst-1 or sygl-1 are fertile and similar to the wildtype, while lst-1 sygl-1 double mutants phenocopy glp-1 null mutants (Fig. 1c) (Kershner et al. 2014). The highly redundant nature of the PUF hub has hampered the identification of its component parts. Indeed, LST-1 and SYGL-1 were not identified using standard forward genetic approaches, but instead were discovered using a candidate gene approach (Kershner et al. 2014), leaving open the possibility that additional
components remain unidentified. For example, the LST-1 or SYGL-1 proteins might work with other unknown redundant factors. Here, we describe the results of mutagenesis screens designed to identify regulators that function redundantly with lst-1 or sygl-1.

**Methods**

**Strain maintenance**

Unless noted otherwise, strains were maintained as previously described (Brenner 1974), at a temperature of 15°C. Balancers used to maintain recovered alleles were hT2[qIs48] (Siegfried and Kimble 2002) and hIn1[unc-54(h1040)] (Zetka and Rose 1992). Table 1 lists the strains used and their genotypes.

**Screen design and phenotype scoring**

We screened for lst-1 or sygl-1 enhancers using a modified ethyl methanesulfonate (EMS) protocol (Brenner 1974). Fourth larval stage (L4) hermaphrodites were soaked in 25 mM EMS (Sigma: M0880) for 4 h at room temperature, washed with M9, and placed on plates. F1 progeny were singled onto individual Petri dishes and allowed to self at 15°C. F2 adult progeny were scored for sterility by dissecting scope, and then L4 larvae were scored for a Glp phenotype using a Zeiss Axioskop compound scope equipped with DIC Nomarski optics, as described (Kershner et al. 2014).

Each screen was done in 2 ways—first with single mutants lst-1(ok814) and sygl-1(tm5040) (Fig. 1d, regimen 1) and then with each of the same mutants carrying a transgenic copy of wildtype glp-1 (Sorensen et al. 2020) in addition to an endogenous copy of glp-1 in F2 strains. Regimen 2 mutagenizes lst-1(lf) or sygl-1(lf) homozygotes and scores for Glp sterility in the F2.

**Allele identification**

Following isolation of a mutant with a Glp phenotype, the starting lst-1 or sygl-1 allele was crossed away to test whether the Glp defect depended on loss of lst-1 or sygl-1. Mutations were then mapped to a chromosome and tested for their ability to complement alleles of likely candidate genes. Mutants that were fertile as single mutants and mapped to chromosome I were tested for complementation with lst-1(ok814) I. Briefly, the double mutant (e.g. mut-x sygl-1) was balanced over the green balancer hT2[qIs48], crossed to lst-1(ok814) sygl-1(tm5040)/hT2[qIs48] males, and nongreen L4 male progeny were scored for the Glp defect. Mutants that were sterile as single mutants and mapped to chromosome III were tested for complementation with the null allele glp-1(q175) III. Briefly, unc-32 glp-1(q175)/hT2[qIs48] males were mated to each suspected glp-1 allele and nongreen male progeny scored for the Glp defect. If an allele failed to complement either lst-1 or glp-1, then Sanger sequencing was used to identify the molecular lesion. The glp-1(q823) allele was sequenced 2,382 bp upstream of the 5′ UTR and 927 bp downstream of the 3′ UTR in addition to the exons and introns, but no lesion was found.

Whole-genome sequencing was used to identify the likely lesion in q831, which was sterile as a single mutant and mapped to the right arm of chromosome I. Briefly, we picked ~570 adult homozygotes, isolated DNA with Puregene Core Kit A (Qiagen ID: 2|G3, 2022, Vol. 12, No. 3)
Table 1. Strains used in study

| Strain                  | Genotype                                                                 | Reference                  |
|------------------------|---------------------------------------------------------------------------|----------------------------|
| N2                     | Wildtype                                                                  | Brenner (1974)             |
| JK2877                 | unc-32(e189) glp-1(q175) III/hT2[qIs48] (I, III)                         | This work                  |
| JK4356                 | lst-1(ok814) I                                                           | Kershner et al. (2014)     |
| JK4774                 | lst-1(ok814) sygl-1(tm5040) I/H1T2[qIs48] (I, III)                        | Kershner et al. (2014)     |
| JK4899                 | sygl-1(tm5040) I                                                         | Kershner et al. (2014)     |
| JK5135                 | sygl-1(tm5040) I; q5s44[Pglp-1::GFP::EYFP::glp-1-3' end] II               | Sorensen et al. (2020)     |
| JK5203                 | lst-1(q821) I, q5s44[Pglp-1::GFP::EYFP::glp-1-3' end] II                  | This work                  |
| JK5209                 | lst-1(q827) sygl-1(tm5040) I/H1T2[qIs48] (I, III)                        | Shin et al. (2017)         |
| JK5277                 | lst-1(q826) I/H1T2[qIs48] (I, III)                                       | This work                  |
| JK5305                 | lst-1(q827) I/H1T2[qIs48] (I, III)                                       | This work                  |
| JK5315                 | lst-1(q826) sygl-1(tm5040) I/H1T2[qIs48] (I, III)                        | This work                  |
| JK5606                 | lst-1(q814) pole-1(q831) I/hIn1[unc-54(h1040)] I                         | This work                  |
| JK5293                 | sygl-1(tm5040) pole-1(q831) I/hIn1[unc-54(h1040)] I                     | This work                  |
| JK5250                 | pole-1(q831) I/hIn1[unc-54(h1040)] I                                    | This work                  |
| JK5268                 | pole(qk49) I/hIn1[unc-54(h1040)] I                                      | This work                  |
| JK5546                 | glp-1(q819) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5547                 | glp-1(q824) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5568                 | glp-1(q818) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5569                 | glp-1(q822) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5570                 | glp-1(q825) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5575                 | glp-1(q817) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5576                 | glp-1(q820) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5577                 | glp-1(q821) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5578                 | glp-1(q823) III/H1T2[qIs48] (I, III)                                     | This work                  |
| JK5293                 | glp-1(q831) I/hIn1[unc-54(h1040)] I                                    | This work                  |

Table 2. Summary of screens and alleles recovered.

| Parental genotypea | Copies of glp-1(+)c | Number of haploid genomes screened | Glp mutants recovereda | Gene identities | Allele identities |
|--------------------|---------------------|-----------------------------------|------------------------|-----------------|------------------|
| lst-1[+] I         | 2                   | 8,749                             | 6                      | 6 glp-1         | q817, q818, q819, q820, q821, q822 n/a |
| lst-1[+] I, q5s44 II| 4                   | 7,922                             | 0                      | n/a             | q823, q824, q825, q831 |
| sygl-1[+] I        | 2                   | 5,504                             | 4                      | 3 glp-1 1 pole-1 | q823, q824, q825, q831 |
| sygl-1[+] I, q5s44 II| 4                 | 3,868                             | 2                      | 2 lst-1         | q826, q827        |

a Alleles were lst-1(ok814) and sygl-1(tm5040).

Animals without q5s44 have 2 endogenous copies of glp-1(+) Animals with q5s44 have 2 endogenous and 2 transgenic copies of glp-1(+) Mutants with Glp phenotype—small germline and sperm to distal end (Austin and Kimble 1987)

Table 3. Genetic characterization of sterile mutants from screens.

| Allele | LGa | Glp | Failure to complementb |
|--------|-----|-----|-------------------------|
| q817   | III | +++ | glp-1(q175)             |
| q818   | III | +++ | glp-1(q175)             |
| q819   | III | +++ | glp-1(q175)             |
| q820   | III | +++ | glp-1(q175)             |
| q821   | III | +++ | glp-1(q175)             |
| q822   | III | +++ | glp-1(q175)             |
| q823   | III | +++ | glp-1(q175)             |
| q824   | III | +++ | glp-1(q175)             |
| q825   | III | +++ | glp-1(q175)             |
| q826   | I   | −   | lst-1(ok814)            |
| q827   | I   | −   | lst-1(ok814)            |
| q831   | I   | +   | pole-1(qk49)            |

+++ 100% penetrance; +, ~30% penetrance; −, not Glp as single mutants.
a LG, linkage group.
bAllele used in complementation test.

158667 following the manufacturer’s directions and submitted the DNA (~100 ng) to the Wisconsin Biotechnology Core for sequencing using an Illumina MiSeq. The genome sequence was uploaded to a Galaxy server and analyzed by CloudMap, as previously described (Minevich et al. 2012). A premature stop codon occurred in 1 gene, F33H2.5, which resides on the right arm of chromosome 1. q831 failed to complement F33H2.5 (qk49) (C. elegans Deletion Mutant Consortium 2012), and the premature stop codon was confirmed by Sanger sequencing of DNA from q831 homozygotes.

Assay for temperature sensitivity of glp-1 and pole-1 alleles

Balanced strains carrying glp-1 or pole-1 alleles were maintained at 15°C, 20°C, or 25°C for at least 1 generation before homozygous glp-1 or pole-1 L4 progeny were scored for a Glp phenotype.

pole-1 phenotype assay

Homozygous pole-1 (q831 or qk49) animals were distinguished from the balancer hIn1[unc-54(h1040)] by their kinked, uncoordinated movement. Homozygous mid-L4 hermaphrodites were raised at 20°C, anesthetized in levamisole, mounted on an agarose pad, and examined using a Zeiss Axioskop compound scope (Crittenden et al. 2017). Vulva formation—wildtype, multivulva, or vuvalvless—was scored in addition to germline defects.

Immunostaining

Strains were maintained at 20°C for immunostaining following published procedure (Crittenden et al. 2017). The SP56 polyclonal antisperm antibody (Ward et al. 1986), a gift from Susan Strome (UCSC, CA, USA), was diluted 1:200. The secondary antibody Alexa Fluor 555 donkey α-mouse (1:1,000, Invitrogen number A31570) was added with DAPI (1 μg/ml) to mark DNA. Gonads were
Table 4. Molecular lesions in alleles recovered from the screen.

| Allele       | Type of mutation | Nucleotide change | Codon change | Amino acid change |
|--------------|------------------|------------------|--------------|------------------|
| glp-1(q817)  | Missense         | C → T            | CGG → UCG    | P1111S           |
| glp-1(q818)  | Nonsense         | C → T            | CAA → UAA    | Q96Stop          |
| glp-1(q819)  | Missense         | C → T            | CAU → UAU    | H1000Y           |
| glp-1(q820)  | Missense         | T → A            | AAU → AAA    | N992K            |
| glp-1(q821)  | Nonsense         | T → G            | CGA → UGA    | R499Stop         |
| glp-1(q822)  | Nonsense         | Not found        | UAU → UAG    | Y176Stop         |
| glp-1(q824)  | Substitution     | AC → CA in intron 4 | n/a         | n/a              |
| glp-1(q825)  | Splice site      | G → A            | n/a          | n/a              |
| lst-1(q826)  | Nonsense         | C → T            | CGA → UGA    | R114Stop         |
| lst-1(q827)  | Splice site      | G → A            | n/a          | n/a              |
| pole-1(q831) | Nonsense         | G → A            | UGG → UGA    | W1899Stop        |

n/a, not applicable.

*See Methods for more details.

184 bp from 5′ splice site.

Table 5. glp-1 alleles and temperature sensitivity.

| Allele       | % Glp 25°C | % Glp 20°C | % Glp 15°C | n  |
|--------------|------------|------------|------------|----|
| N2           | 0          | 0          | 0          | 20 |
| q175         | 100        | 100        | 100        | 20 |
| q817         | 100        | 100        | 100        | 40 |
| q818         | 100        | 100        | 100        | 40 |
| q819         | 100        | 100        | 100        | 40 |
| q820         | 100        | 100        | 100        | 40 |
| q821         | 100        | 100        | 100        | 20 |
| q823         | 100        | 100        | 100        | 20 |
| q824         | 100        | 100        | 100        | 20 |
| q825         | 100        | 100        | 100        | 20 |

n, number germlines scored.

For q817 at 15°C, 38 germlines scored.

mounted in Vectashield (Vector Laboratories number H-1000), sealed with nail polish, and kept in the dark at 4°C until imaging.

Microscopy

DAPI/SP56 stained gonads were imaged with a Zeiss Axioskop compound microscope equipped with a Hamamatsu ORCA-Flash4.0 cMos camera and a 63/1.4 NA Plan Apochromat oil immersion objective. Carl Zeiss filter sets 49 and 43HE were used for the visualization of DAPI and Alexa 555. Images were captured using Micromanager (Edelstein et al. 2010, 2014).

GLP-1 protein conservation

Protein sequences for C. elegans glp-1 orthologs from other Caenorhabditis species were acquired from Wormbase. Sequences of the Ankyrin (ANK) repeats were aligned using M-Coffee to examine amino acid conservation (http://tcoffee.crg.cat/apps/tcoffee) or engineered by CRISPR/Cas9 gene editing (Haupt et al. 2019). Indeed, all 9 failed to complement glp-1(null) (Table 3). Therefore, this mutation must be a lesion in some other gene; its identity is described below.

Results and discussion

 Screens for Glp mutants in lst-1 and sygl-1 single mutant backgrounds

To identify new GSC regulators and perhaps new components of the PUF hub, we conducted genetic screens for mutations that cause a Glp phenotype in a lst-1(0) or sygl-1(0) single mutant background (Fig. 1d). Our initial screens simply mutagenized lst-1(0) and sygl-1(0) single mutants and scored their F2 progeny for the Glp phenotype (Fig. 1d, regimen 1). We screened 8,749 haploid genomes after mutagenesis of lst-1(0) and 5,504 haploid genomes after mutagenesis of sygl-1(0) (Table 2). This first set of screens recovered 10 mutants. However, outcrossing revealed that all 10 mutants generated animals with a Glp phenotype after lst-1(0) or sygl-1(0) was removed. Nine mutations, alleles q817–q825, caused a fully penetrant Glp phenotype and mapped to chromosome III (Table 3). Because the glp-1 locus is large (~7.4 kb) and located on chromosome III, these 9 mutations were likely glp-1 alleles. Indeed, all 9 failed to complement glp-1(null) (Table 3). The 10th allele q831 caused a low penetrance Glp phenotype and was mapped to the right arm of chromosome I, at some distance from both sygl-1 and lst-1 loci. Therefore, this mutation must be a lesion in some other gene; its identity is described below.

The initial screens were heavily biased for the recovery of glp-1 alleles. To limit the isolation of more glp-1 alleles, we introduced a transgenic copy of wildtype glp-1 into the lst-1(0) and sygl-1(0) single mutants (Fig. 1d, regimen 2; Table 2). The glp-1 transgene, qS44 or glp-1(tg), is a single copy insertion of wildtype glp-1 on chromosome II that rescues a glp-1 null mutant (Sorensen et al. 2020). Using the same EMS mutagenesis procedure as before, we screened 7,922 lst-1(0); glp-1(tg) haploid genomes and 3,868 sygl-1(0); glp-1(tg) haploid genomes. No mutants with a Glp phenotype were isolated from lst-1(0); glp-1(tg) but 2 were recovered from sygl-1(0); glp-1(tg) (Table 2). These mutations were subsequently determined to be alleles of lst-1 (see below). Table 3 summarizes the genetic characterization of alleles recovered from the screen, and Table 4 summarizes their molecular lesions. Our failure to recover sygl-1 alleles in the lst-1(0) background shows that our screens were not performed to saturation. However, we note that the sygl-1 locus is relatively small (621 bp coding region) and therefore likely a poor mutagenesis target.

Characterization of lst-1 alleles

The lst-1 locus generates 2 RNA isoforms—one longer, called lst-1L, and 1 shorter, called lst-1S (Fig. 2a; Table 4). Most lst-1 alleles available prior to this work were isolated in deletion screens (Kershner et al. 2014) or engineered by CRISPR/Cas9 gene editing (Haupt et al. 2019). In addition, 1 allele from these screens was previously reported, the nonsense mutant lst-1(q826) (Shin et al. 2017). Here, we report a second allele obtained in the screen, lst-1(q827), which alters the 5′ splice site in lst-1 intron 2 (Fig. 2a; Table 4). As previously reported for lst-1(q826), lst-1(q827) was confirmed by complementation tests and Sanger sequencing. Both alleles are phenotypically similar to previously characterized lst-1(0) mutants: as a single mutant, they appear wildtype (n > 50) and as lst-1 single mutants they were all sterile.
(n > 50) and had the Glp phenotype (n = 10). These lst-1 alleles will prove useful in future studies focused on lst-1 function.

Characterization of glp-1 alleles

We identified the molecular lesions in the glp-1 alleles with Sanger sequencing; q818, q821, and q822 were nonsense mutants; q817, q819, and q820 were missense mutants and q825 altered a 5' splice site (Fig. 2b; Table 4). The q824 allele had a 2bp change (AC → CA) in intron 4 that did not affect the 5' or 3' splice sites or the branch point (Fig. 2b). We failed to determine the lesion in 1 allele, q823, despite sequencing all exons and introns plus 2,382 bp upstream of the transcription start site and 927 bp.
downstream of the 3’ UTR. Nonetheless, the remaining 8 alleles were all previously unreported glp-1 lesions.

The 3 glp-1 missense alleles—q817, q819, and q820—all carry amino acid changes in the ANK repeats (Fig. 2b and c). ANK repeats are conserved across eukaryotes with roles in protein interaction, cell signaling, and disease (Roehl et al. 1996; Mosavi et al. 2004). Many previously identified glp-1 alleles also have changes in this region. Mutations in ANK repeats 1, 2, 4, and 5 all cause a temperature sensitive Glp phenotype (Kodoyianni et al. 1992; Berry et al. 1997; Nadarajan et al. 2009; Dalfo et al. 2010). Our 3 newly identified missense alleles occur in different repeats, ANK 3 (q819 and q820) and ANK 6 (q817) and they are not temperature sensitive (Table 5). All 3 affect conserved residues (Fig. 3). We conclude that the newly identified ANK missense mutations affect residues essential for GLP-1 function. These alleles should prove useful for investigating ANK repeats and their role in Notch signaling.

Characterization of pole-1(q831)

One mutant allele isolated in the sygl-1(lf) background, q831, mapped to the right arm chromosome I. Whole-genome sequencing revealed a nonsense mutation R1899Stop in F33H2.5 (Table 4), which encodes a C. elegans ortholog of the catalytic subunit of DNA polymerase e (Fig. 4a). We confirmed q831 as an allele of F33H2.5 by Sanger sequencing, and by its failure to complement gk49, a deletion allele in F33H2.5 that had been generated by the C. elegans Knockout Consortium (C. elegans Deletion Mutant Consortium 2012). F33H2.5 has been named pole-1 for its DNA polymerase e orthology. The pole-1(q831) mutation was isolated because sygl-1(lf) pole-1(q831) double mutants had a Glp phenotype. During outcrossing, we found that pole-1(q831) single mutants were 100% sterile (Fig. 4d–f). To ask if pole-1 sterility was due to a Glp defect, we examined L4 larvae under DIC/Normaski and also stained dissected gonads with a sperm-specific antibody (SP56) (Ward et al. 1986) and DAPI (Fig. 4b–f) (see Methods). Wildtype L4 gonads contain several hundred germ cells, with undifferentiated cells at the distal end and differentiated sperm at the proximal end (Fig. 4b). glp-1(null) L4 gonads, in contrast, contain only a few germ cells, all of which have differentiated into SP56-positive sperm extending to the distal end (Fig. 4c). Similar to glp-1(null) gonads, the pole-1(q831) gonads were physically smaller than wildtype; however, only ~30% had differentiated sperm extending to the distal end and thus were Glp (Fig. 4d and f). The other ~70% did not have...
sperm extending to the distal end and were designated nonGlp steriles (Fig. 4e and f). We also observed a low penetrance Glp phenotype in the deletion strain pole-1(gk49) (Fig. 4a and f).

Because the Glp phenotype was not fully penetrant at 20°C, we examined pole-1(q831) animals raised at 15°C and 25°C. Indeed, the Glp penetrance increased with the temperature—indicating that the Glp defect is temperature sensitive (Fig. 4f).

In addition to germline defects, pole-1 mutants had a range of other defects, consistent with a broad role in development. For example, pole-1 mutants had vulval defects (Fig. 4f) and were uncoordinated.

DNA polymerase ε pole-1 was not previously been recognized critical for GSC maintenance, though other components of the DNA polymerase ε complex have been linked to GSC function.
replication machinery have been implicated in germ cell proliferation (Yoon et al. 2018). We next asked if the pole-1 Glp phenotype was enhanced by loss of lst-1 or sygl-1. We found that pole-1(q831) single mutants were 30% Glp; lst-1(qf) pole-1(q831) double mutants were 41% Glp; and sygl-1(qf) pole-1(q831) double mutants were 65% Glp (Fig. 4f). Thus, loss of sygl-1 is a clear enhancement of the pole-1 Glp defect, but loss of lst-1 had a more minor increase and is not clearly an enhancement. Finally, pole-1 vulval defects were not enhanced (Fig. 4f). We conclude that sygl-1 is an enhancer of the pole-1 germline defect.

Conclusions and future directions
The goal of the mutant screens in lst-1 and sygl-1 mutant backgrounds was to identify new regulators of GSC self-renewal. In particular, we sought to test the idea that the LST-1 and SYGL-1 proteins might work with other factors that were similarly redundant. The screens identified 9 alleles of glp-1, 2 alleles of lst-1, and 1 allele of pole-1—the C. elegans ortholog of DNA polymerase e. Although the screens were not saturated, identification of pole-1 with a low penetrance Glp phenotype demonstrates that additional genes likely await discovery. Any additional screens in C. elegans null backgrounds should focus on the modified design with transgenic glp-1 to avoid isolation of more glp-1 alleles. Alternatively, overexpression of either lst-1 or sygl-1 causes a germline tumor (Shin et al. 2017) and so one might seek suppressors of those tumors or enhancers of the low penetrance pole-1 Glp phenotype.

Data availability
Strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

Acknowledgments
We thank past and present members of the Kimble and Wickens labs for thoughtful discussions during the screens. We thank Erika Sorensen for sharing glp-1(qf) prior to publication, Jadwiga Forster for technical support, and Sarah Crittenden for help scoring pole-1. The gk49 allele was provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (R01 OD010440).

Author’s Contributions
AK, HS, KH, and JK designed screens and methods for mutant characterization; AK, HS, KH, FK-C, and JK performed screens; HS and KH characterized lst-1 alleles; SR-T characterized glp-1 alleles; AK and SR-T characterized pole-1 alleles; SR-T, AK, HS, KH, and JK wrote the paper.

Funding
SR-T was supported by the NSF Graduate Research Fellowship under Grant DGE-1256259 and NIH Predoctoral Training Grant in Genetics T32GM007133. JK was an Investigator of the Howard Hughes Medical Institute and is now supported by NIH R01 GM134119.

Conflicts of interest
None declared.

Literature cited
Austin J, Kimble J. glp-1 is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. Cell. 1987; 51(4):589–599.
Berry LW, Westlund B, Schell T. Germ-line tumor formation caused by activation of glp-1, a Caenorhabditis elegans member of the Notch family of receptors. Development. 1997;124(4):925–936.
Brenner S. The genetics of Caenorhabditis elegans. Genetics. 1974; 77(1):71–94.
C. elegans Deletion Mutant Consortium. Large-scale screening for targeted knockouts in the Caenorhabditis elegans genome. G3 (Bethesda). 2012;2:1415–1425.
Crittenden SL, Bernstein DS, Bachorik JL, Thompson BE, Gallegos M, Petcherski AG, Moulder G, Barstead R, Wickens M, Kimble J, et al. A conserved RNA-binding protein controls germline stem cells in Caenorhabditis elegans. Nature. 2002;417(6889):660–663.
Crittenden SL, Seidel HS, Kimble J. Analysis of the C. elegans germline stem cell pool. Methods Mol Biol. 2017;1463:1–33.
Dafo D, Priess JR, Schnabel R, Hubbard EJA. glp-1(e2141) sequence correction. Worm Breeder’s Gaz. 2010;18(4).
Edelstein A, Amodaj N, Hoover K, Vale R, Stuurman N. Computer control of microscopes using μManager. Curr Protoc Mol Biol. 2010; Chapter 14:Unit14.20.
Edelstein AD, Tsuchida MA, Amodaj N, Pinkard H, Vale RD, Stuurman N. Advanced methods of microscope control using μManager software. J Biol Methods. 2014;1(2):e10.
Haupt KA, Enright AL, Ferdous AS, Kershner AM, Shin H, Wickens M, Kimble J. The molecular basis of LST-1 self-renewal activity and its control of stem cell pool size. Development. 2019;146(20):dev181644.
Haupt KA, Law KT, Enright AI, Kanzler CR, Shin H, Wickens M, Kimble J. A PUF hub drives self-renewal in Caenorhabditis elegans germline stem cells. Genes. 2020;214:147–161.
Hubbard EJ, Greenstein D. The Caenorhabditis elegans Gonad: a test tube for cell and developmental biology. Dev Dyn. 2000;218(1):2.
Hubbard EJ, Scheld T. Biology of the Caenorhabditis elegans germline stem cell system. Genetics. 2019;213(4):1145–1188.
Kershner AM, Shin H, Hansen TJ, Kimble J. Discovery of two GLP-1/Notch target genes that account for the role of GLP-1/Notch signaling in stem cell maintenance. Proc Natl Acad Sci U S A. 2014;111(10):3739–3744.
Kodymian V, Maine EM, Kimble J. Molecular basis of loss-of-function mutations in the glp-1 gene of Caenorhabditis elegans. Mol Biol Cell. 1992;3(1):1199–1213.
Lissemore JL, Currie PD, Turk CM, Maine EM. Intragenic dominant suppressors of glp-1, a gene essential for cell-signaling in Caenorhabditis elegans, support a role for cdc10/SWI6/ankyrin motifs in GLP-1 function. Genetics. 1993;135(4):1023–1034.
Minevich G, Park DS, Blankenberg D, Poole RJ, Hobert O. CloudMap: a cloud-based pipeline for analysis of mutant genome sequences. Genetics. 2012;192(4):1249–1267.
Mosavi LK, Cammett TJ, Desrosiers DC, Peng Z. The ankyrin repeat as molecular architecture for protein recognition. Protein Sci. 2004;13(6):1435–1448.
Nadarajan S, Govindan JA, McGovern M, Hubbard EJA, Greenstein D. MSP and GLP-1/Notch signaling coordinate yolk and oocyte growth in C. elegans. Development. 2009;136(13):2223–2234.
