Reevaluation of whether a soma–to–germ-line transformation extends lifespan in Caenorhabditis elegans

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The germ lineage is considered to be immortal. In the quest to extend lifespan, a possible strategy is to drive germ-line traits in somatic cells, to try to confer some of the germ lineage’s immortality on the somatic body. Notably, a study in Caenorhabditis elegans suggested that expression of germ-line genes in the somatic cells of long-lived daf-2 mutants confers some of daf-2’s long lifespan. Specifically, mRNAs encoding components of C. elegans germ granules (P granules) were up-regulated in daf-2 mutant worms, and knockdown of individual P-granule and other germ-line genes in daf-2 young adults modestly reduced their lifespan. We investigated the contribution of a germ-line program to daf-2’s long lifespan and also tested whether other mutants known to express germ-line genes in their somatic cells are long-lived. Our key findings are as follows. (i) We could not detect P-granule proteins in the somatic cells of daf-2 mutants by immunostaining or by expression of a P-granule transgene. (ii) Whole-genome transcript profiling of animals lacking a germ line revealed that germ-line transcripts are not up-regulated in the soma of daf-2 worms compared with the soma of control worms. (iii) Simultaneous removal of multiple P-granule proteins or the entire germ-line program from daf-2 worms did not reduce their lifespan. (iv) Several mutants that robustly express a broad spectrum of germ-line genes in their somatic cells are not long-lived.

Together, our findings argue against the hypothesis that acquisition of a germ-cell program in somatic cells increases lifespan and contributes to daf-2’s long lifespan.

Germline | Aging | C. elegans | daf-2 | P granules

Germ and soma are the two most fundamentally different cell types found in multicellular organisms (1). Germ cells are totipotent and constitute the only immortal lineage, capable of generating entire new organisms generation after generation, whereas somatic cells differentiate into specialized cell types and are mortal, senescing and dying each generation. Maintaining the proper identity of these cell types is essential for propagation of species, and molecular barriers at both the transcriptional and translational levels have evolved to ensure the germ–soma distinction (1). Removal of these barriers in germ cells can lead to both expression of somatic factors and sterility (2–4), whereas removal of these barriers in somatic cells is associated with re-entry into the cell cycle and cancer (5, 6). Despite these barriers, it is unknown whether some cell types can tolerate partial fate switching and perhaps adopt traits of other cell types to benefit the organism.

An attractive possibility is that acquisition of a germ-cell program by the somatic body can capture some of the immortality of the germ lineage and extend lifespan (7, 8). Indeed, a study in Caenorhabditis elegans supports that possibility (9). In C. elegans, inhibition of the insulin-signaling pathway by a mutation in daf-2 doubles lifespan through activation of the FOXO transcription factor DAF-16 (10). The findings that implicated somatically expressed germ-line factors in daf-2’s lifespan extension were as follows. (i) daf-2 mutants were observed to ectopically express a pgl-1::gfp transgene under the control of the germ-line–specific pie-1 promoter. (ii) Transcripts for several germ-line–specific genes (pie-1, pgl-1, -2, and -3) were detected in germ-line–less glp-4;daf-2 double mutants by RT-PCR. (iii) RNA interference (RNAi) depletion of individual germ-line genes (pie-1, pgl-1, -2, -3, or mes-4) from young adult worms modestly reduced their lifespan (9).

A striking example of a soma–to–germ transformation is seen in the synMuv (synthetic Multivulva) B mutants (11–13). The synMuv B genes encode chromatin regulators that broadly function in gene repression. These genes, which include C. elegans homologs of heterochromatin proteins (hpl-2), retinoblastoma (lin-35), and the Dp/Rb/Muv (DRM) complex, work in somatic cells to repress expression of germ-line genes. As a result of ectopic germ-line gene expression in the soma, synMuv B mutants arrest as larvae when grown at elevated temperatures. Notably, ectopic germ-line gene expression and larval arrest can be suppressed by inactivating the germ-line transcription program (13).

In this work, we investigated the extent of somatic expression of germ-line components in different C. elegans mutants and the consequences of somatic expression of germ-line components on lifespan. In contrast to what was previously reported, we could not detect expression of germ-line proteins or up-regulation of germ-line transcripts in the soma of long-lived daf-2 mutants. We also found that daf-2 mutants do not use the worm version of germ granules or a master germ-line regulator in their soma to extend lifespan. Finally, we show that multiple synMuv B mutants that are known to ectopically express germ-line factors are not long-lived.

Significance

Understanding the genetic mechanisms that control lifespan is essential for the development of regenerative therapies that seek to reverse the aging process. In the nematode Caenorhabditis elegans, long-lived mutants that are defective in insulin signaling up-regulate a number of stress response genes to promote survival. A study published in 2009 reported that these long-lived mutants also express in their somatic cells factors that are normally restricted to germ cells and that these mutants rely on germ-line factors for some of their lifespan extension. Our studies call these findings into question and instead suggest that expression of certain germ-line factors in the somatic cells of worms is detrimental to the health of worms and reduces lifespan.

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Data deposition: The transcriptome profiling data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE76946).

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Results
daf-2 Mutants Do Not Detectably Express Germ-Line Proteins in Their Soma. Germ granules and the meiotic synaptonemal complex are specific to germ cells and are required for fertility in diverse animals (14, 15). *C. elegans* germ granules, called P granules, are perinuclear ribonucleoprotein complexes that contain PGL-1 and -3 as constitutive components (16). The synaptonemal complex, which assembles between homologous chromosomes during meiosis, contains HTP-3 in its lateral elements (15). To test for somatic expression of P-granule and synaptonemal-complex proteins in *daf-2* mutants, we immunostained *daf-2* L1 larvae for the P-granule protein PGL-3 and the meiotic protein HTP-3. Although robust PGL-3 and HTP-3 staining was observed in somatic cells of synMuv B mutants (e.g., *lin-13*), which are known to ectopically express a germ-line program in their soma (11–13), PGL-3 and HTP-3 were not detected in somatic cells of *daf-2(e1368)* or *daf-2(e1370)* worms (Fig. 1A). As a control, PGL-3 and HTP-3 were brightly stained in the primordial germ cells of all worms examined. To test for somatic expression of a germ-line transgene, we introduced a pgl-1::gfp transgene driven by the pgl-1 promoter into *daf-2(e1370)*. Although robust transgene expression was observed in somatic cells of synMuv B mutants (e.g., *lin-35*), transgene expression was not detected in the somatic cells of *daf-2(e1370)* L2/L3 larvae and adults (Fig. 1B and Fig. S1 A and B). Thus, *daf-2* mutants do not detectably express in their soma the germ-line proteins or transgenes that we tested.

daf-2 Mutants Do Not Up-Regulate Germ-Line Transcripts in Their Soma. P granules and meiotic proteins represent only a fraction of the unique cellular repertoire that defines germ cells. To determine whether the somatic tissues of *daf-2* mutants accumulate mRNAs normally restricted to germ cells, we performed transcript profiling of *daf-2* adults that lack a germ line. We removed the germ line genetically using the mutant, *mes-1*. The *mes-1* gene encodes a predicted receptor tyrosine kinase that is important for maintaining early germ-line blastomere fate (17). At elevated temperature, in the majority of embryos (68% at 25 °C) from *mes-1* mothers, the primordial germ cell P4 is incorrectly specified as a muscle precursor; such embryos develop into viable adults that lack a germ line (18). We predicted that if *daf-2* animals ectopically express germ-line genes in their soma, then we would observe increased accumulation of germ-line transcripts in germ-line–less *mes-2* mes-1 double mutants compared with germ-line–less *mes-1* single mutants.

From populations of *daf-2* mes-1 and *mes-1* adults, we collected germ-line–less animals and sequenced their rRNA-depleted RNA using next-generation sequencing. We compared mapped reads across genes in several categories: ubiquitously expressed, enriched expression in the germ line, expressed specifically in the germ line, or expressed specifically in somatic cells (Fig. 1C). We previously defined these categories using microarray and serial analysis of gene expression (SAGE) data from different isolated tissues and whole worms with and without a germ line (refs. 3 and 19–21; Materials and Methods). Surprisingly, *daf-2* mes-1 double mutants did not display increased levels of either genes with germ-line–enriched expression (*P = 4.8 × 10^{-15}* or germ-line–specific genes (*P = 2.2 × 10^{-10}* compared with *mes-1* single mutants (Fig. 1C and Fig. S2). Based on our significance criteria, a multiple-hypothesis corrected *P value of <0.05 and a fold change >2 in either direction, 273 genes were up-regulated and 496 genes were down-regulated in *daf-2* mes-1 compared with *mes-1*. None of the 273 up-regulated genes were classified as germ-line–specific, and only 7 of the 273 were classified as germ-line–enriched; a total of 27 germ-line–enriched genes would have been expected by chance (Fig. S2 A and B).

![Fig. 1.](image-url)
Gene Ontology analysis of the 273 significantly up-regulated genes revealed genes involved in lipid glycosylation, aging, and dauer larval development. No categories specific to germ-line processes were observed (Fig. S2C). We infer from these findings that daf-2 mutant worms do not significantly up-regulate germ-line transcripts in their soma. Our findings do not rule out the possibility that daf-2 mutants produce low levels of germ-line transcripts in a subset of somatic cells and that these low levels confer some lifespan extension.

Removing P Granules or Disabling the Germ-Line Program in daf-2 Mutants Does Not Reduce Lifespan. To test whether the long lifespan of daf-2 mutants depends in part on low-level expression of germ-line genes, specifically those encoding P-granule components, we depleted important P-granule components from daf-2 animals and measured their lifespan. Among P-granule components, members of the PGL and germ-line helicase (GLH) families are constitutive components that serve roles in promoting granule formation (PGLs) and granule association with the nuclear periphery (GLHs) (22). It was previously observed that treatment of daf-2 young adult worms with RNAi targeting single P-granule genes (pgl-1, -2, or -3) resulted in a slight decrease in lifespan, suggesting that these components play a role in the lifespan extension mechanisms used by daf-2 (9). We repeated that experiment, treating daf-2 young adults with RNAi against pgl-1, but did not observe a reduction in lifespan (Fig. S3A). We wondered whether simultaneously depleting multiple components of P granules would affect daf-2 lifespan. We used a single RNAi construct that simultaneously depletes the four most important pgl and glh genes (pgl-1, -3, glh-1, and -4) (4). We did not observe a decrease in lifespan of P-granule–depleted daf-2 young adults compared with untreated daf-2 young adults (Fig. 2A). Treatment of wild-type young adults with this P-granule RNAi did not affect lifespan (Fig. 2A). We also treated daf-2 worms with P-granule RNAi from hatching; this treatment more effectively reduced P-granule levels in the soma of lin-35 mutant worms than did treatment by P-granule RNAi from adulthood (Fig. S4), but it also did not result in a reduction in daf-2 lifespan (Fig. 2B). Thus, it daf-2 worms do express P-granule proteins in their soma at below detectable levels, these proteins do not play a role in extending daf-2’s lifespan.

Treatment of daf-2 young adults with mes-4 RNAi was also reported to modestly decrease lifespan (9). Based on analysis of synMuv B mutants (see below), MES-4 promotes expression of a germ-line program in somatic cells (11–13). Importantly, MES-4 affects germ-line development and expression of germ-line genes in the soma of synMuv B mutants in a maternal-effect manner (13, 23). Thus, we reasoned that an effect on lifespan would be seen in the offspring of mes-4 mutant mothers, unless MES-4 works in a novel manner in daf-2’s soma. We investigated a possible role of MES-4 in lifespan extension by first repeating the single RNAi knockdown of mes-4 in daf-2 young adults; we did not observe a reduction in lifespan (Fig. S3B). We then took a genetic approach by introducing mes-4(bn85) into a daf-2(e1370) background and measuring the lifespans of the fertile M+Z− mes-4 mutants [maternal supply (M+) but no zygotic synthesis (Z−) of MES-4] and sterile M−Z− mes-4 mutants [neither maternal supply (M−) nor zygotic synthesis (Z−) of MES-4]. Only the M−Z− generation completely lacks the capacity to express a germ-line program (13, 23). Compared with daf-2(e1370) single mutants, daf-2(e1370);mes-4 M+Z− double mutants did not display an altered lifespan, whereas daf-2(e1370);mes-4 M−Z− double mutants showed a dramatic increase in lifespan (Fig. 2C). The increase in lifespan in daf-2;mes-4 M−Z− worms is likely due to the synergistic effects of reduced insulin signaling and absence of germ cells (24). Interestingly, germ-line–less mes-4 M−Z− single mutants were not more long-lived than wild type (Fig. 2C and Fig. S5A), probably because mes-4 mutant worms are defective in the somatic gonad signal that extends lifespan in germ-line–less worms (SI Results and Fig. S5 B and C). We also generated a daf-2(e1368);mes-4(bn85) double mutant and again observed that M+Z− worms did not display an altered lifespan compared with daf-2(e1368) single mutants (Fig. S5D). daf-2(e1368);mes-4(bn85) M+Z− worms rarely produced viable M−Z− progeny, so that generation’s lifespan was not assessed. Together, our results strongly argue that expression of germ-line proteins in daf-2’s somatic cells does not contribute to daf-2’s long lifespan.
synMuv B Mutants Ectopically Express Germ-Line Factors but Are Not Long-Lived. As another approach to testing whether expression of a germ-line program in somatic cells extends lifespan, we analyzed the synMuv B mutants. synMuv B mutants express in their soma numerous germ-line genes, including genes encoding P-granule components and meiotic factors (11–13). The degree of germ-line gene expression in somatic cells is strongly influenced by temperature: synMuv B mutant animals grown at high temperatures (e.g., 26 °C) have higher levels of ectopic germ-line gene expression than animals grown at lower temperatures (e.g., 20 °C) (ref. 13; Fig. 3 A and B). Most synMuv B mutants reared at high temperature arrest as L1 larvae, a result of somatic expression of germ-line genes, because treatment with RNAi, which abrogates ectopic germ-line gene expression, suppresses this larval arrest (13). Different synMuv B mutants display different degrees of somatic expression of germ-line genes. For example, some mutant larvae (e.g., hpl-2 and lin-13) display germ-line–like perinuclear staining of P granules in their intestine, whereas other mutants (e.g., lin-9 and -35) display diffuse P-granule staining, and others lack P-granule staining (e.g., lin-61) (Fig. 3B). These differences between synMuv B mutants provide an opportunity to test whether lifespan is extended by somatic expression of germ-line genes and, if so, whether extension correlates with the degree of ectopic germ-line gene expression.

We performed lifespan assays on six synMuv B mutants that show varying levels and patterns of somatic expression of germ-line genes. At 20 °C, three of the six mutants, lin-9, -13, and -35, displayed ectopic germ-line proteins, as assessed by PGL-1 and HTP-3 staining (Fig. 3A). None of these mutants displayed an increased lifespan compared with wild type (Fig. 3C and Fig. S6A). Only hpl-2 mutants reared at 20 °C showed an increase in lifespan. However, we could not detect ectopic PGL-1 or HTP-3 in these worms. hpl-2 mutants were previously reported to have a modest increase in lifespan and to express genes that control dauer formation, lipid metabolism, and longevity in addition to germ-line genes in their soma (25). Raising synMuv B mutants at 24 °C caused a dramatic increase in the level of ectopic germ-line gene expression, but did not result in an increase in lifespan (Fig. 3 B and D and Fig. S6B). These results show that mutants that ectopically express germ-line genes in their somatic cells are not longer-lived and, in most cases, are shorter-lived than wild type.

Discussion

Our data challenge a previous report (9) that somatic acquisition of a germ-line program contributes to lifespan extension in C. elegans. In the report from Curran et al. (9), certain findings were puzzling. First, given that MES-4 and PGL-1 function in a strict maternal-effect manner (23, 26), we were surprised by the Curran et al. finding that depleting daf-2 adults of those factors via RNAi reduced lifespan in the same generation. Second, Curran et al. (9) observed that RNAi of pgl-2, a dispensable P-granule factor, resulted in the same degree of lifespan reduction as RNAi of the important pgl family members pgl-1 and -3. Third, Curran et al. (9) used a pgl-1:gfp transgene driven by the pie-1 promoter to
detect a soma-to-germ transformation in *daf-2* larvae and dauer worms. This transgene is expressed in the germ line, but has also been reported to be expressed in non-germ-line tissues such as the hypodermis (27), complicating interpretation of somatic expression of the transgene as being due to transformation of somatic cells toward germ line. In our reevaluation of the Curran et al. (9) findings, we took precautions to control for genetic background effects, which have been shown to influence lifespan (28), and we addressed key questions using multiple approaches, including immunostaining, genome-wide transcript profiling, and depletion of proteins by both RNAi and mutants. We found that *daf-2* mutants do not detectably express germ-line proteins or transcripts in their somatic cells. Additionally, our lifespan analyses indicate that *daf-2* mutants do not use P granules or the master germ-line chromatin regulator MES-4 to extend lifespan. Finally, we found that multiple synMuv B mutants that misexpress germ-line factors in their somatic cells are not long-lived.

Expression of germ-line genes in somatic cells has been linked to tumorigenesis in multiple organisms. In *Drosophila*, mutation of the *l(3)mbt* gene causes development of brain tumors that express numerous germ-line genes (6). Interestingly, preventing expression of certain germ-line genes, including *vasa* and *pwi*, prevented development of these tumors, suggesting that certain germ-line gene products promote tumor formation and/or growth. Recently, the human homologs of these germ-line genes were found to be expressed in a wide variety of human cancers, including ovarian and brain cancers (29). Although cancer cells may hijack the mechanisms used by germ cells to proliferate and survive, other cells, such as the somatic cells in *C. elegans*, are negatively affected by expression of germ-line proteins. An ectopic germ program in *C. elegans* somatic cells causes developmental arrest at elevated temperature and, as we have shown, shorter lifespan.

Nearly all proteins found in P granules are predicted to serve some role in RNA metabolism (14, 16). Although the functions of these proteins are only starting to be discovered, a recent study showed that P granules serve an important role in maintaining germ cell identity: Depleting important P-granule components (PGL-1 and GLH1 proteins) from the C. elegans germ line causes misexpression of neural and muscle fate markers (4). An attractive hypothesis is that P granules repress somatic fates in germ cells by preventing translation of mRNAs that promote somatic development. It is the case, then, one possibility is that expressing P-granule components in somatic cells impairs the translation of normally expressed somatic transcripts, thereby compromising cellular function and integrity.

The mechanisms used by *daf-2* mutants to extend lifespan have been extensively characterized and include turn-on of specific transcriptional programs that promote stress resistance and longevity (10). These gene-expression programs are controlled by various transcription factors, including DAF-16, HSF-1, and SKN-1 (10). To date, these transcription factors have not been linked to maintaining appropriate gene expression in the germ line. Lifespan extension in *daf-2* mutants has also been reported to require the autophagy pathway, which operates in a DAF-16–independent manner to recycle proteins and organelles (30, 31). Interestingly, P granules are normally removed from the somatic blastomeres in embryos through autophagy (32), which likely clears them from the somatic cells of *daf-2* mutants.

Our results do not rule out the possibility that the germ line in wild-type animals and the somatic cells of long-lived mutants use similar mechanisms to protect themselves from stresses. For example, RNAi has been shown to function as a defense system in the germ line, protecting it from viruses and transposons (33–35). *daf-2* mutants have been shown to have enhanced RNAi, which may serve a protective role in their somatic cells (36). Although our data challenge the claim that germ-line proteins in the soma contribute to *daf-2*-2's extended lifespan, they do not rule out the possibility that particular germ-line factors or combinations of germ-line factors may be capable of improving somatic health to promote lifespan extension. Identifying such factors would likely require a genome-wide misexpression approach coupled with lifespan analysis.

Although a topic of intense interest and study, the factors and mechanisms that underlie germ-line immortality and totipotency are not fully understood. Future research will likely reveal how germ-line proteins influence these unique characteristics of the transcriptome and proteome levels. As the functions of various germ-line factors become clearer, a better understanding of the effects of misexpressing those factors in the soma will become clearer as well.

**Materials and Methods**

**Strains and Worm Husbandry.** *C. elegans* strains were maintained at 15 °C on OP50 bacteria as described (37). Strains used in this study are listed in Table S1.

**Immunocytochemistry and Microscopy.** For antibody staining, worms were permeabilized by using the freeze–crack method, fixed for 10 min in methanol and 10 min in acetone, and stained as described (38). Antibody dilutions were 1:3,000 rabbit anti–P–81 (26), 1:500 guinea pig anti–HTP–3 (39), 1:50,000 rat anti–P–3 (40), 1:200 rabbit anti–CEH–18 (41), and 1:150 Alexa Fluor secondary antibodies (Life Technologies). Images shown in Fig. 1 A and B and Fig. S1 were acquired on a Solamere spinning disk confocal system controlled by Micro- Manager software (42); setup was as follows: Yokogawa CSU–1 scan head, Nikon ECL Plan Apo 60× oil immersion, Hamamatsu ImageEM x2 camera, and Plan Apo 60×/1.4–n.a. oil objective. Images shown in Fig. 3 A and B and Fig. S5 were acquired on a Perkin-Elmer Volocity spinning disk confocal system controlled by Volocity software; setup was as follows: Yokogawa CSU–10 scan head, Nikon TE2000-E inverted stand, Hamamatsu C9100 EMCCD camera, and Plan Apo 60×/1.4–n.a. oil objective. Images shown in Fig. S4 were acquired on a Leica MZ16F fluorescence stereomicroscope with a Q imaging Retiga-2000R CCD camera. Fig. 18 and Figs. S1 and S5 contain montages generated by splicing together contiguous images acquired by using identical settings. All images were processed with ImageJ and Adobe Illustrator.

**Lifespan Assays.** Young adult (first day of adulthood) worms were allowed to lay embryos for 6 h, resulting in a fairly synchronous population of F1 progeny (day 0). These F1 worms were scored every 2 d for death by prodding with a platinum pick. Worms that had ruptured, crawled off the plate, or died as a result of their progeny hatching inside the mother worms were removed from analysis. To control for genetic background effects, each mutant was back-crossed to the laboratory wild-type strain at least six times, and all lifespan assays were performed by using strains that were propagated continuously on food (i.e., not allowed to starve) for at least three generations. All worms were maintained at 15 °C to prevent formation of dauer larvae by strains containing a *daf-2* mutation. Lifespan assays were performed on *C. elegans* bacteria except those that involved RNAi, in which case HT115 bacteria were used as the food source. For RNAi controls, we used HT115 bacteria harboring an empty vector (EV RNAi) plasmid. For experiments using dafachronic acid (SI Results), 1 mM (255–1)-dafachronic acid from AdipoGen was added to bacteria on normal Nematode Growth Media plates as described (43). At least two replicates were performed for each lifespan analysis shown in the figures in the main text and in Fig. S3. Only one replicate was performed for the lifespan analyses shown in Figs. S3 and S5 A and C. For each replicate, significance was calculated by using a log-rank test, and graphs were generated with GraphPad Prism software (Version 6.0). All replicates, including those shown in the figures, can be found in Dataset S1.

**Transcriptome Profiling and Analysis.** *mes-1*(bn84ts) single and *daf-2*(e1370); *mes-1*(bn84ts) double-mutant adults were allowed to lay embryos overnight at 22 °C. The next day (day 1), embryos were downshifted to 15 °C to prevent formation of dauer larvae. On day 5, the adult worms were upshifted back to 24 °C. Our reasoning for upshifting adults back to 24 °C was because ectopic expression of germ-line genes has been shown to be higher at elevated temperatures (13). On day 6, a total of 100 sterile, germ-line–less adult worms (that visually lacked embryos) were manually picked into TRIzol, and total RNA was extracted. Ribosomal RNA was depleted by using an NEBNext RNA Depletion Kit (human/mouse/rat; catanata no. E6310), and libraries were constructed by using an NEBNext Ultra RNA Library Prep Kit for Illumi na sequencing (catalog no. E7530). Three biological replicates were performed for each genotype. Libraries were sequenced at the Vincent J. Coates.
Classification of Gene Categories. Gene categories were defined by using published microarray and SAGE datasets that profiled specific tissues or whole worms that contained or lacked a germ line, as described (3, 48). Ubiquitous genes (1,895 genes) are genes that are expressed (tag > 0) in all SAGE datasets that profiled germ-line, muscle, neural, and gut tissue, but that are not in the germ-line–enriched category. Germ-line–enriched genes (2,230 genes) are genes with transcripts enriched in adults with a germ line compared with adults lacking a germ line as assessed by microarray analysis (19). Germ-line–specific genes (169 genes) are genes whose transcripts are expressed exclusively in the adult germ line and accumulate in embryos strictly by maternal contribution. These genes were defined by using multiple datasets and have been described (48). Soma-specific genes (1,181 genes) are genes expressed (tag > 4) in muscle, neural, and/or gut tissue, but not expressed (tag = 0) in germ-line SAGE datasets and also not in the germ-line–enriched category.

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**Supporting Information**

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**SI Results**

Although germ-line–less *daf-2(e1370)*;*mes-4*(bn85) M–Z– double mutants were more long-lived than *daf-2* mutants, germ-line–less *mes-4*(bn85) M–Z– and *mes-4*(bn73) M–Z– single mutants were not more long-lived than wild type (Fig. 2C and Fig. S5A). Previous studies have shown that lifespan extension by germ-line removal is dependent on the presence of a functional somatic gonad (24). We tested whether sterile *mes-4* M–Z– worms lack somatic gonad cells by staining for the gonadal sheath-specific transcription factor CEH-18 (41). *mes-4* M–Z– mutant worms displayed CEH-18 staining in eight nuclei per gonad arm, identifying them as somatic sheath nuclei (Fig. S5B). The somatic gonad promotes lifespan extension, in part, through the DAF-12/nuclear hormone receptor pathway (43, 49). As evidence, supplementing germ-line–less worms that also lack a functional somatic gonad with DAF-12 ligands, called dafachronic acids, increases lifespan (43). Supplementing *mes-4* M–Z– mutants with Δ7-dafachronic acid resulted in a slight increase in lifespan (Fig. S5C), suggesting that somatic gonad signaling of DAF-12 in *mes-4* M–Z– worms is defective or absent.

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**Fig. S1.** *daf-2* L1 larvae, L2/L3 larvae, and adults do not ectopically express a P-granule transgene in their soma (related to Fig. 1B). (A) Wild-type, *lin-35*(n745), and *daf-2(e1370)* L1 and L2/L3 larvae containing a *pgl-1p::pgl-1::gfp* transgene were upshifted to 25 °C for 8 h and then imaged. Primordial germ cells (Z2 and Z3) are indicated by an arrow in L1 larvae, and proliferated germ lines are indicated by brackets in L2/L3 larvae. Examples of nontransgene, autofluorescent gut granules are indicated by arrowheads in wild-type and *daf-2* L2/L3 larvae. All worms were imaged at the same exposure. To show entire worms, figure contains montages of spliced-together images: four, two, three, seven, five, and eight images in the Top Left, Middle Left, Bottom Left, Top Right, Middle Right, and Bottom Right, respectively. (B) Brighter images of worms presented in Fig. S1, better showing the germ-line GFP in wild-type and *daf-2(e1370)* adults. As noted in the legend to Fig. 1B, the figure contains montages of spliced-together images: 12, 10, and 11 images in the Top, Middle, and Bottom, respectively. Brackets indicate distal regions of the germ line, and asterisks indicate distal tips (when visible and not obstructed by the intestine). Examples of nontransgene, autofluorescent gut granules are indicated by arrowheads in wild-type and *daf-2* adults.
Germ-line genes are not up-regulated in germ-line–less daf-2;mes-1 adults (related to Fig. 1C). (A and B) MA plots showing differential expression of all genes in daf-2;mes-1 vs. mes-1 adults. Gray dots show all genes. Significantly up- or down-regulated genes are indicated by red dots. Germ-line–specific genes (A) or germ-line–enriched genes (B) are indicated by blue dots. The total numbers of significantly up- or down-regulated genes are in red, and the numbers of significantly up- or down-regulated germ-line–specific (A) or germ-line–enriched (B) genes in daf-2;mes-1 are in blue. (C) Gene Ontology analysis of the 273 up-regulated genes in daf-2;mes-1 adults shows gene categories involved in aging and dauer formation. These analyses did not identify germ-line–associated processes.

Fig. S3. daf-2 worms do not rely on MES-4 or PGL-1 to extend lifespan (related to Fig. 2). Knockdown of pgl-1 (A) or mes-4 (B) from daf-2(e1370) adults did not alter lifespan at 25 °C. pgl-1 or mes-4 RNAi was initiated on day 3 after hatching (first day of adulthood).
Fig. S4. Somatic expression of the pgl-1p::pgl-1::gfp transgene in lin-35 mutants is more effectively reduced if worms are treated with P-granule RNAi from hatching rather than from young adulthood (related to Fig. 2 A and B). (A) lin-35(n745) pgl-1p::pgl-1::gfp worms were fed from hatching on control (empty vector) RNAi bacteria. Some worms were transferred on day 3 after hatching (first day of adulthood) to P-granule (pgl-1p, -3p, glh-1, and -4p) RNAi bacteria, and worms were imaged on subsequent days. Depletion of P granules starting in young adults resulted in a slight reduction of somatic expression of the P-granule transgene. (B) lin-35(n745) pgl-1p::pgl-1::gfp worms were fed from hatching on either control (empty vector) RNAi or P-granule RNAi bacteria. Depletion of P granules starting at L1 resulted in greater reduction of somatic expression of the P-granule transgene expression than depletion starting in young adults.
Fig. S5. mes-4 M−Z− mutants possess somatic gonad cells but are defective in dafachronic acid signaling (related to Fig. 2C). (A) Germ-line–less mes-4(bn73) M−Z− worms do not have a significantly different lifespan than wild-type worms. (B) mes-4(bn85) M−Z− gonads stain positively for the somatic gonad marker CEH-18. To show an entire gonad arm, B contains a montage of three spliced-together images. Asterisks indicate the distal tip of the gonad. (C) Supplementing mes-4(bn85) M−Z− adults with Δ7-dafachronic acid (DA) slightly increases lifespan compared with ethanol control (vehicle). (D) daf-2(e1368);mes-4(bn85) M+Z− double-mutant lifespan is not significantly different from daf-2(e1368) single-mutant lifespan.
Fig. S6. Representative lifespan curves of synMuv B mutants grown at 20 °C (A) or 24 °C (B) (related to Fig. 3 C and D).
Table S1. Strains used in this study

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Genotype</th>
<th>Notes</th>
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<tbody>
<tr>
<td>SS1174</td>
<td>Wild type</td>
<td>Bristol N2 isolate</td>
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<tr>
<td>SS1226</td>
<td>lin-9(n112)III</td>
<td>Back-crossed MT112 six times to SS1174</td>
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<td>SS1135</td>
<td>lin-13(n770)III</td>
<td>Back-crossed MT8838 six times to SS1174</td>
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<td>lin-35(n745)I</td>
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<td>SS1136</td>
<td>lin-37(n758)III</td>
<td>Back-crossed MT5470 six times to SS1174</td>
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<td>lin-61(n3809)I</td>
<td>Back-crossed MT12833 six times to SS1174</td>
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<td>hpl-2(tm1490)III</td>
<td>Back-crossed PFR40 six times to SS1174</td>
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<td>SS1197</td>
<td>daf-2(e1368)III</td>
<td>Back-crossed DR1572 six times to SS1174</td>
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<tr>
<td>SS1146</td>
<td>daf-2(e1370)III</td>
<td>Back-crossed CB1370 six times to SS1174</td>
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<td>SS1147</td>
<td>mes-4(bn85)V/DnT1-GFP[unc(n754)let qIs51] (IV;V)</td>
<td>Back-crossed SS0803 six times to SS1174</td>
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<td>SS1175</td>
<td>mes-4(bn73)V/DnT1-GFP[unc(n754)let qIs51] (IV;V)</td>
<td>Back-crossed SS1095 six times to SS1174</td>
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<td>SS1159</td>
<td>daf-2(e1370)III;mes-4(bn85)V/DnT1-GFP[unc(n754)let qIs51] (IV;V)</td>
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<td>mes-1(bn84ts)X</td>
<td>Crossed SS0415 and SS1146</td>
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<td>daf-2(e1370)III;mes-1(bn84ts)X</td>
<td>Crossed SS1124 and TH206</td>
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<td>TH206</td>
<td>pgl-1p::PGL-1::TY1::EGFP::3xFLAG + Cbr-unc-119(+)</td>
<td>Crossed SS1124 and TH206</td>
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<td>SS1227</td>
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<td>Crossed SS1146 and TH206</td>
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</table>

Other Supporting Information Files

Dataset 1 (XLSX)
Dataset 2 (XLSX)