HOP-1 Presenilin Deficiency Causes a Late-Onset Notch Signaling Phenotype That Affects Adult Germline Function in *Caenorhabditis elegans*

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ABSTRACT Functionally redundant genes present a puzzle as to their evolutionary preservation, and offer an interesting opportunity for molecular specialization. In *Caenorhabditis elegans*, either one of two presenilin genes (*sel-12* or *hop-1*) facilitate Notch activation, providing the catalytic subunit for the γ secretase proteolytic enzyme complex. For all known Notch signaling events, *sel-12* can mediate Notch activation, so the conservation of *hop-1* remains a mystery. Here, we uncover a novel "late-onset" germline Notch phenotype in which HOP-1-deficient worms fail to maintain proliferating germline stem cells during adulthood. Either SEL-12 or HOP-1 presenilin can impart sufficient Notch signaling for the establishment and expansion of the germline, but maintenance of an adult stem cell pool relies exclusively on HOP-1-mediated Notch signaling. We also show that HOP-1 is necessary for maximum fecundity and reproductive span. The low-fecundity phenotype of *hop-1* mutants can be phenocopied by switching off *glp-1*/Notch function during the last stage of larval development. We propose that at the end of larval development, dual presenilin usage switches exclusively to HOP-1, perhaps offering opportunities for differential regulation of the germline during adulthood. Additional defects in oocyte size and production rate in *hop-1* and *glp-1* mutants indicate that the process of oogenesis is compromised when germline Notch signaling is switched off. We calculate that in wild-type adults, as much as 86% of cells derived from the stem cell pool function to support oogenesis. This work suggests that an important role for Notch signaling in the adult germline is to furnish a large and continuous supply of nurse cells to support the efficiency of oogenesis.

KEYWORDS presenilin; Notch signaling; gene redundancy; germline; oogenesis; proliferation

PARALOGOUS genes often display functional redundancy, whereby the gene products perform overlapping functions. Evolutionary theory suggests that slight differences in function and/or expression could explain the maintenance of apparent genetic redundancy (Thomas 1993; Nowak *et al.* 1997), but experimental dissection of functional differences between redundant genes is complicated by the similarity of their mutant phenotypes. Here, we address the apparent functional redundancy of two *Caenorhabditis elegans* presenilin genes, *sel-12* and *hop-1*, which share 50% amino acid similarity and 31% amino acid identity. These presenilin paralogs have been conserved since at least the origin of the *Caenorhabditis* clade, divergence within which is comparable to the divergence across the chordate phylum (Kiontke *et al.* 2004). Other animal species, including humans, also maintain two presenilin paralogs that exhibit partial genetic redundancy, raising the interesting possibility of molecular specialization that could be afforded by two alternative presenilin subunits for the γ secretase complex. Differences in gene expression and mutant phenotypes for the two mouse presenilin genes suggest that mammalian presenilin redundancy has been preserved through the existence of unique roles for each presenilin in addition to their common roles, but little is known about the division of labor between these two genes with regard to specific cellular events that require presenilin function (Lee *et al.* 1996; Donoviel *et al.* 1999; Herreman *et al.* 1999).

doi: https://doi.org/10.1534/genetics.117.300605
Manuscript received September 14, 2017; accepted for publication December 11, 2017; published Early Online December 13, 2017.
Supplemental material is available online at www.genetics.org/lookup/suppl/doi:10.1534/genetics.117.300605/-/DC1.
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An essential cellular role of presenilin proteins is to mediate the final activation step in the Notch signaling pathway by catalyzing the intramembranous cleavage of the Notch Receptor in cells that respond to Notch ligand [for review see Kopan and Ilagan (2009)]. The presenilin protein is the catalytic subunit of γ secretase, the large membrane-resident enzyme that mediates this proteolytic cleavage and consists of four essential subunits in a 1:1:1:1 stoichiometry [for review see Zhang *et al.* (2014)]. In *C. elegans*, the presenilin subunit is the only one that is encoded by two alternative gene paralogs. Since all *Caenorhabditis* species have conserved a *sel-12* and a *hop-1* ortholog, there must be evolutionary pressure to maintain both genes. It is possible that both genes contribute additively to the overall amount of presenilin needed in a cell, or that the two presenilins may have unique functions in addition to their overlapping functions. Until now, analysis of *sel-12* and *hop-1* mutants has identified unique roles for the *sel-12* presenilin, but none for *hop-1*, leaving the existence of the *hop-1* presenilin a mystery.

Multiple Notch signaling events and their biological outcomes are well characterized throughout *C. elegans* development. On this foundation, the SEL-12 and HOP-1 presenilins provide a tractable system in which to compare the contributions of two presenilin proteins *in vivo* (Li and Greenwald 1997; Westlund *et al.* 1999). The *sel-12* and *hop-1* genes have been found to be largely genetically redundant, in that Notch signaling can be mediated by either presenilin, but is blocked if both presenilins are absent. Thus, *hop-1*; *sel-12* double mutants display a collection of mutant phenotypes that correspond to loss-of-function Notch phenotypes. For example, *hop-1*; *sel-12* homozygous hermaphrodites display maternal-effect embryonic lethality similar to that caused by hypomorphic mutations in the *glp-1*/Notch receptor. These double mutants also display a severe egg-laying defect characterized by the lack of ventral uterine cell fate induction in the somatic gonad, similar to that caused by null mutations in the *lin-12*/Notch receptor. Furthermore, *hop-1*; *sel-12* homozygotes that also lack maternally supplied *sel-12* display the stark germline sterility that is observed in *glp-1*/Notch null mutants, in which germ cells fail to proliferate and instead enter meiosis prematurely (Li and Greenwald 1997; Westlund *et al.* 1999). Lastly, RNA interference (RNAi) of *hop-1* into a *sel-12* mutant background also reveals the larval lethal phenotype that is characteristic of animals that lack both Notch receptors (Li and Greenwald 1997). In contrast, *hop-1* or *sel-12* single mutants do not display these phenotypes, indicating that in a variety of cellular contexts, either *sel-12* or *hop-1* presenilin is sufficient to drive successful Notch signaling, characteristic of genetic redundancy.

The SEL-12 presenilin mediates at least one Notch signaling event for which HOP-1 is not available or is otherwise ineffective: *sel-12* mutant hermaphrodites display an egg-laying defect similar to that of *lin-12*/Notch hypomorphic mutants, in which uterine precursor cells do not respond to Notch ligand presented by the somatic gonadal anchor cell (Cinar *et al.* 2001). The near exclusive use of the *sel-12* presenilin in this context appears to result from lack of *hop-1* expression in the uterine cells, as evidenced by rescue of the *sel-12* egg-laying defect with a *hop-1* transgene expressed under the control of *sel-12* regulatory sequences (Li and Greenwald 1997). Additional somatic phenotypes have been characterized in *sel-12* single mutants, but the cause of these cellular defects has not yet been explained (Wittenburg *et al.* 2000; Eimer *et al.* 2002).

An interesting clue as to the possible role of *hop-1* comes from its chromosomal location compared to its paralog: *hop-1* resides on an autosome, while *sel-12* is located on the X chromosome. In *C. elegans*, genes on the X chromosome have been found to have reduced germline expression relative to that of autosomal genes (Reinke *et al.* 2000, 2004). Furthermore, the X chromosome is disproportionally devoid of genes that encode products necessary for germline development or function (Kamath *et al.* 2003). Maciejowski *et al.* proposed that duplicated gene pairs, in which one partner resides on an autosome and the other on the X chromosome, might evolve germline *vs.* somatic roles, respectively, if the X-linked gene is shut down during germline development (Maciejowski *et al.* 2005). We hypothesized that the existence of the *hop-1* presenilin paralog might provide previously unrecognized but essential presenilin function in the germline, and so we undertook a detailed characterization of germline development and function in a *hop-1* null mutant. Our analysis demonstrates that *hop-1* does not merely contribute additively with *sel-12* to the overall level of presenilin, but that *hop-1* has a unique role in the adult germline, providing the only source of presenilin for Notch activation, and thus facilitates Notch-dependent germline maintenance in the adult. Analysis of *hop-1* mutant hermaphrodites affords a unique opportunity to assess germline function in the adult, and highlights the important role of Notch signaling in supporting the process of oogenesis.

Materials and Methods

C. elegans handling and genetic alleles

All strains were derived from the Bristol N2 strain and maintained according to standard procedures at 20° unless otherwise noted (Brenner 1974). All strains were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN), or constructed from these strains using standard genetic techniques (Brenner 1974). Mutant alleles are listed by linkage group (LG):

LGI: *hop-1*(*ar179*) (Wen *et al.* 2000), *hop-1*(*gk862343*), *hop-1*(*gk751127*) (Thompson *et al.* 2013), *unc-13*(*e1091*), and *dpy-5*(*e61*).
LGII: *dpy-10*(*e128*), *unc-4*(*e120*), and *tra-2*(*e1095*).
LGIII: *glp-1*(*bn18*) (Qiao *et al.* 1995), *glp-1*(*ar202*) (Pepper *et al.* 2003), and *unc-32* (*e189*).
LGIV: *him-8*(*e1489*).
LGV: *fog-2*(*q71*) and *him-5* (*e1490*).
LGX: *unc-1*(*e538*) and *sel-12*(*ok2078*).

The *hop-1*(*ar179*) allele (Wen *et al.* 2000) has an internal 716-bp deletion that is predicted to cause a reading frameshift after the first transmembrane domain, deleting the coding sequences for the next five transmembrane domains before a premature stop codon is encountered after 57 residues. The *hop-1*(*gk862343*) and *hop-1*(*gk751127*) alleles were generated as part of the Million Mutations Project and correspond to G275E and V280E, respectively (Thompson *et al.* 2013), while the *sel-12*(*ok2078*) allele (generated by the *C. elegans* Gene Knockout Project at the Oklahoma Medical Research Foundation, which was part of the International *C. elegans* Gene Knockout Consortium) has a 1524-bp deletion that removes the coding sequences for transmembrane domains 2–6 (http://www.wormbase.org), and was outcrossed four times before use here. Unless specifically noted, homozygous *hop-1* and *sel-12* single mutants lack both zygotic and maternal gene products, since they are derived from homozygous hermaphrodite parents.

Worm synchronization and developmental timing

Synchronized and staged populations were obtained following the hatch-off protocol described by Pepper *et al.* (2003), except that worms were grown at 20° unless otherwise noted, and a hatching interval of 1.5 hr was used. Time zero corresponds to the time at which hatched L1 larvae were transferred to a new plate. Thus, we use "60 hr posthatch" to refer to a population of worms that hatched 60–61.5 hr previously. Time course studies following vulval development demonstrated that *hop-1*(*ar179*) hermaphrodites develop with comparable timing to that of N2 controls. For synchronized populations prepared as above at 20°: < 10% of animals in a 45 hr posthatch population reached L4 lethargus for both N2 (n = 35) and *hop-1*(*ar179*) (n = 35); in a 47 hr posthatch population, 54% of N2 (n = 24) and 77% of *hop-1*(*ar179*) (n = 27) were in L4 lethargus; and in a 48 hr posthatch population, 70% of N2 (n = 29) and 82% of *hop-1*(*ar179*) (n = 38) were in L4 lethargus. In a 55 hr posthatch population, 66% of N2 (n = 24) and 70% of *hop-1*(*ar179*) (n = 20) contained mature oocytes. Thus, N2 and *hop-1*(*ar179*) appeared to have similar developmental timing, and we considered worms at the 50 hr posthatch time point to be finishing the L4/adult molt, and worms at the 55 hr posthatch time point to be at the beginning of adulthood. A similar analysis at 15° for N2, *hop-1*(*ar179*), and *glp-1*(*bn18*)^{ts} identified 85–90 hr posthatch as the time by which the majority of worms reached the L4/adult molt for all three phenotypes (data not shown).

Brood measurements

Self-brood size was determined by counting all progeny derived from isolated L4 hermaphrodites transferred once or twice a day until no more progeny were produced. Progeny were counted either as eggs (within 6–12 hr of being laid) or as L3–L4 live progeny. Each strain was checked for embryonic lethality by counting unhatched eggs 20–24 hr after moving a mother. In all strains used for brood counts, embryonic death (as determined by unhatched embryos) accounted for < 3% of a brood (data not shown). We have found that for some genotypes, the age of a mother can influence the size of the brood produced by her progeny (R. Z. Solomon and C. Goutte, unpublished data), and so, to eliminate this potential variable, here we use only "early born" animals for brood assessments (animals laid in the first day of the mother's laying period). Mated brood size was determined as above, except that hermaphrodites or *fog-2*(*q71*) females were exposed to males either as young adults for 48 hr or as day 4 adults for the duration of the experiment; 4–5 young males were used in each mating. Student's t-test was used for statistical analysis.

Sperm counts

Whole-mounts of young adult hermaphrodites (55 hr posthatch) were prepared according to Pepper *et al.* (2003), staged worms were harvested with M9 buffer, fixed with 95% ethanol, stained with DAPI (VectaShield Mounting Medium with 1.5 μg/ml DAPI), and mounted on a 2% agarose pad for viewing with a Nikon (Garden City, NY) Eclipse Ti confocal laser-scanning microscope with epifluorescence at 600× magnification. Z-stacks of the spermathecas were obtained with a 0.75 μm step size. Spermatids, spermatocytes, and embryos were labeled in PhotoShop CS6 and analyzed in ImageJ. Each spermatheca underwent two independent analyses, and the final counts were averaged. For N2, the average difference between the two analyses was 3.40% (n = 7), and for *hop-1*(*ar179*), the average difference between the two analyses was 8.40% (n = 34). Total sperm per gonad was calculated as: #spermatids + 4 × (#spermatocytes) + 1/2(#embryos); total sperm count per hermaphrodite can be estimated by doubling the total sperm count per gonad. The Student's t-test was used for statistical analysis.

The presence of unutilized sperm in hermaphrodites that had finished producing progeny was determined by harvesting hermaphrodites that were in the fourth or sixth day of adulthood (days 4 and 6 correspond to 120 and 168 hr posthatch populations, respectively), and proceeding as above, except that spermatids were visualized and counted using a Axioscope microscope (Zeiss [Carl Zeiss], Thornwood, NY). The entire gonad region of the worm was searched for small DAPI-staining nuclei that had spermatid-like morphology. Worms were scored as having either 0, 1–10, 11–20, or > 20 visible spermatids. Results for day 4 and 6 were not significantly different, and therefore were combined.

Reproductive span

N2 and *hop-1*(*ar179*) hermaphrodites were followed for 10 days of adulthood. At the end of each day, the hermaphrodites (and males if present) were transferred to a new plate, and the prior plate was scored for the presence of progeny (for the graph shown in Figure 1C, a cut off of ≥ 1 progeny was used). To minimize physical trauma to the hermaphrodites (Pickett *et al.* 2013), exposure to males was limited to 2 days: either day 1 and day 2 of adulthood, or day 3 and day

4 of adulthood. These two protocols did not yield differences in the observed reproductive span for either N2 or *hop-1*(*ar179*) worms, and therefore the results are combined in the graph shown in Figure 1C.

Gonad proliferative zone measurements

Gonads were dissected from synchronized staged animals (see above) using a protocol modified from that of Francis *et al.* (1995): synchronized worms were harvested from a single NGM plate with M9 buffer, washed twice with 10–15 ml of M9 buffer, and resuspended in ~200 μl M9 with 0.25 mM levamisole. Worms were transferred to a poly-L-lysine- coated (Sigma [Sigma Chemical], St. Louis, MO) microscope slide, and cut between the pharynx and the midregion using a 25 gauge × 1-in. needle. An eyelash was used to help position the extruded gonads as they stuck to the slide. The slide was then bathed in 95% ethanol for 10 min. Excess ethanol was removed, and 6 μl of VectaShield Mounting Medium with DAPI and a coverslip was added. Gonads were analyzed under 400–1000× magnification with a Zeiss Axioscope equipped with epifluorescence, and photographed with a digital Spot Camera (Diagnostic Instruments). The morphology of DAPI-stained germ cell nuclei was used to distinguish mitotically dividing nuclei, early meiotic nuclei, and late meiotic nuclei. Nuclei with a distinct crescent-shaped morphology have been identified as early meiotic nuclei, based on the expression of meiotic markers and the absence of mitotic markers (Hansen *et al.* 2004); these nuclei define the "transition zone," in which nuclei are in early meiotic prophase. The size of the proliferative zone can be quantified by measuring the distance between the distal boundary of the transition zone and the distal tip of the gonad, or by counting the total number of nuclei in this region (Hansen *et al.* 2004; Killian and Hubbard 2005). For our analysis, we defined the boundary between the proliferative zone and the transition zone as the distal-most region that contained at least two crescent-shaped nuclei, and we counted all germ nuclei distal to this boundary as a measure of proliferative zone size. For dissections of males or hermaphrodites from a population of mixed genotype, desired worms were isolated 1–5 hr before the time of harvest. The Student's *t*-test was used for statistical analysis.

Notch/glp1(ar202) gain-of-function temperature shift

Each strain was synchronized at 15° as described above. Worms were shifted to 25° after 123 hr at 15°, which corresponds to the beginning of day 2 of adulthood at 20°. After 24 or 48 hr at 25°, worms were harvested and prepared for whole-mount DAPI staining as described above. The morphology of the DAPI-stained nuclei and their distribution in the gonad was used to categorize gonads into the three categories of late-onset tumors (as distinct from proximal tumors that do not develop under this late temperature-shift regime; Berry *et al.* 1997; Pepper *et al.* 2003), as described in the figure legend of Figure 3. The phenotype of the two gonads of a single animal did not necessarily correlate, and therefore were scored independently.

Notch/glp1(bn18) loss-of-function temperature shift

glp-1(*bn18*)^{ts} hermaphrodites were grown at 15° until the designated time of shift to 25°; at this point, worms were each transferred by picking onto a preequilibrated 25° plate; every 8–12 hr, each worm was transferred to a new 25° plate, so that embryos could be counted within 20 hr of being laid [because of the temperature-sensitive embryonic lethal phenotype caused by the *glp-1*(*bn18*)^{ts} allele, all embryos laid at 25° fail to hatch (Kodoyianni *et al.* 1992). Transfers were continued until no embryos were observed on at least two successive plates.

Embryo-laying rates

Synchronized worms were plated to individual temperature-equilibrated plates just before the start of adulthood (50 hr for 20° and 80 hr for 15°). Once egg laying had begun, worms were transferred every 6–12 hr (for 20° as in Figure 8A) or every 6–18 hr (for 15° as in Figure 8B) to fresh plates. Upon transfer, the number of laid embryos were counted, and this process was repeated until no embryos were observed on at least two successive plates. The embryo-laying rate (embryos/hr) for each window was calculated as the number of embryos divided by the number of hours the mother had spent on the plate. The Student's *t*-test was used for statistical analysis. We noted that the final brood sizes attained in these experiments were slightly smaller than usual for both N2 and *glp-1*(*bn18*)^{ts}, and may be the result of the frequent transfers required for rate measurements. Average brood size in the 15° embryo-laying rate experiment was 277 ± 37 for N2 (n = 11) and 260 ± 24 (n = 17) for *glp-1*(*bn18*), compared to average brood size in the 15° experiment with only daily transfers of 321 ± 45 for N2 (n = 8) and 300 ± 17 (n = 10) for *glp-1*(*bn18*).

Oocyte size measurements

Synchronized hermaphrodites of the appropriate age were placed in M9 buffer on a 2% agarose pad containing 5 mM sodium azide to immobilize the worms. The volume of the most mature oocyte (−1) in each visible gonad was estimated according to McCarter *et al.* (1999). Comparisons of N2 and *hop-1*(*ar179*) were done in quadruplicate, yielding similar results. An example of the average oocyte volumes obtained in one experiment is as follows: $N2$ = 15,480 ± 2950 μm^3 (n = 15); *hop-1*(*ar179*) = 19,640 ± 2500 μm^3 (n = 15); and *sel-12*(*ok2078*) = 15,580 ± 3620 μm^3 (n = 28). The difference between the N2 and *hop-1*(*ar179*) mean oocyte volumes is statistically significant (P = 0.0003), but not that between N2 and *sel-12*(*ok2078*) (P = 0.924, Student's *t*-test). To measure penetrance of the enlarged oocyte (Looc) phenotype, we used the criteria of Nadarajan *et al.* (2009); a gonad is scored as Looc if the −1 oocyte volume is ≥ 20% larger than the corresponding mean volume for N2 in the same set of experiments (Nadarajan *et al.* 2009). All oocyte measurements were done on worms raised at 20°, except for *glp-1*(*bn18*)^{ts} animals, which were grown at 15° until the L4

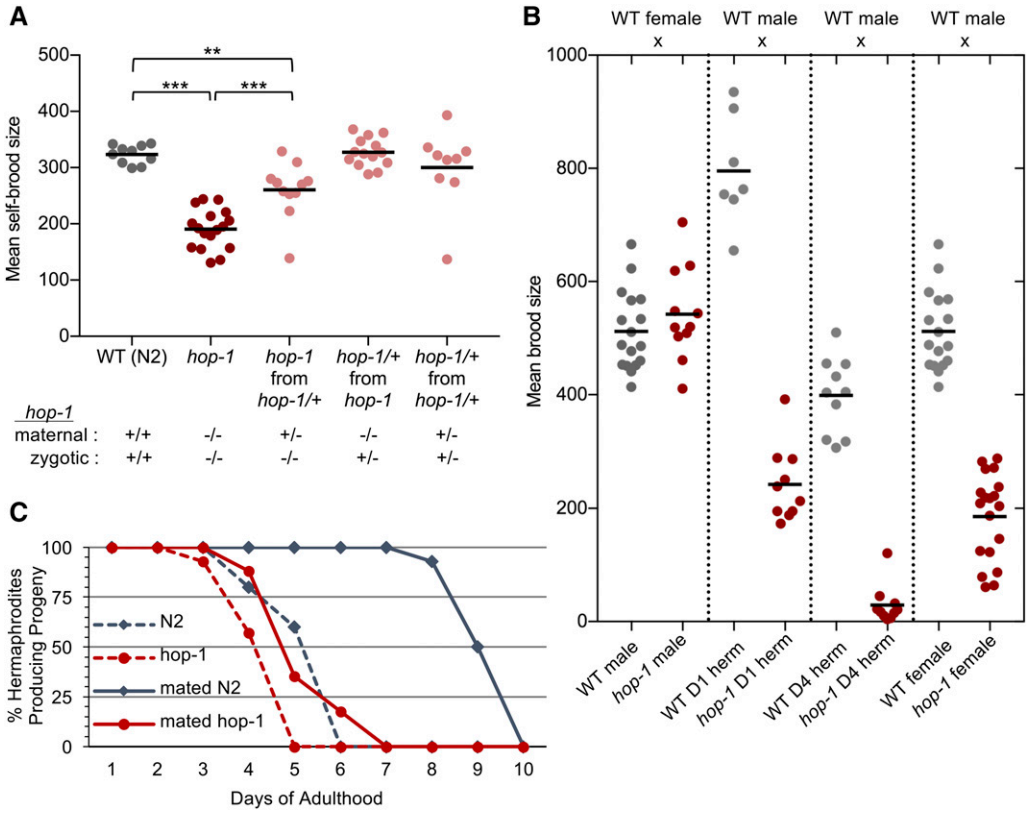

Figure 1 HOP-1 is required for maximum fecundity and duration of reproductive span. (A) Plot of self-brood size for hermaphrodites with reduced *hop-1* function. *hop-1*(+) activity is reduced in the hermaphrodite being assayed (zygotic genotype) as well as her mother (maternal genotype), as indicated; "−" represents the *hop-1*(*ar179*) null allele and "+" represents the wild-type (WT) allele. Full genotypes are (left to right): N2, *hop-1*(*ar179*), *hop-1*(*ar179*) derived from *hop-1*(*ar179*)/*dpy-5*(*e61*) *unc-13*(*e1091*) mothers, *hop-1*(*ar179*)/+; *unc-32*(*e189*)/+; *him-5*(*e1490*)/+ from *hop-1*(*ar179*); *unc-32*(*e189*) mothers mated to *him-5*(*e1490*) males, and *hop-1*(*ar179*)/*dpy-5*(*e61*) from *hop-1*(*ar179*)/*dpy-5*(*e61*) mothers. Horizontal lines indicate mean; *n* values are (left to right): 10, 17, 11, 14, and 9. Difference between the last two groups and WT are not statistically significant ($P > 0.3$); ** $P < 0.005$ and *** $P < 0.001$. (B) Plot of brood size for mated hermaphrodites (herms) or females. For each experimental pair, the animal indicated above the plot was mated with either *hop-1*(*ar179*) (red) or control *hop-1*(+) (gray) worms. Pair 1: total cross progeny brood produced by mating to *fog-2*(*q71*) females to indicated *him-8*(*e1489*) males. Pair 2: total brood produced by mating *him-8*(*e1489*) males to indicated young adult (day 1) hermaphrodites. Pair 3: cross progeny brood produced by mating *him-8*(*e1489*) males to indicated adult day 4 hermaphrodites after exhaustion of self-progeny [data are derived only from animals that successfully produced progeny, which was 90% for N2 day 4 hermaphrodites ($n = 30$) and 30% for *hop-1* day 4 hermaphrodites ($n = 33$)]. Pair 4: cross progeny brood produced by mating *him-8*(*e1489*) males to indicated *fog-2*(*q71*) females. The control data for pair 1 and pair 4 (WT female × WT male) are the same, replotted for ease of comparison. Horizontal lines indicate mean; *n* values are (left to right): 17, 11, 7, 10, 10, 10, 17, and19. Differences between the two genotypes assayed within each pair are all statistically significant ($P < 1 \times 10^{-6}$), except for pair 1 ($P = 0.32$). (C) Graph of percentage of unmated or mated hermaphrodites that produce progeny (≥ 1) each day over 10 days of adulthood. *hop-1*(*ar179*) hermaphrodites in red and N2 hermaphrodites in blue; $n = 10$ for unmated N2, $n = 14$ for unmated *hop-1*(*ar179*), $n = 14$ for mated N2, and $n = 17$ for mated *hop-1*(*ar179*).

stage, shifted to 25°, and then observed after 16 hr at 25° (Nadarajan *et al.* 2009).

Data availability

All strains not provided by the *Caenorhabditis* Genetics Center are available upon request.

Results

HOP-1 is necessary for maximum fecundity in hermaphrodites

hop-1 mutant hermaphrodites are self-fertile and produce viable progeny (Li and Greenwald 1997); however, the extent of germline function in these animals had not been investigated. We first probed germline output in HOP-1-deficient hermaphrodites by measuring the number of self-progeny produced by hermaphrodites that lacked zygotic and maternal *hop-1*(+) activity. For our analyses, we used the *hop-1*(*ar179*) allele, which contains an internal deletion that removes most of the coding sequence (see *Materials and Methods*). On average, *hop-1*(*ar179*) hermaphrodites were found to produce 56% the number of self-progeny produced by wild-type hermaphrodites in similar conditions (Figure 1A). The reduced self-brood size of *hop-1*(*ar179*) is a recessive phenotype, and either zygotically or maternally supplied *hop-1*(+) activity can contribute to increasing brood size, with the largest effect coming from the zygotic contribution (Figure 1A). Two independent missense alleles of *hop-1* also cause a reduced fecundity phenotype: *hop-1*(*gk862343*) hermaphrodites have an average brood size of 172 ± 40 ($n = 9$) and *hop-1*(*gk751127*) hermaphrodites have an average brood size of 259 ± 40 ($n = 14$).

Decreased fecundity of hop-1 mutants can be attributed to decreased oocyte production and/or function

The self-brood of a hermaphrodite is normally limited by the number of sperm produced, so we compared sperm production in wild-type and HOP-1-deficient hermaphrodites. Sperm are stored in two spermathecas, one in each of two gonad arms. At the beginning of adulthood, *hop-1*(*ar179*) hermaphrodites contained an average of 152 ± 31 sperm per gonad arm ($n = 34$), which is not significantly different from that of wild-type hermaphrodites [161 ± 16 ($n = 7$);

$P = 0.278$] and should be sufficient to produce a wild-type sized brood (2×152 progeny). Thus, the fully penetrant fecundity defect of *hop-1*(*ar179*) hermaphrodites cannot be explained by insufficient sperm production.

To test the functionality of sperm produced in the absence of *hop-1*(+) function, we turned to *hop-1*(*ar179*) males. Although this is not a direct test of hermaphrodite sperm function, sperm development proceeds similarly in hermaphrodites and males, and therefore we reasoned that a major role for HOP-1 in sperm development might be revealed by this assay. Young *hop-1*(*ar179*) males were found to sire broods to equivalent size as those sired by wild-type males under the same mating conditions, indicating that there was no significant defect in the functionality of the sperm produced by HOP-1-deficient males (Figure 1B, first pair).

To eliminate the role of sperm in brood size, exogenously supplied wild-type sperm were introduced into *hop-1*(*ar179*) hermaphrodites by mating. If the *hop-1* brood defect were caused by defective hermaphrodite sperm, then *hop-1*(*ar179*) animals would be expected to yield wild-type broods when mated to wild-type males. We found the opposite result: the unlimited supply of sperm allowed wild-type hermaphrodites to achieve a mean brood size of 796 ± 98 ($n = 7$), while mated *hop-1*(*ar179*) hermaphrodites yielded an average brood size of only 242 ± 66 ($n = 10$) (Figure 1B, second pair).

To completely remove the contribution of hermaphrodite-produced sperm, brood production was analyzed for two types of mated *hop-1* animals that lacked their own sperm: either *hop-1*(*ar179*) hermaphrodites that had exhausted their own sperm supply (day 4 adults) or female *hop-1*(*ar179*) animals that produce only oocytes due to the feminizing *fog-2*(*q71*) mutation. In both cases, the fecundity of *hop-1*(*ar179*) animals was dramatically reduced relative to otherwise wild-type animals (Figure 1B, third and fourth pairs), suggestive of a defect in oocyte production or function.

In wild-type hermaphrodites, progeny production continues until the sperm supply is exhausted. We reasoned that if oocyte production is compromised in *hop-1*(*ar179*) animals, then *hop-1*(*ar179*) hermaphrodites that had ceased to produce progeny might contain unutilized sperm. To this end, we analyzed DAPI-stained gonads from day 4 and day 6 adult hermaphrodites. At this age, almost all wild-type hermaphrodite gonads are devoid of spermatids (1.8% N2 gonads contain > 10 spermatids; $n = 109$). In contrast, 43% of *hop-1*(*ar179*) gonads contain > 10 spermatids ($n = 106$), and most of these have > 20 spermatids, which was never observed among the wild-type gonads. Although we cannot eliminate a possible role for HOP-1 in hermaphrodite sperm function, our results demonstrate an important role for HOP-1 in oocyte production and/or function, and defects in this role could fully account for the fecundity defect of *hop-1*(*ar179*) hermaphrodites.

HOP-1-deficient animals have reduced reproductive span

Optimal oocyte production/function could require HOP-1 function throughout the life of a hermaphrodite. However, an alternative model is suggested by the above mating experiments, in which the hermaphrodite reproductive span was extended by the presence of an unlimited sperm supply. Under these conditions, the need for HOP-1 function is more pronounced in older animals than in younger animals. For example, day 4 adult *hop-1*(*ar179*) hermaphrodites showed the most severe defect in fecundity: only 30% produced cross progeny upon mating, and most of these produced < 25 progeny ($n = 33$). In contrast, 90% of wild-type day 4 adult hermaphrodites produced cross progeny, and most produced > 300 progeny ($n = 30$) (Figure 1B). These results suggest that the role of HOP-1 in oocyte production/function might become increasingly important with age, and thus play a key role in allowing an extended reproductive span in the presence of unlimited sperm.

To directly assess reproductive span, we followed *hop-1*(*ar179*) hermaphrodites over the course of 10 days of adulthood in the presence of wild-type males, and measured the proportion of animals that produce progeny each day. Because males are present, sperm are no longer limiting, and the duration of the reproductive span can be measured. In these experiments, $> 90\%$ of control wild-type hermaphrodites continued producing progeny through 8 days of adulthood. In contrast, none of the *hop-1*(*ar179*) hermaphrodites continued to produce progeny after the sixth day, and most produced their last progeny on the fourth day of adulthood (Figure 1C). This analysis demonstrates that HOP-1 function allows a roughly twofold extension of the reproductive span of hermaphrodites.

HOP-1 is necessary for sustained germ cell proliferation in the adult

In hermaphrodites, sperm and oocytes are derived from a progenitor stem cell population that proliferates in the distal end of each of two gonad arms. Proliferation begins during early larval development and continues throughout adulthood, generating > 1000 germ cell progenitors per gonad arm (Kimble and White 1981). Maintenance of a proliferative germline stem cell pool is dependent on Notch signaling from the somatic distal tip cell (DTC), which surrounds the distal region of the gonad (Austin and Kimble 1987; Henderson *et al.* 1994). In response to Notch ligand, the underlying germ cell progenitor cells maintain an undifferentiated mitotic fate, but as they move proximally through the gonad, they evade the effects of Notch signaling from the DTC and transition to meiosis. The first 40 germ cells to enter meiosis do so during the L3–L4 stages, and ultimately differentiate into sperm. All germ cells that enter meiosis subsequently will take on an oogenic fate, which includes oocytes and nurse cells [for review see Hansen and Schedl (2013), Pasternak *et al.* (2004)]. The adult gonad contains an assembly line of germ cells from the most distal pool of proliferating stem cells to the most proximal mature oocyte. Morphological changes in early meiotic prophase nuclei can be used to estimate where the transition to meiosis occurs along the distal–proximal axis of the gonad. The region distal to this transition zone is referred to as the proliferative zone, and contains proliferating

germ cell progenitors as well as germ cells in meiotic S phase in preparation for entry into meiotic prophase (Hansen *et al.* 2004; Crittenden *et al.* 2006; Maciejowski *et al.* 2006; Cinquin *et al.* 2010; Fox *et al.* 2011). Excessive Notch signaling increases the size of the proliferative zone, as meiotic entry is delayed to a more proximal position (Berry *et al.* 1997; Pepper *et al.* 2003). Conversely, decreased Notch signaling results in a smaller proliferative zone because germ cells enter meiosis prematurely, diminishing the size of the stem cell pool (Austin and Kimble 1987; Michaelson *et al.* 2010; Fox and Schedl 2015). In extreme cases, where no GLP-1/Notch receptor activity is present, germline proliferation fails to occur, and instead the first several larval germ cells enter meiosis and differentiate into sperm. In this case, no other germ cells—mitotic or meiotic—populate the adult gonad, resulting in a sterile gonad (Austin and Kimble 1987). The same phenotype is observed upon depletion of both HOP-1 and SEL-12 presenilins (Li and Greenwald 1997; Westlund *et al.* 1999; Francis *et al.* 2002), demonstrating the essential role of presenilin function in germline Notch signaling.

The fact that both sperm and oocytes are produced by HOP-1-deficient hermaphrodites indicates that sufficient Notch signaling occurs in the distal gonad to prevent meiotic entry and allow at least some germ cell proliferation. However, the reduced fecundity of *hop-1*(*ar179*) hermaphrodites led us to hypothesize that either the level, or the duration, of Notch signaling might be reduced in a HOP-1-deficient gonad, resulting in a smaller number of proliferating germ cells. We measured the size of the proliferative zone in gonads dissected from *hop-1*(*ar179*) hermaphrodites over the course of adulthood, and compared them to those of similarly aged wild-type hermaphrodites. Almost all *hop-1*(*ar179*) hermaphrodites began adulthood with a gonad proliferative zone that was comparable in size to that of wild-type young adults; however, all *hop-1*(*ar179*) gonads failed to maintain this proliferative zone through adulthood (Figure 2, A and B and Supplemental Material, Figure S1 in File S1). Within the first 5–10 hr of adulthood, the mean proliferative zone size of *hop-1* gonads dropped to only 53% that of similarly aged wild-type gonads (60 hr posthatch). At this time point, only 52% of *hop-1*(*ar179*) young adult gonads had a proliferative zone that contained $>$ 100 germ cell progenitors (compared to 100% for N2 gonads), and 29% lacked a proliferative zone all together [$n = 21$ for *hop-1*(*ar179*) and $n = 24$ for N2; see Figure S1 in File S1]. The penetrance of this phenotype increased as the *hop-1*(*ar179*) hermaphrodites aged, such that by day 3 of adulthood, no *hop-1*(*ar179*) gonads contained $>$ 50 nuclei in the proliferative zone, and most gonads had morphologically distinguishable meiotic nuclei in the distal-most region of the gonad, indicating premature meiotic entry of germ cell progenitors (Figure 2A and Figure S1 in File S1). In comparison, wild-type day 3 or day 4 adult hermaphrodites all maintained a proliferative zone of $\geq$ 100 nuclei (Figure 2, A and B and Figure S1 in File S1). These results demonstrate that the key contribution of the HOP-1 presenilin is not in the establishment of a germ cell proliferative population, but rather in the maintenance of this proliferating pool through adulthood.

Analysis of male *hop-1*(*ar179*) gonads also revealed HOP-1 dependence for the maintenance of an adult stem cell population. *hop-1*(*ar179*) male gonads successfully established a proliferative zone during larval development, but the proliferative zone was quickly depleted during the end of larval development and early adulthood (Figure 2C). The gonads of almost all day 2 adult males contained only differentiated spermatocytes and spermatids, even in the distal-most region of the gonad, where Notch signaling would normally maintain the undifferentiated germ cell progenitor fate. In contrast, similarly aged wild-type adult males maintained a distal population of undifferentiated germ cells.

The absolute need for HOP-1 function to maintain the adult germline stem cell pool demonstrates that SEL-12 and HOP-1 are not functionally redundant for this role, but it is possible that they are both additively required, and that SEL-12-deficient gonads might also display a similar adult depletion of the gonad proliferative zone. We analyzed the gonads of young adult *sel-12*(*ok2078*) hermaphrodites and found that the proliferative zone was comparable to wild-type (average of 235 $\pm$ 36 nuclei for 55 hr and 240 $\pm$ 49 nuclei for 60 hr; $n = 21$ for each time point; $P > 0.1$ for N2 *vs.* *sel-12* at both time points). Unfortunately, we were unable to accurately assess the persistence of this proliferative zone through adulthood because the *sel-12* egg-laying defect caused accumulating embryos to impinge upon the gonad, ultimately leading to matricide. Instead, to directly compare SEL-12- and HOP-1-deficient gonads through adulthood, we turned to male gonads. Unlike male *hop-1*(*ar179*) gonads, *sel-12*(*ok2078*) male gonads were found to maintain a proliferative zone of wild-type size through at least day 4 of adulthood (Figure 2, C and D). These results demonstrate that the SEL-12 presenilin is entirely dispensable for maintaining germ cell proliferation in adulthood, and indicate that the HOP-1 presenilin is both necessary and sufficient for this role.

HOP-1 is necessary for GLP-1/Notch signal transduction in the adult gonad

A likely explanation for the loss of germ cell proliferation in adult *hop-1*(*ar179*) gonads is that the Notch receptor cannot be activated in adult HOP-1-deficient gonads. We tested this hypothesis by monitoring the ability of a hyperactive *glp-1*/Notch allele to induce late-onset "germline tumors" in adult HOP-1-deficient gonads. The *glp-1*(*ar202*) point mutation causes temperature-sensitive hyperactivity of the *glp-1*/Notch receptor that is still dependent on γ secretase activity (Pepper *et al.* 2003). Excessive Notch activation throughout the gonad leads to ectopic germ cell proliferation that, in extreme cases, is described as a germline tumor because the gonad becomes filled with proliferating germ cells at the expense of differentiating germ cells. To focus our inquiry on adult *glp-1*/Notch activation, we allowed *hop-1*(*ar179*); *glp-1*(*ar202*) hermaphrodites to complete larval development and day 1 of adulthood at the permissive temperature, and then shifted them to the restrictive temperature to

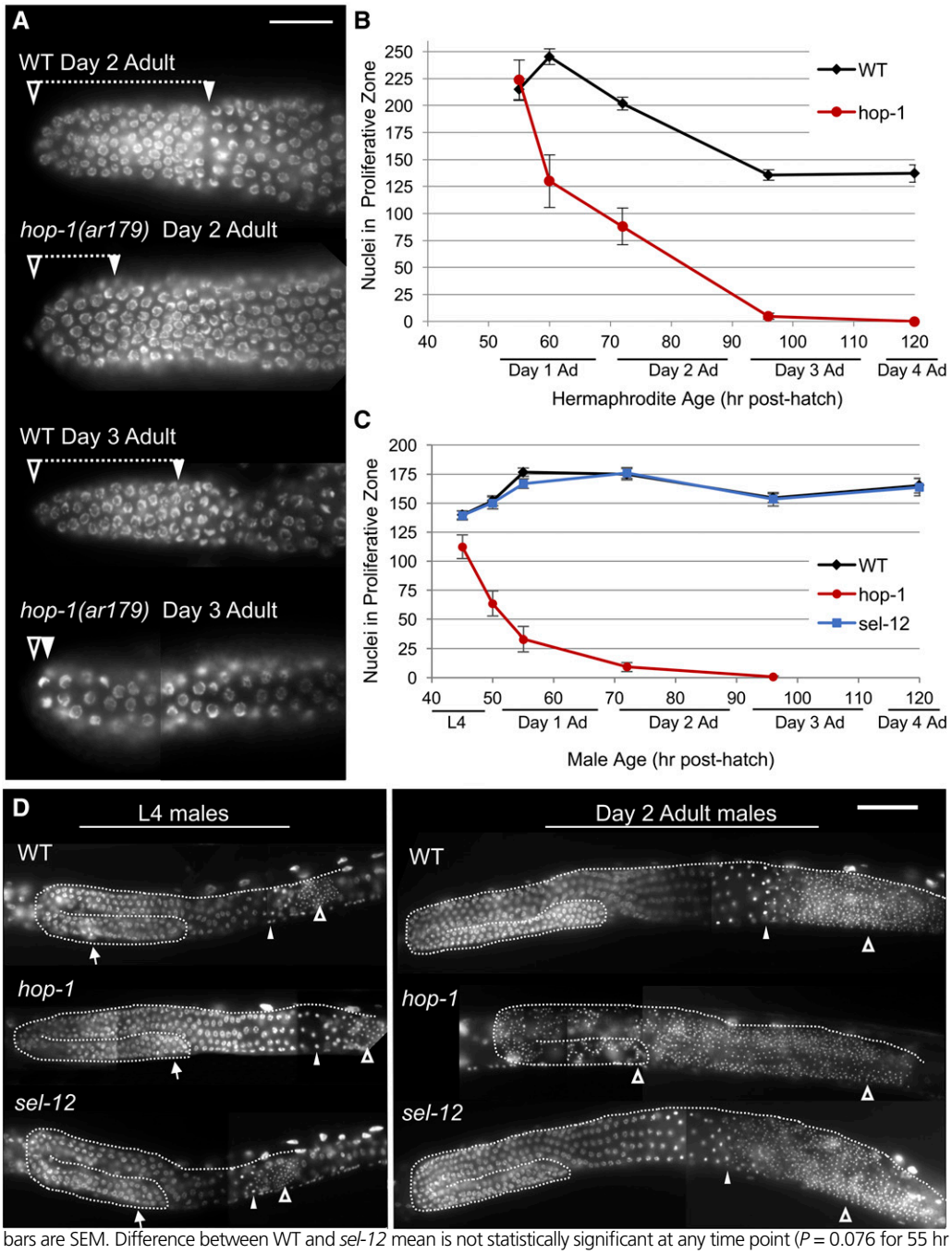

Figure 2 HOP-1 is required to maintain the germline proliferative zone in adulthood. (A) Representative hermaphrodite gonads dissected from day 2 (72 hr posthatching) and day 3 adults (96 hr posthatching); only the distal portion of each gonad arm is shown. Open arrow indicates distal tip of the gonad and closed arrow indicates distal boundary of the transition zone at which overt meiotic differentiation is recognized by crescent-shaped leptotene/zygotene nuclei. Bar, 20 μm. (B) Mean size of proliferative zone (number of nuclei) for gonads dissected from wild-type (WT) N2 and *hop-1*(*ar179*) hermaphrodites at different time points through adulthood at 20°. Under these conditions, hermaphrodites [WT and *hop-1*(*ar179*)] reach the L4/adult molt by 48–50 hr posthatching, and begin adulthood by 55 hr posthatching (see *Materials and Methods* for developmental timing measurements). $N \geq 20$ dissected gonads for each data point except *hop-1* day 4 adult (Ad), for which $n = 15$. Error bars are SEM. Difference between *hop-1* and N2 mean is statistically significant at each time point except 55 hr ($P = 0.66$ for 55 hr and $P < 0.0001$ for all other time points). The penetrance of the *hop-1*(*ar179*) defect at each time point is shown in distribution charts in Figure S1 in File S1. (C) Mean size of proliferative zone (number of nuclei) for gonads dissected from WT, *hop-1*(*ar179*), and *sel-12*(*ok2078*) males at different time points from L4 through adulthood at 20°. All strains carry *him-8*(*e1489*). $N \geq 20$ dissected gonads for each data point except day 3 Ad ($n \geq 16$) and *sel-12* day 4 Ad ($n = 9$). Error bars are SEM. Difference between WT and *sel-12* mean is not statistically significant at any time point ($P = 0.076$ for 55 hr and $P > 0.78$ at all other time points). Difference between WT and *hop-1* mean is statistically significant at all time points ($P = 0.018$ for 45 hr and $P < 0.0001$ for all other time points). (D) Representative gonads of WT, *hop-1*(*ar179*), and *sel-12*(*ok2078*) males [all *him-8*(*e1489*)]. The gonads of all L4 males contain mitotically dividing germ cells in the most distal region (the distal gonad is reflexed against the remainder of the gonad; examples of cells in the brief mitotic metaphase are indicated with arrows). Germ cells in the bend region of the gonad have entered meiosis, and those more proximally have developed into spermatocytes (solid arrowhead) and mature spermatids (open arrowhead). The same organization persists in day 2 Ad gonads of WT and *sel-12* males, but not in *hop-1* gonads where all germ cells have switched to meiosis and developed into spermatids. All animals are positioned with their anterior to the left, and gonads are outlined with a dotted line. Bar, 50 μm.

induce the hyperactivity of the *glp-1*(*ar202*) allele in day 2 adults. The effect of adult *glp-1*(*ar202*) induction can be seen by comparing gonads after 24 and 48 hr at the restrictive temperature. In an otherwise wild-type background, the percentage of gonads exhibiting excessive proliferation at the expense of meiotic differentiation increased from 21% at 24 hr to 66% after 48 hr; similarly, the percentage of tumorous gonads increased from 5.7% at 24 hr to 24% at 48 hr (Figure 3). In contrast, in a HOP-1-deficient background, the penetrance of these phenotypes changed very little between 24 and 48 hr (Figure 3), indicating that in HOP-1-deficient adults, *glp-1*(*ar202*) cannot be activated.

In these experiments, we cannot fully eliminate residual *glp-1*(*ar202*) hyperactivation that might have occurred during larval development at the permissive temperature. Nonetheless, the ability of the *hop-1*(*ar179*) mutation to suppress *glp-1*(*ar202*)-induced late-onset germline tumors supports the conclusion that Notch signal transduction in adult germ cells requires the HOP-1 presenilin.

The hop-1 mutant phenotype is a late-onset glp-1/Notch mutant phenotype

We propose that the *hop-1*(*ar179*) phenotype represents a novel late-onset *glp-1/Notch* phenotype, in which Notch signaling is functional in the early gonad but nonfunctional later in adulthood. This scenario would explain how *hop-1* animals can successfully establish and expand a germline, but cannot maintain it in adulthood. In contrast, SEL-12-deficient gonads can both establish and maintain the germ cell stem cell fate, and gonads that lack both HOP-1 and SEL-12 function fail to establish a proliferative germline at all (Li and Greenwald 1997; Westlund *et al.* 1999). Given these results, we propose that the wild-type germline normally switches from a dual presenilin mode, in which either HOP-1 or SEL-12 executes Notch activation, to an exclusive HOP-1 presenilin mode, thus accounting for the late-onset Notch phenotype of HOP-1-deficient gonads.

If a late-onset Notch defect is responsible for the *hop-1* fecundity defect, then we predicted that a *hop-1*-like brood could be phenocopied in a wild-type hermaphrodite by experimentally turning off Notch signaling at the time at which the gonad normally switches from dual presenilin to HOP-1 dependence. We used a loss-of-function temperature-sensitive *glp-1*(*bn18*)^{ts} allele (Kodoyianni *et al.* 1992; Qiao *et al.* 1995) to test this idea, and to estimate the developmental timing of the hypothesized switch. *glp-1*(*bn18*)^{ts} hermaphrodites were shifted from permissive (15°) to restrictive temperature (25°) at different times through development and adult life, and fecundity was then assessed by measuring self-broods. The results of this analysis reveal a clear linear relationship between the amount of time spent at permissive temperature (functional Notch signaling) and fecundity (Figure 4). This correlation is consistent with the well-established relationship between Notch signaling and the persistence of the stem cell pool (Austin and Kimble 1987), and establishes the minimum time required for Notch signaling to allow hermaphrodite self-fertility. Since all animals shifted during late L3 (60 hr post-hatching at 15°, $n = 19$) generated ≥ 14 progeny, with an average brood size of 40 progeny, we conclude that Notch signaling through the L3 stage is sufficient to support self-fertilization in the hermaphrodite gonad (Figure 4).

To estimate the developmental time point at which animals normally switch from dual presenilin to HOP-1, we asked which of the temperature-shifted populations (represented in Figure 4) most closely recapitulated a *hop-1*-like brood. Additional analysis of *glp-1*(*bn18*)^{ts} animals maintained at the permissive temperature (see below and Figure S2 in File S1) revealed that the embryo output rate was slightly slower

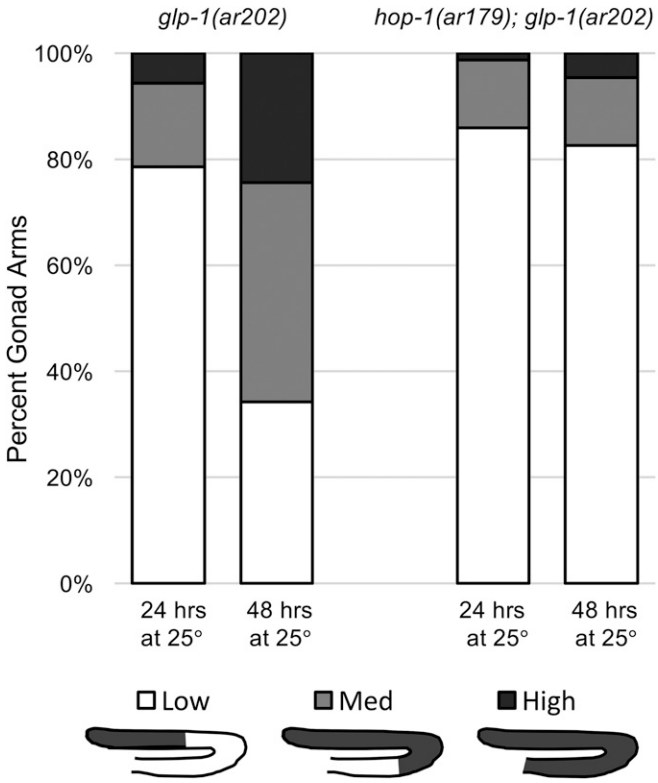

Figure 3 *hop-1*(*ar179*) suppresses the late-onset tumor caused by *glp-1* (*ar202*). *glp-1*(*ar202*) and *hop-1*(*ar179*); *glp-1*(*ar202*) hermaphrodites were raised at the permissive temperature (15°) through day 1 of adulthood, and then shifted to the restrictive temperature (25°) to induce *glp-1*(*ar202*) hyperactivity on day 2 of adulthood. The penetrance of the late-onset tumor phenotype, characterized by excess accumulation of undifferentiated germ cells, is plotted for *glp-1*(*ar202*) and for *hop-1*(*ar179*); *glp-1*(*ar202*) gonad arms, as assayed either 24 or 48 hr after temperature shift to 25°. Gonad arms were characterized according to the following criteria: Low = proliferative zone is normal or slightly enlarged, and meiotic cells are present in the mid- and proximal gonad; Med = proliferative zone extends well past the gonad bend, but meiotic cells are present in the proximal gonad; and High = proliferative zone fills entire gonad, and no meiotic cells are visible in the gonad ("full tumor"). *N* = 70, 82, 78, and 86 gonad arms (left to right).

than that of *glp-1*(+) animals at the same temperature, and thus for our interpretation, we adjusted the expected *hop-1*-like brood size accordingly. By the time that wild-type hermaphrodites produce 190 progeny [the mean brood size of *hop-1*(*ar179*) animals at 15° is 190 ± 38, $n = 8$], *glp-1*(*bn18*)^{ts} animals have produced an average of 115 progeny (Figure S2 in File S1); thus, we used 115 as our benchmark for the size of brood that should be attainable by *glp-1*(*bn18*) animals before switching to HOP-1 dependence. By these criteria, the low-fecundity phenotype of *hop-1* was best phenocopied by *glp-1*(*bn18*)^{ts} animals that were shifted to restrictive temperature during the mid-L4 stage (80 hr posthatch at 15°), pinpointing mid-L4 as the developmental time point for the switch between the dual presenilin and HOP-1 modes.

The large range of brood sizes and proliferative zone measurements observed for *hop-1*(*ar179*) hermaphrodites may reflect variation in the exact timing of the switch to HOP-1 dependency (compare wild-type and *hop-1* distribution, and

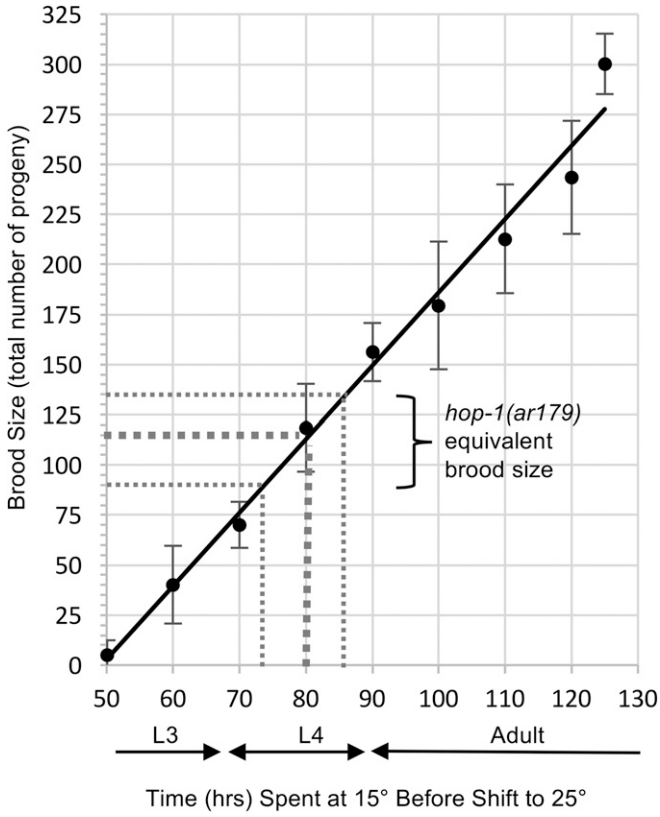

Figure 4 Correlation of GLP-1/Notch activity with hermaphrodite fecundity. Mean brood size is plotted for *glp-1*(*bn18*)^{ts} animals shifted from 15 to 25° at the indicated time. *N* = 10–20 animals for each time point; error bars are SEM. The last data point represents continuous maintenance at 15° without a shift to 25°. Relative developmental stage of animals is indicated below the plot, as determined by examining the progression of vulval and gonad morphogenesis of additional *glp-1*(*bn18*)^{ts} animals at the indicated time points (see *Materials and Methods*). Horizontal dashed lines represent the comparable *hop-1* brood size expected under these conditions: mean brood of 115 progeny (thick line) and range of 88–140 (thin lines) (see *Materials and Methods* and Figure S2 in File S1). Vertical dashed lines indicate corresponding times at which a temperature shift of *glp-1*(*bn18*)^{ts} animals would be expected to yield the *hop-1*-like brood size.

error bars in Figure 1A and Figure 2, B and C). Using a *glp-1*(*bn18*)^{ts} temperature-shift graph (Figure 4) and a *glp-1*(*bn18*)^{ts} /*glp-1*(+) conversion graph (Figure S2 in File S1), we predicted that loss of Notch signaling in early L4 would result in a brood of roughly 125 progeny, while loss of Notch signaling at the end of L4 would result in a brood of roughly 250 progeny. In our experiments, the range of observed *hop-1*(*ar179*) broods at 15° was 150–267 progeny (n = 8), consistent with a loss of Notch signaling occurring at slightly different times within the L4 stage.

Declining SEL-12 presenilin availability/activity in the gonad may cause the switch to HOP-1 dependency

A switch to HOP-1 dependence during the L4 stage might indicate that at this developmental time, the SEL-12 presenilin normally becomes unavailable or inactive in the germline. Several observations support this hypothesis. First, reducing the *sel-12*(+) dosage (maternal and zygotic) by half in *hop-1*(*ar179*) animals reduces the average brood size by 34% [126 ± 37, n = 18 for *hop-1*(*ar179*); *sel-12*(*ok2078*)/+ *vs.* 191 ± 35, n = 17 for *hop-1*(*ar179*)]. This diminished brood size is consistent with an earlier cessation of Notch signaling, as predicted by the above *glp-1* temperature-shift experiment. Second, comparison of *hop-1*(*ar179*) males and hermaphrodites reveals an earlier loss of germ cell proliferation in males as compared to hermaphrodites (compare Figure 2, B and C). This difference could be caused by a variety of factors, one of which is the fact that males have only one genetic dose of *sel-12*, compared to the two doses of *sel-12* in a hermaphrodite. *sel-12* is one of the X chromosome genes that escapes dosage compensation, and is expressed twofold higher in hermaphrodites compared to males (Jans *et al.* 2009). To further explore this possibility, we measured the persistence of the gonad proliferative zone in *hop-1*(*ar179*) *tra-2*(*e1095*) pseudomales that have two copies of the X chromosome, and therefore double the genetic dosage of *sel-12* compared to normal males. The proliferative zone was found to persist longer in *hop-1*(*ar179*) *tra-2*(*e1095*) XX pseudomales compared to *hop-1*(*ar179*) XO males (Figure 5). Reciprocally, *hop-1*(*ar179*) males that had reduced *sel-12* function exhibited proliferative zone loss earlier in development than did *hop-1*(*ar179*) males with normal *sel-12* activity (Figure 5). Although these experiments are not a direct demonstration of the influence of *sel-12*, they are consistent with the idea that the timing of the switch to HOP-1 dependence reflects a developmental time at which *sel-12* function declines below a certain threshold.

Further support for this model and specification that *sel-12* reduction might occur at the mRNA level comes from analysis of *sel-12* and *hop-1* expression data (Figure 6) from genome-wide RNA-sequencing (RNA-Seq) expression analysis conducted by ModENCODE (Gerstein *et al.* 2010). The partial genetic redundancy of *sel-12* and *hop-1* predicts that these genes have overlapping expression patterns; however, our results predict that in the L4 gonad, *sel-12* activity becomes limiting while *hop-1* is readily available in the gonad. Indeed, *sel-12* mRNA measurements reveal that at the L4 stage, detectable *sel-12* expression is almost entirely somatic rather than germline, and does not increase significantly upon transition to adulthood. In stark contrast, the *hop-1* gene shows a steep increase in expression after the L2 stage, with the highest expression attained in adults. Furthermore, *hop-1* expression appears to be almost entirely germline expression, as sterile L4 animals show very little *hop-1* expression. Independently, Wang *et al.* identified 4699 genes expressed in dissected gonads from adult hermaphrodites, and *hop-1*, but not *sel-12*, is represented in the collection (Wang *et al.* 2009). Together, these expression data are consistent with a switch in the L4 gonad from dual presenilin mode to exclusive HOP-1 mode, and further suggest that this switch can be at least partially explained by presenilin mRNA levels.

HOP-1-deficient animals produce enlarged oocytes

The *hop-1*(*ar179*) phenotype presents a unique opportunity to analyze oogenesis independently of germline development, because a germline stem cell pool is established and

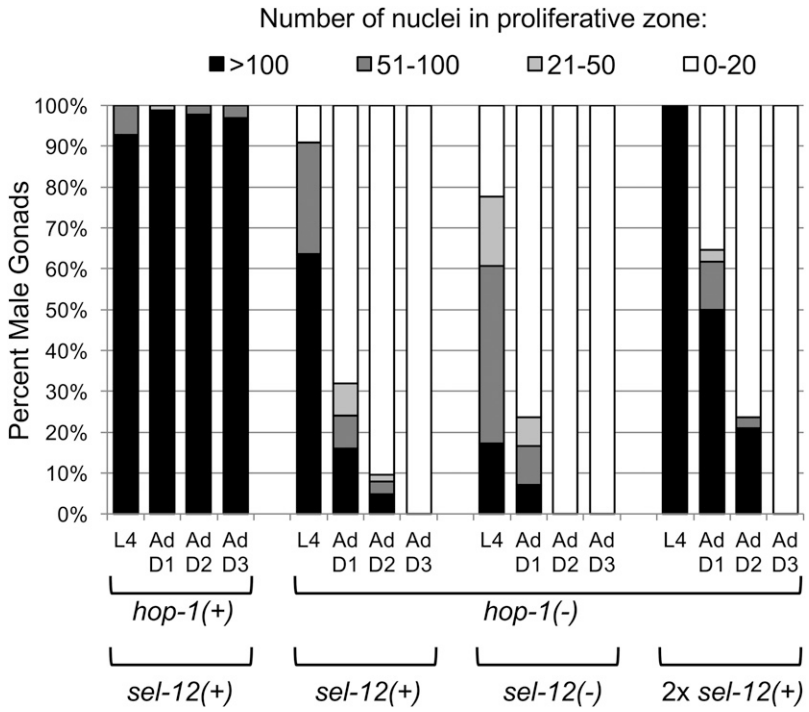

Figure 5 Timing of *hop-1* phenotype onset is modified in animals with altered *sel-12* dosage. Penetrance of male gonad proliferation defect is plotted at four different ages for each of four different genotypes that vary presenilin dosage. Dissected gonads are categorized by severity of phenotype according to size of the proliferation zone (number of nuclei distal to the meiotic transition zone). The *sel-12* gene is located on the X chromosome and is not dosage compensated (Jans *et al.* 2009), so males are hemizygous, except for *tra-2* pseudomales, which have two X chromosomes. $n \geq 22$ gonad arms for each genotype at each time point. Age (left to right) for each genotype: 45, 55, 72, and 96 hr posthatching (20°). Full genotypes (left to right): *him-8*(*e1489*); *sel-12*(+)/ø, *hop-1*(*ar179*); *him-8*(*e1489*); *sel-12*(+)/ø, *hop-1*(*ar179*); *him-8*(*e1489*); *sel-12*(*ok2078*)/ø [*no zygotic sel-12*(+), *and half dose of maternal sel-12*(+)] *hop-1*(*ar179*); *tra-2*(*e1095*); *sel-12*(+)/*sel-12*(+). *D*, *day*.

sufficient sperm and oocytes are generated to yield self-fertility. Without HOP-1 presenilin, we have demonstrated that germ cell proliferation ceases and fecundity is reduced. We observed two additional defects in the *hop-1*(*ar179*) adults. First, as has been documented for other Notch signaling mutants (Nadarajan *et al.* 2009), the mature oocytes of *hop-1*(*ar179*) hermaphrodites are often abnormally large (Figure 7). In 41% of *hop-1*(*ar179*) gonads, the most mature oocyte was ≥ 50% larger in volume than the average size of the corresponding oocyte in a wild-type gonad; by comparison, only 3.4% of N2 gonads exhibited such large oocytes [$n = 76$ for *hop-1*(*ar179*) and $n = 58$ for N2]. In contrast, *sel-12*(*ok2078*) gonads rarely contained such large oocytes (3.6% $n = 28$). Although Notch signaling has been shown to be required to promote timely oocyte cellularization and appropriate oocyte size, the mechanism for this influence remains unknown (Nadarajan *et al.* 2009). Our results demonstrate that the HOP-1 presenilin, and not the SEL-12 presenilin, is essential for this role of Notch signaling in oogenesis.

Notch signaling is necessary in the adult gonad for high embryo output rate

As an indicator of oogenesis productivity, we analyzed the rate of embryo production by *hop-1*(*ar179*) animals over the course of the hermaphrodite egg-laying period. Oogenesis is the rate-limiting step to embryo production in wild-type adult hermaphrodites with sperm, and thus changes in oogenesis output should be reflected in the rate of embryo output (McCarter *et al.* 1999). We expected embryo productivity to cease prematurely in *hop-1*(*ar179*) hermaphrodites because of the diminishing pool of germ cell progenitors, but were surprised to find a reduced embryo output rate throughout adulthood (Figure 8A). Continuous monitoring at 15° revealed greater resolution in this comparison: young adult *hop-1*(*ar179*) animals begin producing embryos at a rate comparable to that of wild-type hermaphrodites, but while wild-type animals dramatically increase output rate through the first 2 days of laying, *hop-1* animals fail to increase production rate beyond the rate attained in the first half-day of laying (Figure 8B).

To determine whether the reduced embryo output rate of *hop-1* hermaphrodites reflects a defect in Notch signaling, we also analyzed the embryo output rate of hermaphrodites with reduced *glp-1*/Notch function. A temperature of 15° is semi-permissive for *glp-1*(*bn18*)^{ts} function, allowing enough Notch signaling in the distal gonad to generate and maintain a proliferative zone, but one that is only 70% the size of a wild-type proliferative zone at 15° (Michaelson *et al.* 2010; Fox *et al.* 2011; and C. Goutte, unpublished results). We found that throughout adulthood at 15°, *glp-1*(*bn18*)^{ts} hermaphrodites exhibited a distinct reduction in embryo output rate relative to wild-type (Figure 8B). Despite the reduced rate, embryo production increased gradually through the first 2 days of laying, closely paralleling the changes in wild-type embryo output over the course of adulthood. Surprisingly, *glp-1*(*bn18*)^{ts} animals continued to produce embryos for a longer time than wild-type, ultimately producing close-to-wild-type-sized broods (Table 1). This result has important general implications for the study of fecundity, because it demonstrates that a reduced rate of embryo production is not necessarily indicative of reduced fecundity. For the purposes of this study, the reduced embryo output rate of *glp-1*(*bn18*)^{ts} animals corroborates the suggestion that Notch signaling is necessary for efficient oogenesis.

Interestingly, the two different temporal profiles of *hop-1* and *glp-1* embryo production rate mimic the temporal profiles

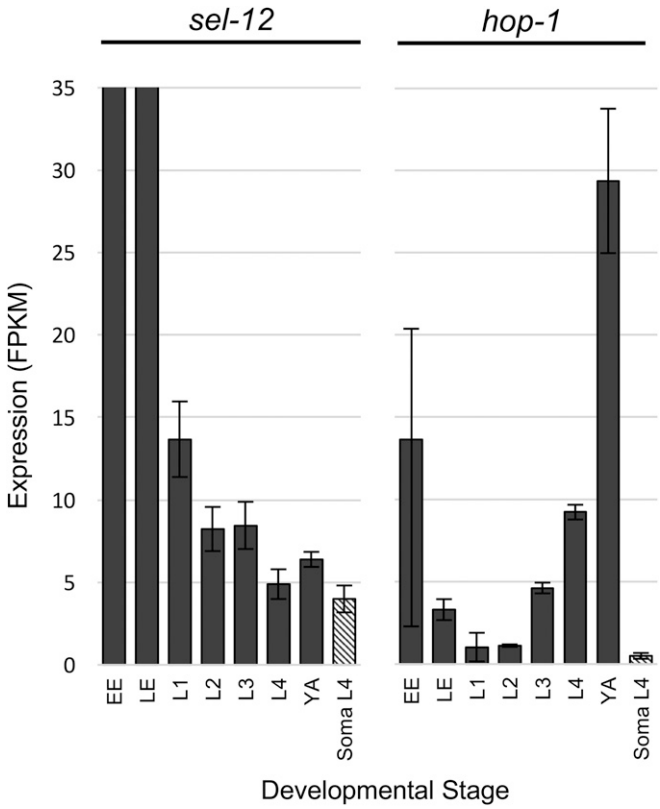

Figure 6 Expression dynamics of *sel-12* and *hop-1* through development. RNA-sequencing expression data from ModENCODE (Gerstein *et al.* 2010) was used to calculate expression values (FPKM) by WormBase (http://www.wormbase.org, release WS260, date Aug 2017). The FPKM values for each experiment in each library in each developmental stage were averaged, and the median average FPKM value for two (or four for EE) independent poly-A RNA-Seq libraries is plotted here, with error bars representing the range of average FPKM values for each stage. The "soma L4" strain has no germline due to the *glp-1*(*q224*) mutation, which prevents germline expansion during larval development. EE, early embryo; LE, late embryo; FPKM, fragments per kilobase of transcript per million mapped reads.

of their defects in germline proliferation: *hop-1* gonads begin adulthood with a normal proliferative zone, which then becomes depleted over the course of the adult laying period, and the embryo production rate is initially comparable to wild-type but then becomes progressively more and more reduced relative to wild-type. In contrast, *glp-1*(*bn18*)^{ts} hermaphrodites produce embryos at a rate that is roughly 70% that of wild-type, regardless of the age of the animal, and the proliferative zone size is 70% that of wild-type animals. This correlation raises the possibility of a simple, yet unrecognized, link between the size of the proliferative zone and the rate of oocyte production.

The reduced embryo production rate of *hop-1*(*ar179*) cannot be explained simply by a reduced number of available oocytes generated by the proliferative zone, because such a model would predict a delay of at least 50 hr (the time required for a germ cell to progress from meiotic entry to mature oocyte; McCarter *et al.* 1999; Jaramillo-Lambert *et al.* 2007) between the onset of the proliferative zone phenotype and the embryo output phenotype. Instead, reduced embryo output is observed in *hop-1* animals at the beginning of adulthood, nearly coincident with the diminishment of the proliferative zone (Figure 2B). As an alternative explanation, we considered the function of germ cells upon exiting the proliferative zone. In a wild-type hermaphrodite gonad, germ cells in early meiotic prophase are transcriptionally active and function as nurse cells to provide material that is taken up by older oocytes developing in the proximal gonad (Wolke *et al.* 2007). It is estimated that at least half of these cells are later culled from the gonad by apoptosis (Gumienny *et al.* 1999; Jaramillo-Lambert *et al.* 2007). We hypothesized that limited nurse cell function could provide an alternative explanation for the reduced *hop-1* embryo output rate, and would better account for the close timing of this phenotype with the reduction in the proliferative zone.

To gauge the contribution of the stem cell pool to nurse cell production, we used our analysis of N2 and *glp-1*(*bn18*)^{ts} to estimate the percentage of germ cell progenitors that take on a nurse cell role rather than develop into an oocyte. We reasoned that nurse cell production in each gonad could be quantified by calculating the number of germ cells that enter meiosis but do not give rise to oocytes. To do this, we used proliferative zone output rates that have been calculated for N2 and *glp-1*(*bn18*)^{ts} at 15° by Fox and Schedl (2015) and compared them to the embryo output rates that we measured at 15°. Since each mature oocyte gives rise to an embryo, the embryo output rate per gonad (half the total embryo output rate) was used to represent the number of oocytes produced per gonad per hour, and was subtracted from the total number of germ cells that enter meiosis each hour (Table 1). These calculations indicate that, for roughly 15 germ cells that enter meiosis per hour at 15°, two will become oocytes and 13 will function only as nurse cells. Thus, at 15°, nurse cells represent 86% of the proliferative zone output in a wild-type hermaphrodite gonad, and the proliferative zone must supply a total of 7.4 germ cells to generate each oocyte. Repeating this analysis for *glp-1*(*bn18*)^{ts} animals indicates that roughly 1.5 oocytes and 11 nurse cells are contributed by the proliferative zone each hour at 15°. Despite the 31% reduction in proliferative zone output rate, *glp-1* animals maintained roughly the same ratio of germ cells to oocyte as wild-type (Table 1). These calculations indicate that there is a high demand on the proliferative zone to generate roughly seven germ cells for each oocyte, and support a direct relationship between the size of the proliferative zone and the rate of oogenesis.

Discussion

The HOP-1 presenilin has a unique function not shared with its paralog SEL-12

We have uncovered a unique role for the HOP-1 presenilin in the adult germline. Previous work demonstrated that hermaphrodites that lack both HOP-1 and SEL-12 presenilins exhibit the sterile Notch loss-of-function phenotype, in which germ cell progenitors fail to proliferate and instead enter meiosis prematurely. Since either single mutant is fertile and successfully establishes a

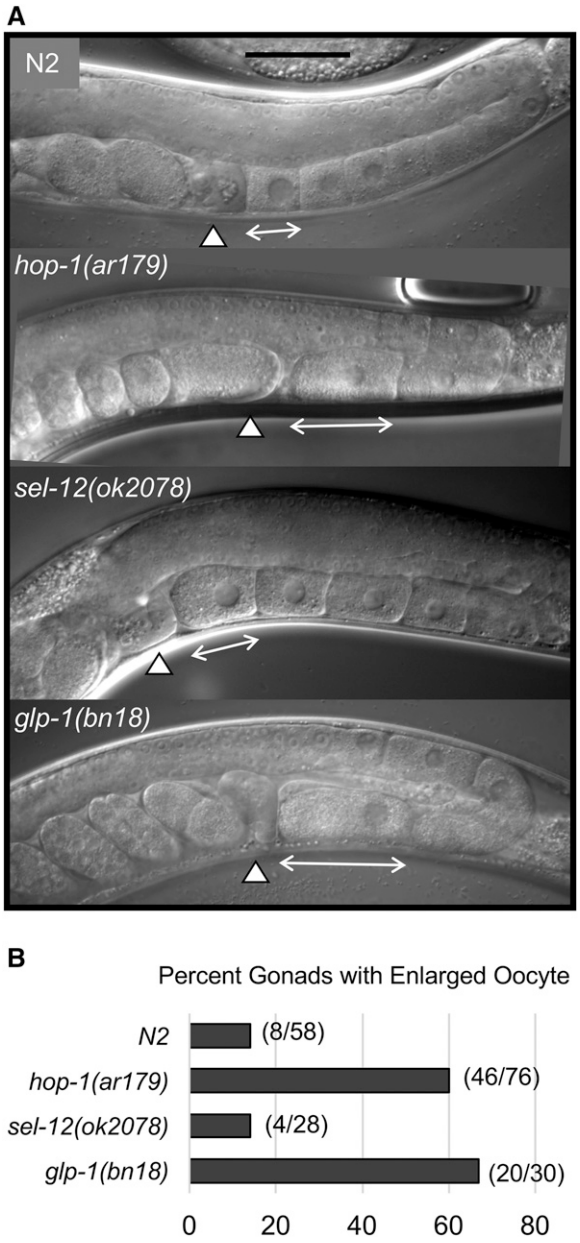

Figure 7 *hop-1*(*ar179*) hermaphrodites produce enlarged oocytes. (A) Representative DIC images of live day 1 adult animals of indicated genotype (only one gonad arm is shown). The length of the most mature oocyte (−1 oocyte, double-headed arrow) and the adjacent spermatheca (arrowhead) are indicated. For reference, a gonad from a temperature-shifted *glp-1*(*bn18*)^{ts} animal [according to Nadarajan *et al.* (2009)] displays the enlarged oocyte phenotype (Looc). Black Bar, 50 μm. (B) Penetrance of the Looc phenotype, using the criteria of Nadarajan *et al.*, whereby a gonad is considered to have the Looc phenotype if the volume of the −1 oocyte is at least 20% greater than the corresponding average wild-type volume (Nadarajan *et al.* 2009).

productive germline, the roles of the HOP-1 and SEL-12 presenilin were concluded to be functionally redundant with respect to Notch signaling in the germline (Li and Greenwald 1997; Westlund *et al.* 1999). These findings suggested that either the *hop-1* or *sel-12* gene could provide the presenilin subunit necessary for γ secretase to cleave the Notch receptor in response to Notch ligand. In this study, we focus on adult gonads and demonstrate that after larval development, the two presenilins are no longer functionally redundant and HOP-1 has an essential role in maintaining gonad productivity in adulthood. Without HOP-1 function, overall fecundity and reproductive span are diminished, and both hermaphrodite and male adult gonads are unable to maintain a germ cell progenitor stem cell population. In contrast, SEL-12-deficient males, which can be analyzed late into adulthood, continue to maintain a wild-type-sized stem cell pool. Thus, in the *C. elegans* germline, the presenilin paralogs have overlapping functions during larval development, but not during adulthood, and the observation that HOP-1 is required to achieve maximum fecundity offers a previously unrecognized evolutionary advantage to preserving the *hop-1* presenilin paralog.

HOP-1 deficiency causes a late-onset Notch phenotype that reveals a switch from dual presenilin mode to HOP-1 mode

The depletion of the germ cell stem cell pool in *hop-1* adults is reminiscent of the phenotype obtained when animals carrying a temperature-sensitive *glp-1*/Notch allele are shifted to restrictive temperature during adulthood (Austin and Kimble 1987). Given the key role of presenilins in Notch signal transduction, we propose that the *hop-1* phenotype reflects a late-onset Notch phenotype, such that Notch-mediated germ cell proliferation proceeds successfully through larval development, but then ceases in adulthood because Notch receptor can no longer be activated in HOP-1-deficient germ cells. In support of this hypothesis, we found that HOP-1-deficient gonads are unable to support late adult Notch induction by the hyperactive *glp-1*(*ar202*) allele. Furthermore, the *hop-1* fecundity defect can be phenocopied by switching off GLP-1/Notch function during the L4 stage in an otherwise wild-type hermaphrodite. Based on these findings, we propose that in the *C. elegans* germline, there is normally a developmental switch from a dual presenilin phase to an exclusive HOP-1 phase, such that germ cells initially have access to either HOP-1 or SEL-12 for Notch activation, but later rely exclusively on HOP-1.

The onset of *hop-1* phenotypes in early adulthood suggests that in a wild-type gonad, dependence on HOP-1 must begin just before, or at the onset of, adulthood. The variability from gonad-to-gonad in the timing of proliferation zone loss suggests that the timing of the switch to HOP-1 dependence is not under tight developmental regulation. Furthermore, the fact that many *hop-1* gonads from day 1 adults have reduced, but not depleted, proliferative zones suggests that in *hop-1*-deficient gonads, total germline Notch signaling decreases gradually rather than abruptly. Our combined analysis of *hop-1*(*ar179*) and *glp-1*(*bn18*) hermaphrodites suggests that Notch signaling through the L3 stage of development is sufficient for hermaphrodite self-fertility, while Notch signaling after L3 serves to increase fecundity. The *hop-1* low-fecundity defect was best phenocopied by switching off Notch signaling during the L4 larval stage, thus confirming the end of larval development as the developmental time

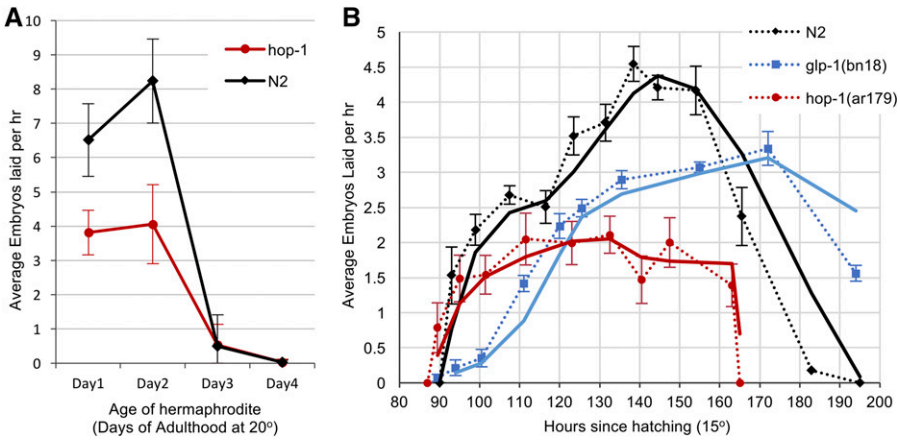

Figure 8 *hop-1*(*ar179*) and *glp-1*(*bn18*)^{ts} hermaphrodites have a slower rate of embryo production. (A) Mean embryo-laying rate for N2 and *hop-1*(*ar179*) hermaphrodites at 20°, as measured in a 6-hr window once a day for the first 4 days of adulthood. Error bars are SD; n = 17 for *hop-1* and n = 12 for N2. (B) Continuous monitoring of embryo-laying rate for N2, *hop-1*(*ar179*), and *glp-1*(*bn18*)^{ts} hermaphrodites at 15°, as measured in 6–18-hr successive windows throughout the laying period. Error bars are SEM; n = 11 for N2, n = 14 for *hop-1*(*ar179*), and n = 17 for *glp-1*(*bn18*)^{ts}. Dashed lines connect average values for each time point; solid lines represent moving average with a period of two data points. Embryo-laying rates at 15° are roughly half of those at 20°.

during which HOP-1 is likely to become essential for germline Notch signaling.

The hop-1 late-onset phenotype may reflect a depletion of germline SEL-12

A logical explanation for the switch to HOP-1 dependence in the wild-type germline is that the alternative presenilin, SEL-12, becomes unavailable or nonfunctional toward the end of larval development, leaving germ cells dependent on the HOP-1 presenilin for Notch activation. In support of this hypothesis, the onset of proliferative zone loss in HOP-1-deficient gonads is earlier among animals that have reduced *sel-12* dosage, and delayed among animals that have elevated *sel-12* dosage. Genome-wide gene expression studies further support this model because germline expression of *sel-12* is low, or undetected, in L4 and adult animals, whereas *hop-1* germline expression increases dramatically during larval development and accounts for most, if not all, *hop-1* expression in the last larval stage before adulthood.

At least part of the *sel-12* gene product used during larval germline development can be supplied maternally. This conclusion is deduced from the observation that *hop-1*; *sel-12* double mutants can successfully establish a larval germline if, and only if, maternal *sel-12* is available (Westlund *et al.* 1999). *hop-1* is also supplied maternally, as evidenced by the partial maternal rescue of the *hop-1* fecundity defect (Figure 1A); however, maternal *hop-1* is not sufficient to allow *hop-1*; *sel-12* double mutants to establish a germline (Westlund *et al.* 1999). Several examples of maternally supplied gene products are known to be essential for germline development. It is not clear how long maternally supplied products can persist in the developing germline, but they must undergo significant dilution during larval germline expansion, since the two founding germ cell progenitors give rise to > 500 germ cells by the end of larval development. Thus, for *sel-12*, which may have only minimal zygotic germline expression, the amount of SEL-12 protein available in germline stem cells may drop below a critical threshold after maternal product is diluted during larval germline expansion. In a developing wild-type gonad, the diminishing supply of maternal *sel-12* product may normally be buffered by the rapid increase in the expression of its paralog, the *hop-1* gene, during larval development.

A molecular difference between Notch signaling for germline expansion vs. germline maintenance

We have postulated two modes for germline Notch signaling: a dual presenilin mode during larval development and an exclusive HOP-1 mode during adulthood. Interestingly, these two phases correspond roughly with two distinct phases of germline development. During larval development, Notch signaling drives expansion of the germline from an initial pool of ~10 germ cell progenitors in the first larval stage (L1) to a total of > 500 germ cells by the end of larval development (Kimble and White 1981). During midlarval development, Notch ligand becomes limited to the distal region of the gonad, thus restricting germline expansion to a distal subset of germ cell progenitors, while more proximal germ cell progenitors enter meiosis. This organization persists in the adult, such that Notch signaling continuously maintains a distal pool of proliferating germ cell progenitors in each gonad. Although these two phases of germline development (larval expansion and adult maintenance) are subject to different cellular environments and different physiological influences (Hubbard *et al.* 2013), it has been assumed that the core components of Notch signal transduction are the same in these two circumstances. Here, we reveal a molecular difference between the core Notch signal transduction machinery used in larval germline expansion *vs.* that involved in adult germline maintenance.

Alternative presenlin subunits offer the opportunity for differential regulation of Notch signal transduction during the two phases of germline development. For example, the efficiency or sensitivity of HOP-1-mediated Notch signaling may be different from that of SEL-12-mediated Notch signaling because of biochemical differences between the two paralogs. Although the HOP-1 and SEL-12 proteins are predicted to have similar topology, and are functionally redundant for several Notch signaling events, they share only 31% amino acid identity, leaving open the possibility for differences in their stability, function, or susceptibility to regulation by other molecules. HOP-1 appears to have evolved under more relaxed

Table 1 Hermaphrodite gonad output at 15°

	Wild-type (N2)	*glp-1*(*bn18*)^{ts}
Germ cells entering meiosis[a] (per hr)	15.4 ± 3.1	10.7 ± 1.9
Embryo production[b] (oocyte production)[c] (per hr)	2.08 ± 0.45 (n = 44)	1.49 ± 0.22 (n = 34)
Calculated nurse cell production[d] (per hr)	13.3	9.2
Nurse cells as fraction of germ cells entering meiosis	86.5%	86.1%
Total germ cells required per oocyte	7.4	7.2
Time required for proliferative zone to supply nuclei for one oocyte[e]	28 min	41 min
Fecundity (progeny per self-brood)	321 ± 45 (n = 8)	300 ± 17 (n = 10)

[a] Proliferative zone output rate from Fox and Schedl (2015; see Table 2b).
[b] Embryo production rate for day 2 adult animals (130–160 hr posthatch, see Figure 8) is divided by 2 to obtain the contribution of each gonad.
[c] For the purposes of this calculation, the number of mature oocytes produced per gonad is estimated to be the same as the number of embryos produced per gonad (< 1% of laid eggs are unfertilized oocytes).
[d] Nurse cells are defined here as any germ cell fate other than oocyte (the difference of lines 1 and 2).
[e] The proliferative zone output rate (line 1) is used to calculate the time required to produce sufficient germ cell nuclei to support the production of one oocyte [average of 7.3 germ cell nuclei (line 5)].

selection than SEL-12, which has maintained more conservation with presenilins from distant species, such as the mammalian Presenilin-1 and Presenilin-2 proteins (Li and Greenwald 1997). Greater constraints on SEL-12 function than on HOP-1 function may have allowed HOP-1 to evolve biochemical differences that affect its performance as the γ secretase catalytic subunit. For example, the demands of rapid germline expansion during larval development may require maximum γ secretase function, which may be better afforded by the SEL-12 presenilin. In contrast, the demands on adult germline maintenance may prioritize responsiveness to physiological or environmental changes rather than maximum output. Molecular comparisons of SEL-12 and HOP-1 presenilin function are needed to determine whether these proteins differentially affect Notch signal transduction in a way that impacts the behavior of the germline during its expansion phase *vs.* its maintenance phase.

Genetic redundancy of autosome/X chromosome gene pairs

If the SEL-12 and HOP-1 presenilins do not impart different effects on Notch activation, then the question remains as to why the *sel-12* gene is unable to fulfill the role of adult germline presenilin. One answer may come from differences in gene expression, which may be partially dictated by chromosomal location. The lower germline expression of X chromosome genes may constrain the amount of *sel-12* presenilin available in germ cells, while the autosomal *hop-1* gene is highly expressed in the germline, as indicated by gene expression studies. In this sense, the important germline role of *hop-1* provides a specific example of the germline/soma specialization that Maciejowski *et al.* (2005) postulated for gene duplicates that have autosome/X chromosome locations. However, unlike the clear germline/soma subfunctionalization of the gene pairs described by Maciejowski *et al.*, a strict division of labor between germline and soma is not apparent for the presenilins until adulthood. We propose that the delay for observing this germline/soma subfunctionalization could be caused by the presence of maternal contributions during early development: only after maternal sources are exhausted can the germline-specific role of the autosome paralog (*hop-1*) be revealed. Maciejowski *et al.* identified 128 X-linked genes that have an autosomal paralog and are likely involved in cell-essential functions, but for which no phenotype had been reported by large-scale RNAi studies. They found that for 16% of these, the autosomal paralog appeared to have germline-specific function, as suggested by associated sterility or embryonic lethality (Maciejowski *et al.* 2005). Our analysis of HOP-1/SEL-12 germline function offers a possible explanation for the lack of apparent germline function for some or all of the remaining 84% gene pairs: maternal contribution of these gene products could mask the germline/soma subfunctionalization until after depletion of the maternal product. If this is the case, depletion of the autosomal paralog, like *hop-1*, may not cause outright sterility, but instead a late-onset sterility after the exhaustion of maternal sources. Thus, our analysis of adult presenilin-deficient males and hermaphrodites points to the importance of analyzing mutants late into adulthood to reveal different roles for genes that appear to be functionally redundant. It will be interesting to see if this type of temporal analysis will uncover adult roles for other redundant genes, particularly those with an X chromosome paralog.

Relationship between germ cell proliferation and adult oogenesis

HOP-1-deficient animals provide a unique opportunity to separate the role of Notch signaling during germline development *vs.* its involvement in adult oogenesis. Because of the early role of Notch signaling in establishing and expanding the germline, it has been difficult to characterize Notch signaling roles that occur during adult reproduction. To this end, temperature-sensitive alleles of *glp-1*/Notch have provided the only tool to uniquely perturb adult germline Notch function (Austin and Kimble 1987; Pepper *et al.* 2003; Fox and Schedl 2015). The correlation between GLP-1/Notch activity and germ cell proliferation in the distal adult hermaphrodite is well established (Austin and Kimble 1987; Pepper *et al.* 2003; Fox and Schedl 2015), but possible relationships

between germ cell proliferation and oogenesis have not been explored.

In addition to the cessation of germ cell progenitor proliferation, HOP-1-deficient adult gonads revealed two other defects: enlarged oocytes and a reduced rate of embryo output. The fact that all three *hop-1* phenotypes are observed within the first day of adulthood indicates that HOP-1 function is necessary not just to produce a large number of oocytes, but also to support efficient oocyte development, a process that takes 2 days to progress from mitotic exit to mature oocyte (McCarter *et al.* 1999; Jaramillo-Lambert *et al.* 2007). Similar oogenesis defects were observed in hermaphrodites with compromised *glp-1*/Notch function. Although we have not directly tested the underlying cause of these oogenesis defects, our results are consistent with a very simple, yet unexplored, model, in which the size of the germline proliferative zone directly influences the progress of oogenesis. This model is supported by the observation that the extent and timing of proliferative zone defects in *hop-1* and *glp-1* mutants correlates with the extent and timing of the apparent rate of oogenesis in these animals.

In addition to oocytes, the germline proliferative zone generates germ cells that function transiently as nurse cells to nourish developing oocytes. Little is known about nurse cell identity or function in *C. elegans*, but their removal by apoptosis in the pachytene region of the gonad has been investigated, and used to estimate that roughly half of all oogenic germ cells function as transient nurse cells (Gumienny *et al.* 1999). More recently, fluorescent-nucleotide labeling of S phase germ cells demonstrated that more than half of the cells generated in the proliferative zone are culled from the gonad, pointing to a significant population of transient nurse cells in the gonad (Jaramillo-Lambert *et al.* 2007). By comparing proliferative zone output (Fox and Schedl 2015) to embryo output, we offer a third approach for the quantification of nurse cells, and estimate that 86% of oogenic germ cells that enter meiosis function exclusively as nurse cells.

Our calculations suggest that at 15°, roughly seven germ cells are generated for the production of each oocyte, thus placing a high demand on the proliferative zone to maintain a constant supply of germ cells that facilitate oogenesis. The observation that this ratio did not change for *glp-1* animals that have a reduced proliferative zone leads us to suggest a simple model, in which the availability of germ cells exiting the proliferative zone is a key limiting factor for the rate of oogenesis. This model predicts that a reduced stem cell population in the distal gonad would attenuate the rate of oogenesis, thereby maintaining the same ratio of germ cells per oocyte.

This proposed relationship between the proliferative zone size and the rate of oogenesis offers a possible explanation for previously observed oogenesis defects. Gonads with defective Notch signaling have been shown to have enlarged oocytes, delayed oocyte cellularization, and increased cytoplasmic streaming (Nadarajan *et al.* 2009). These phenotypes could be manifestations of slowed oogenesis that is caused by reduced proliferative zone output. For example, if the rate of oogenesis is slowed, then developing oocytes may take longer to cellularize and spend extended time absorbing material from the shared gonad cytoplasm, resulting in enlarged oocytes. Nadarajan *et al.* (2009) proposed an alternative model in which Notch signaling has a novel role in oogenesis that is separable from its effect on germ cell proliferation. This interpretation was based partly on the observation that the enlarged oocyte phenotype appeared before depletion of the gonad proliferative zone. Our analysis suggests that prior to complete loss of a proliferative zone, an incremental reduction in proliferative zone output could reduce the rate of oogenesis. Thus, it is possible that the oogenesis defects observed by Nadarajan *et al.* (2009) may have resulted from diminished (but not yet eliminated) stem cell pools, as might be expected in temperature-sensitive mutants or RNAi treatments. Another result that appeared to support separate functions for *glp-1* in germ cell proliferation *vs.* oocyte growth was the observation that preventing germline cell death suppresses the *glp-1* enlarged oocyte defect, but not the *glp-1* proliferation defect. Our model offers a possible explanation for this result: if the availability of nurse cells is a limiting factor for the rate of oogenesis, then perhaps reducing the efflux of nurse cells (by preventing germline cell death) might compensate for a reduced influx of nurse cells (from reduced proliferative zone output), thus allowing a more normal rate of oogenesis and suppression of the enlarged oocyte phenotype, despite a reduced proliferative zone output. Our model does not preclude the existence of additional roles for Notch signaling during oogenesis, but offers a simple explanation whereby defects in oogenesis could represent indirect consequences of the germline proliferation phenotype of Notch mutants.

Our analysis highlights an unappreciated connection between germline stem cells and progeny production. We suggest that the rate of oogenesis is dependent upon the rate at which new germ cells are supplied from the distal stem cell pool, and that the majority of these cells function as nurse cells to support the development of older oocytes. The idea that changes to the size of the germline stem cell pool may directly alter the rate of oogenesis has important implications for the interpretation of all oogenesis defects. For example, some oogenic phenotypes that are characteristic of "reproductive aging" (Luo and Murphy 2011) could be, in part, caused by reduced rates of oogenesis that reflect diminishing supplies of germ cells from the proliferative zone. Indeed, older hermaphrodites have been found to have diminished proliferative zones (Killian and Hubbard 2005), but this phenotype has not been connected to oogenesis defects described in older hermaphrodites. In addition to Notch signaling and age, other physiological influences, such as food availability, are known to influence the size of the proliferative zone (Hubbard *et al.* 2013), perhaps representing additional scenarios in which oogenesis is modulated through the control of proliferative zone size. Although much remains to be elucidated in the intervening steps between germ cell progenitors

and mature oocytes, the proposed relationship between the size of the stem cell pool and the efficiency of oogenesis has broad implications for our understanding of germline homeostasis.

Acknowledgments

We are grateful to Gary Williams of WormBase for help with RNA-Seq data; the *C. elegans* Gene Knockout consortium for generating *sel-12(ok2075)*; Brian Brady, Mariana Brenna, Shelton Cochran, Jennifer Mackinnon Krems, and David Brinkley for contributions to this work; and Bernard Lakowski, Steven L'Hernault, David Fitch, and Jane Hubbard, for helpful discussions. Some *C. elegans* strains were provided by the *Caenorhabditis* Genetics Center, which is funded by the National Institutes of Health Office of Research Infrastructure Programs (P40 OD-010440). This work was supported by the Gregory S. Call Student Research Fund and an Amherst College Faculty Research Award from the H. Axel Schupf '57 Fund for Intellectual Life.

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Communicating editor: M. Sundaram