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Authors

Knutson, Andrew Kekūpa'a
Rechtsteiner, Andreas
Strome, Susan

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

Andrew Kekūpa'a Knutson[a], Andreas Rechtsteiner[a], and Susan Strome[a,1]

[a]Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, CA 95064

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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.

germ line | 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 reentry 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-like 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-toward-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 transcriptional 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).

[1]To whom correspondence should be addressed. Email: sstrome@ucsc.edu.

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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. 1*A*). 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. 1*B* 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 P_4 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 *daf-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. 1*C*). 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 ($P = 4.8 \times 10^{-15}$) or germ-line–specific genes ($P = 2.2 \times 10^{-16}$) compared with *mes-1* single mutants (Fig. 1*C* 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*).

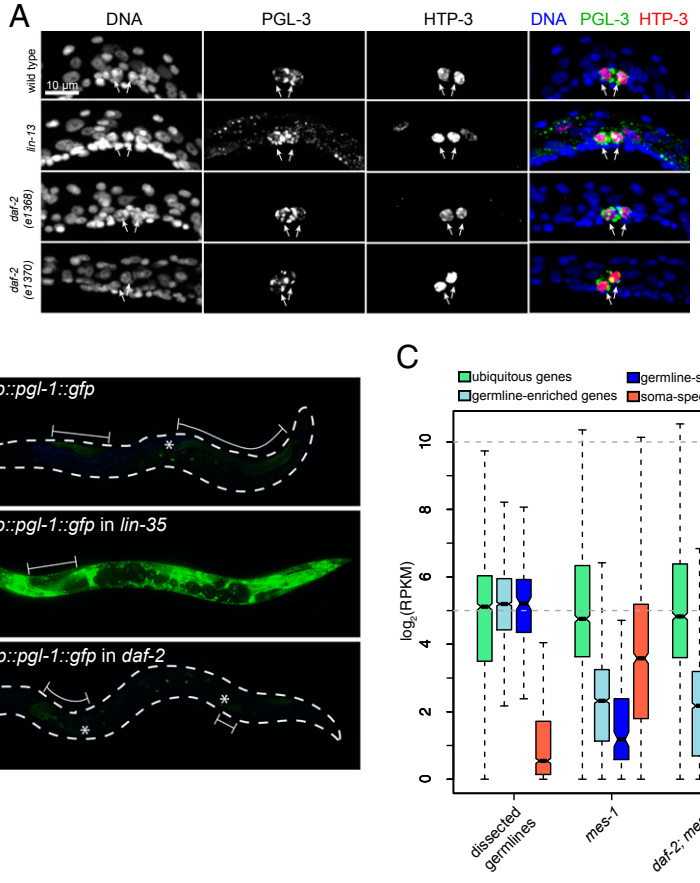

Fig. 1. *daf-2* mutant worms do not accumulate germ-line proteins or transcripts in their somatic cells. (*A*) Wild-type, *lin-13*, *daf-2(e1368)*, and *daf-2(e1370)* L1 larvae grown at 25 °C were immunostained for the P-granule protein PGL-3 (green), the meiotic protein HTP-3 (red), and DNA (blue). Primordial germ cells (Z2 and Z3) are indicated by arrows. (*B*) *pgl-1p::pgl-1::gfp* transgene expression in wild-type, *lin-35(n745)*, and *daf-2(e1370)* adults upshifted to 25 °C for 8 h. To show entire worms, panels contain montages of spliced-together images: 12, 10, and 11 images in *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). See Fig. S1*B* for brighter images. (*C*) Boxplots of normalized RNA-sequencing expression values [$\log_2$(RPKM)] for ubiquitously expressed genes (green), genes with germ-line–enriched expression (light blue), germ-line–specific genes (dark blue), and soma-specific genes (red) in dissected germ lines, *mes-1(bn84ts)* germ-line–less mutant adults, and *daf-2(e1370);mes-1(bn84ts)* germ-line–less double-mutant adults. Expression values reflect three biological replicates for each sample. Each box extends from the 25th to 75th percentile of the $\log_2$(RPKM) values. The whiskers extending from each box indicate the 2.5th and 97.5th percentiles. Wedges around the median indicate 95% confidence intervals for the medians.

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. S2*C*). 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 those 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. S3*A*). 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. 2*A*). Treatment of wild-type young adults with this P-granule RNAi did not affect lifespan (Fig. 2*A*). 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. 2*B*). Thus, if *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. S3*B*). 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. 2*C*). 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. 2*C* and Fig. S5*A*), probably because *mes-4* mutant worms are defective in the somatic gonad signal that extends lifespan

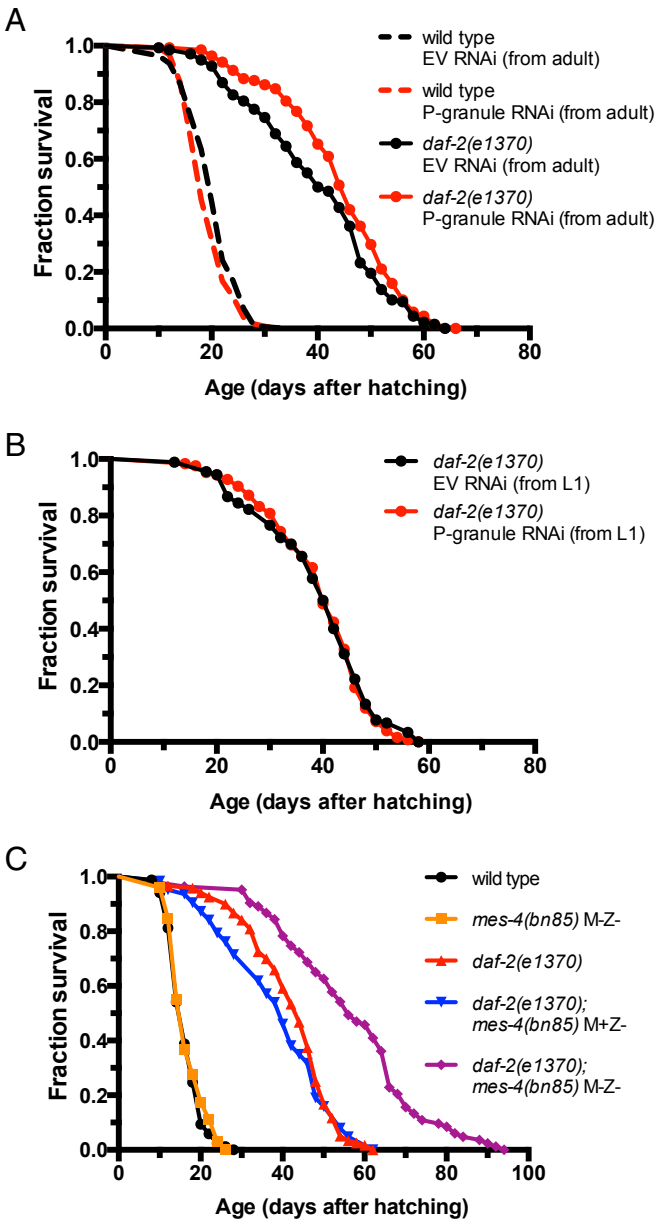

Fig. 2. Knockdown of P granules or inactivation of the germ-line program by loss of MES-4 does not decrease *daf-2* lifespan at 20 °C. (*A*) Lifespan analysis of wild type (dashed lines) and *daf-2(e1370)* mutants (solid lines) treated with control RNAi [empty vector (EV) black] or P-granule (*pgl-1*, *-3*, *glh-1*, and *-4*) RNAi (red) from adulthood (day 3 after hatching). (*B*) Lifespan analysis of *daf-2(e1370)* mutants treated with control RNAi or P-granule (*pgl-1*, *-3*, *glh-1*, and *-4*) RNAi from hatching (day 0). (*C*) Lifespan analysis of wild type (black circles), *mes-4(bn85)* M−Z− (yellow squares), *daf-2(e1370)* (red triangles), *daf-2(e1370);mes-4(bn85)* M+Z− (blue inverted triangles), and *daf-2(e1370);mes-4(bn85)* M−Z− (purple diamonds) mutants. Replicates of lifespan analyses are in Dataset S1.

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. S5*D*). *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 factors, because treatment with *mes-4* 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. 3*B*). 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. 3*A*). None of these mutants displayed an increased lifespan compared with wild type (Fig. 3*C* and Fig. S6*A*). 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. S6*B*). 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

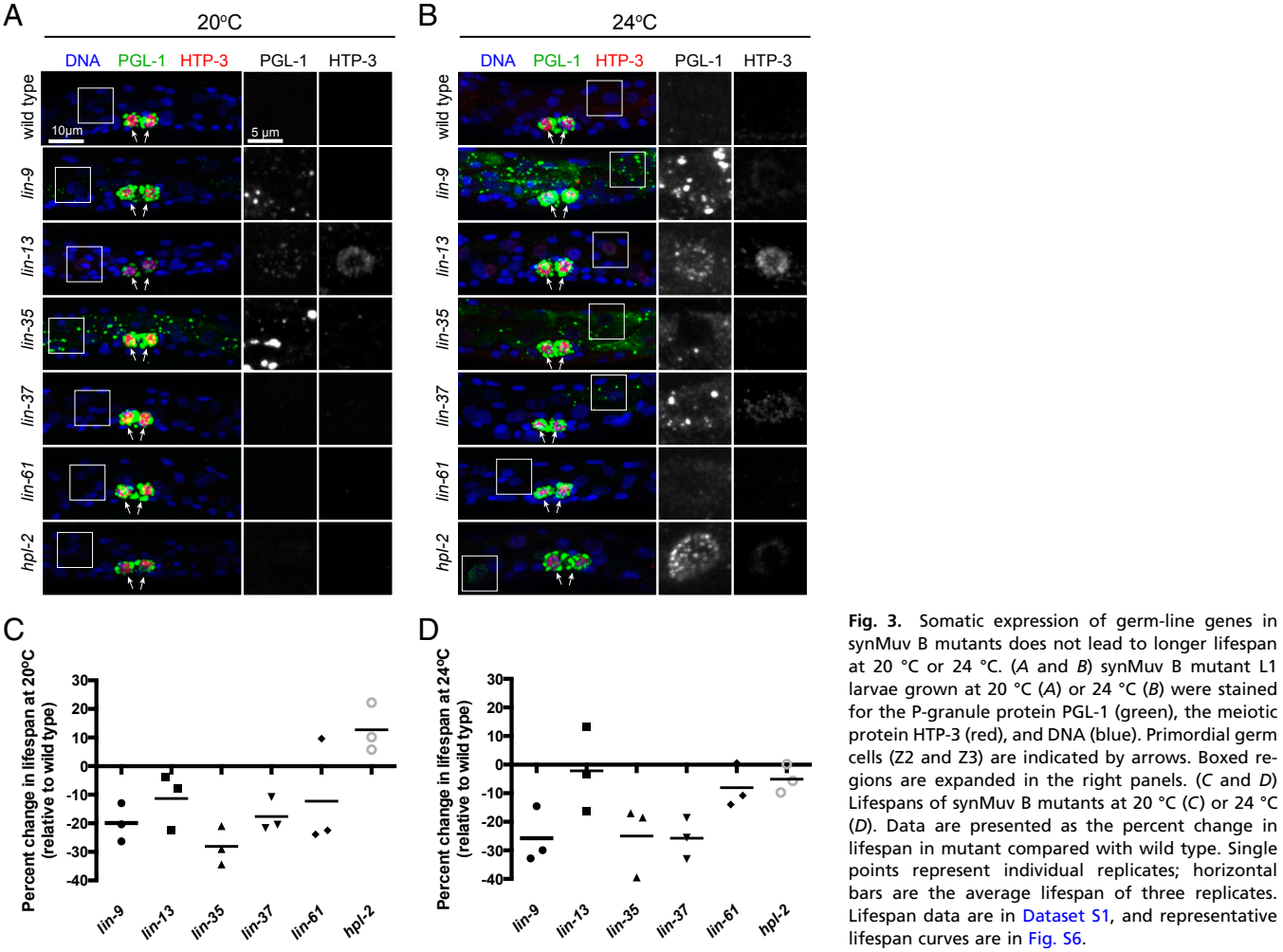

Fig. 3. Somatic expression of germ-line genes in synMuv B mutants does not lead to longer lifespan at 20 °C or 24 °C. (*A* and *B*) synMuv B mutant L1 larvae grown at 20 °C (*A*) or 24 °C (*B*) were stained for the P-granule protein PGL-1 (green), the meiotic protein HTP-3 (red), and DNA (blue). Primordial germ cells (Z2 and Z3) are indicated by arrows. Boxed regions are expanded in the right panels. (*C* and *D*) Lifespans of synMuv B mutants at 20 °C (*C*) or 24 °C (*D*). Data are presented as the percent change in lifespan in mutant compared with wild type. Single points represent individual replicates; horizontal bars are the average lifespan of three replicates. Lifespan data are in Dataset S1, and representative lifespan curves are in Fig. S6.

detect a soma-to-germ transformation in *daf-2* larvae and dauers. 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 *piwi*, 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 and GLH 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 RNAs that promote somatic development. If this 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*'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 at 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 1:30,000 rabbit anti–PGL-1 (26), 1:500 guinea pig anti–HTP-3 (39), 1:50,000 rat anti–PGL-3 (40), 1:200 rabbit anti–CEH-18 (41), and 1:500 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 CSUX-1 scan head, Nikon TE2000-E inverted stand, Hamamatsu ImageEM ×2 camera, and Plan Apo 60×/1.4-n.a. oil objective. Images shown in Fig. 3 *A* and *B* and Fig. S5*B* 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. 1*B* and Figs. S1 and S5*B* 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 backcrossed 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 OP50 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 (25S)-Δ^7-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. S5*D*. 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 24 °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 rRNA Depletion Kit (human/mouse/rat; catalog no. E6310), and libraries were constructed by using an NEBNext Ultra RNA Library Prep Kit for Illumina sequencing (catalog no. E7530). Three biological replicates were performed for each genotype. Libraries were sequenced at the Vincent J. Coates

Genomics Sequencing Laboratory at the University of California, Berkeley, by using the Illumina HiSeq 2000 platform. Raw sequences were mapped to transcriptome version WS220 by using Tophat2 (44). Only reads with one unique mapping were allowed; otherwise, default options were used. Reads mapping to ribosomal RNAs were removed from further analysis. DESeq2 (45) was used for differential expression analysis. A Benjamini–Hochberg multiple hypothesis-corrected *P* value cutoff of 0.05 was used as a significance cutoff. Transcript length and sequencing depth normalized read counts per transcript (RPKM) as displayed in Fig. 1*C* were obtained by using Cuffdiff2 (46). To minimize the effects of highly expressed genes in the different samples in Fig. 1*C*, the RPKM values for this figure were further normalized with Upper Quartile normalization (47). Gene Ontology analysis was carried out by using the DAVID Bioinformatics Resource (Version 6.7). Processed data including RPKM and fold change values can be found in Dataset S2.

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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