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A soma-to-germline transformation in long-lived *C. elegans* mutants

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Abstract

Unlike the soma which ages during the lifespan of multicellular organisms, the germline traces an essentially immortal lineage. Genomic instability in somatic cells increases with age, and this decline in somatic maintenance might be regulated to facilitate resource reallocation toward reproduction at the expense of cellular senescence. We report here that *C. elegans* mutants with increased longevity exhibit a soma-to-germline transformation of gene expression programs normally limited to the germline. Decreased insulin-like signaling causes the somatic misexpression of germline-limited *pie-1* and *pgl* family of genes in intestinal and ectodermal tissues. DAF-16/FoxO, the major transcriptional effector of insulin-like signaling, regulates *pie-1* expression by directly binding to the *pie-1* promoter. The somatic tissues of insulin-like mutants are more germline-like and protected from genotoxic stress. Gene inactivation of components of the cytosolic chaperonin complex that induce increased longevity also cause somatic misexpression of PGL-1. These results suggest that the acquisition of germline characteristics by the somatic cells of *C. elegans* mutants with increased longevity contributes to their increased health and survival.

Keywords

aging; RNAi; soma-to-germline transformation; essential genes; lethal; *daf-2*; *daf-16*; *pie-1*; *pgl-1*; *cct-4*; *cct-6*; cytosolic chaperonin complex; insulin signaling; germline; stem cells; longevity; *C. elegans*

In genetic and functional genomic screens for gene inactivations that increase *C. elegans* lifespan, decreases in the insulin-like signaling pathway confer the largest increase in longevity. Inactivation of essential genes that mediate core functions such as RNA transcription and splicing, translation initiation, and chromatin-remodeling, constitute the second tier of potent longevity enhancing pathways¹. In addition to regulating longevity,

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daf-2/insulin receptor(InsR) mutants as well as mutations in downstream signaling components such as the PI-3 kinase *age-1*, and the serine/threonine kinases *akt-1*, and *pdk-1* exhibit enhanced immune responses to bacterial pathogens² and enhanced RNA interference (RNAi) response in somatic cells³. Because RNAi constitutes an immune response in *C. elegans* ⁴, it is reasonable that the stress resistance pathway triggered by low insulin-like signaling would intensify RNAi. The molecular basis of how decreased *daf-2/InsR* signaling enhances RNAi is unknown, but it is mediated by the major transcriptional output of insulin like signaling, the FoxO transcription factor DAF-16, because mutations in *daf-16* suppress the enhanced RNAi of insulin signaling deficient mutants³. Another major pathway that negatively regulates *C. elegans* RNAi is the synMuvB pathway of chromatin factors that also regulate the repression of germline fates in somatic cells⁵. Given that the germline genome is much more protected than the soma from viral invasion and mutational events by both small RNA-mediated and other genome surveillance pathways^{6–9}, we explored whether the enhanced RNAi phenotypes of insulin signaling mutants and other long-lived mutations engage germline versus somatic cell specification programs.

Mutants in the synMuvB class of genes, which include the *lin-35* retinoblastoma homologue, display enhanced response to dsRNA and somatic misexpression of the normally germline-limited P-granule component PGL-15. This expression of germline genes in somatic tissues has been linked to the enhanced RNAi phenotype since germline cells are remarkably proficient at RNAi. *pgl-1* encodes an RNA binding protein that is localized to P-granules and is essential for gene silencing by cosuppression in the germline¹⁰, supporting the model that somatic misexpression of *pgl-1* contributes to enhanced somatic cell RNA interference. Thus, increased longevity mutants and germ cells both have exceptional longevity, are resistant to cellular stress, and are enhanced for RNAi. If a soma-to-germline transformation phenotype were present in the insulin-like signaling mutants then the germline quality of somatic cells could explain the enhanced RNAi phenotype and potentially contribute to the remarkable lifespan extension (Fig. S1).

Germ cell character in somatic tissues

We tested whether the insulin-like-signaling mutants induce a soma to germline transformation of cell fate. A GFP::PGL-1 protein fusion expressed under the control of the germline-specific *pie-1* promoter was monitored in *daf-2(e1370)* (the insulin like receptor) or *age-1(mg305)* (the downstream PI-3 kinase) mutant strains. *pie-1* and *pgl-1* are exclusively expressed in the germline of wild type animals (Fig. 1a–c, Fig. S2)^{11, 12}. Decreased insulin-like signaling caused strong misexpression of GFP::PGL-1 in hypodermal and intestinal somatic tissues of dauers (Fig. 1d, e) and late larval stage animals (Fig. 1f–g, Fig. S2).

To monitor *in vivo* regulation of endogenous *pie-1* transcript levels, we performed quantitative RT-PCR (qRT-PCR) from *daf-2(e1370)* and *daf-2(e1368)* dauers. This stage was chosen because GFP::PGL-1 expression from the *pie-1* promoter was the strongest in dauers (Fig. 1d) and germline transcripts would be attenuated because the dauer germline is less developed (Table 1, Table S1; Fig. S5, Fig. S6)¹³. Comparison of both *daf-2(e1368)* and *daf-2(e1370)* dauers to age-matched wild-type L2/L3 larvae showed a 2.1 ± 0.1 -fold and

4.5±1.0-fold increase in *pie-1* transcript levels, respectively (Table 1, Table S1). The FoxO transcription factor DAF-16 is the most downstream transcriptional effector of the insulin-like signaling pathway^{14,15}. DAF-16 nuclear localization is activated in low insulin-like signaling conditions. Loss of the FOXO transcription factor DAF-16 abrogates the longevity and enhanced RNAi phenotypes caused by defects in insulin-like signaling^{3,14,16}. In agreement, the *daf-2(e1370); daf-16(mgDf47)* double mutant had reduced *pie-1* transcript levels (Table 1, Table S1). We looked for *pie-1* misexpression in other longevity promoting manipulations that function independent of insulin-like signaling, such as mitochondrial *clk-1(RNAi)*¹⁷ and dietary restriction via an *eat-2(ad465)*¹⁸ mutation and food deprivation¹⁹ (Fig. S7). None of these measures induced the misexpression of germline genes observed in the insulin-like signaling mutants.

There are potential DAF-16 binding sites in the *pie-1* promoter region, predicting that the misexpression of the germline-specific transgene and endogenous *pie-1* transcript would occur at the level of DAF-16 binding to the *pie-1* promoter element. We examined the ability of purified recombinant DAF-16 (Fig. S3) to bind to regions of the *pie-1* promoter that contain predicted DAF-16 binding/associated elements (DBE and DAE) by electrophoretic mobility gel shift assays (EMSA) (Fig. 2a,b)^{20–22}. DAF-16 was capable of binding to most fragments of the *pie-1* promoter tested (Fig. 2b lane 2, Fig. S4). This binding was specific to the purified DAF-16 protein as shown by supershift analysis with a monoclonal antibody that recognizes a FLAG epitope tag engineered into the C-terminus of the recombinant protein (Fig. 2b, lane 3) and by competition of binding with a cold dsDNA oligo containing multiple DAEs (Fig. 2b, lane 4).

Under conditions of low insulin-like signaling DAF-16/FoxO has been shown to occupy the promoters of stress resistance and detoxifying enzymes such as *sod-3*²² and *mtl-1*²³. We tested for *in vivo* binding of DAF-16/FoxO to the *pie-1* promoter using chromatin immunoprecipitation (ChIP) with a DAF-16::GFP fusion gene that is only expressed in somatic tissues. To induce DAF-16 nuclear localization we inactivated *daf-2/InsR* by RNAi. Notably, we found significant enrichment of DAF-16/FoxO at the somatic *pie-1* promoter (Fig. 2c).

The finding that *pie-1* mRNA is increased during dauer diapause meshes the observations that the transcriptional activity of dauers is reduced ~85% compared to other larval stages, and that dauers are exceptionally long-lived²⁴. PIE-1 functions as a global repressor of RNA polymerase II transcription in the germline during early embryonic development²⁵, and could have a similar role in maintaining a transcriptional repressed state in the soma of a dauer animal.

Ectopic somatic expression of *pie-1* causes misexpression of germline genes in somatic cells, a phenotype very similar to the misexpression of germline genes induced in the synMuvB mutant class^{5,26}. Analysis by qRT-PCR of the *pgl* family of germline-limited genes revealed that they too were expressed at higher levels in *daf-2* mutants in a *daf-16* dependent manner (Table 1, Table S1). Since the endogenous mRNA contribution from an L3 germline compared to that of a dauer germline is 2 to 3-fold higher¹³, this analysis could systematically underestimate the extent of soma to germline transformation of cell fate in the

daf-2 mutants. To eliminate germline contribution to these whole animal mRNA assays, we genetically ablated the germline of a *daf-2* mutant using a *glp-4* germline proliferation defective mutation²⁷ and analyzed the expression of these germline-specific genes by qRT-PCR. Comparison of *pie-1*, *pgl-1*, *pgl-2*, and *pgl-3* expression levels between wild type and *glp-4* also shows that these genes are germline-specific in wild type and therefore expressed at a far lower level in the *glp-4* mutant (Table 1). The *glp-4* mutant has little or no lifespan change relative to wild type²⁸. The somatic expression of *pie-1*, *pgl-1*, *pgl-2*, and *pgl-3* transcripts was much more dramatic comparing *daf-2*; *glp-4* animals to the *glp-4* control (Table 1).

Somatic protection of insulin-like signaling mutants

The somatic misexpression of germline genes suggests that the somatic cells of an insulin-like-signaling mutant are more germline-like and this transformation may have functional consequences. The *C. elegans* germline is exceptionally proficient at RNAi and perhaps for this reason, the somatic tissues of insulin-like signaling mutants are enhanced for RNAi³. We hypothesized that germline-transformed somatic cells, like germ cells, may engage additional protective pathways that prevent or slow genomic destabilization, which could increase the ability to respond to stress and extend lifespan^{29,30}.

We tested the ability of *daf-2(e1368)* mutants to protect somatic tissues from genomic instability by RNAi-depleting genes previously shown to protect the genome^{31,32}. Gene inactivation of *M04F3.1/rpa-2*, *W07A8.3/srxa-6/.5/dnJ-25*, or F49E12.6 causes increased spontaneous mutation rate and reduced viability in wild type, presumably due to increased somatic mutation (Fig. S8). *daf-2(e1368)* animals fed the same RNAi clones showed much lower levels of developmental arrest than wild type animals, suggesting that *daf-2* mutants were better protected from this form of genotoxic stress than wild type (Fig. S8). These same gene inactivations in wild type induce DNA damage and DNA strand slippage of a tandem repeat as measured by the somatic expression of a normally out-of-frame lacZ-GFP transgene³². Inactivation of *W07A8.3/srxa-6/.5/dnJ-25* by RNAi causes strong intestinal expression of the out of frame lacZ fusion gene in wild type, while far fewer *daf-2(e1368)* mutant animals reanimated this out of frame fusion gene, suggesting a reduction in genomic instability in the somatic cells of the insulin-like signaling mutants (Fig. S9). The fact that *daf-2* mutants are enhanced for RNAi suggests that the resistance to somatic DNA damage is not a consequence of an attenuated response to the RNAi treatment, but is instead an increased ability of these somatic cells to respond to deficits in pathways that regulate genomic surveillance.

Soma-to-germline transformation promotes cell survival

If the misexpression of germline genes in somatic tissues contributes to the longevity phenotype of insulin-like signaling mutants then inactivation of these genes by RNAi should suppress the longevity phenotype. To test this we inactivated several germline genes misexpressed in the somatic cells of *daf-2/InsR* mutants. To rule out pleiotropic effects, we also performed a similar analysis on wild type animals. Depletion of these misexpressed germline genes in the *daf-2* mutant reduced the health of these normally long-lived animals.

The same gene inactivations in wild type caused a slight increase in lifespan (Fig. 3, Table S2). This phenotype is likely due to the reduction in the proliferating germ cell population within the gonad, which has been previously shown to increase lifespan in wild type animals³³. Although none of the gene inactivations could suppress *daf-2* lifespan as well as inactivation of *daf-16/FoxO*, it is interesting that these gene inactivations changed sign for longevity control in this genetic background compared to wild type. The somatic expression of germline features in the *synMuvB* mutants is suppressed by loss of function mutations in the chromatin remodeling ATPase, *isw-1* and the SET domain containing protein, *mes-4*³⁴. Similarly, RNAi inactivation of *isw-1* and *mes-4* could partially suppress the enhanced longevity of *daf-2/InsR* mutants.

Mechanism of soma-to-germline transformation

An RNAi screen for essential gene inactivations that increase lifespan identified a large number of longevity genes that regulate gene expression and possibly RNAi. We tested whether any of the other genes identified in our post-developmental RNAi screen to extend lifespan also displayed expression of germline genetic markers in somatic tissues by tracking the expression of endogenous PGL-1. PGL-1 is normally restricted to the germline where it forms perinuclear punctate structures around mitotic and meiotic germ cells (Fig. 4a). Gene inactivation by RNAi of two components of the cytosolic chaperonin complex *cct-4* and *cct-6* that increase longevity, caused somatic expression of PGL-1 as detected by antibody staining (Fig. 4a, Fig. S10). The somatic PGL-1 protein localized to punctate perinuclear rings in hypodermal cells and also produced intestinal cytoplasmic granules. It was notable that these are the same tissues where we observe misexpression of *pie-1* in the insulin-like signaling mutants.

The cytosolic chaperonin complex negative regulates the activity of intestinal *skn-1*³⁵. Loss of cytosolic chaperonin complex function causes SKN-1-dependent transcriptional activation of detoxifying enzymes and stress response genes targets such as *gst-4*^{35,36}. In addition, intestinal *skn-1* mediates the longevity phenotype of insulin-like signaling mutants parallel to *daf-16*³⁷. In support of the intestine acting as an important tissue for this regulation, the strongest misexpression of PGL-1 was detected in the intestine of *cct-4* and *cct-6* RNAi treated animals. Together these data support a model where *C. elegans* longevity-promoting mutations such as decreased insulin-like signaling or reduction of the cytosolic chaperonin complex causes the misexpression of germline specific genes in somatic tissues and this germline-like quality contributes to the increased survival and health of these animals (Fig. 5).

Discussion

Our data reveals that many *C. elegans* longevity mutants exhibit a soma-to-germline transformation that contributes to their enhanced survival. The somatic protection stemming from the soma-to-germline transformation likely plays a role both during the pre-reproductive dauer diapause stage, where *C. elegans* can survive for months, and post-reproductive adulthood to promote longevity. This phenotype although surprising makes intuitive sense as germ cells employ protective mechanisms to ensure genetic integrity. The

recruitment of these normally germline-protective pathways in somatic tissues provides a mechanism for the finding that long-lived mutants exhibit resistance to genotoxic stress. Germ cells are tremendously resistant to numerous stresses, which renders them immortal and capable of regeneration on a scale not shared by somatic tissues. We hypothesize that the transformation of somatic cells to a more germ cell-like state provides increased genomic stability and may be an important pathway facilitating lifespan extension.

Alternatively, misregulation of germline genes in the soma may result in the ability of these post-mitotic cells to reinstate a proliferative state. *chk-1* and other checkpoint proteins regulate postmitotic cell survival³⁸. In the embryo the CHK-1 pathway is suppressed so that cell divisions can occur properly. If in the insulin-like signaling mutants the somatic tissues were more proliferative this could also result in increased lifespan. Intriguingly, *smk-1/rad-2* regulates the silencing of *chk-1* and SMK-1 is a component of the insulin-like signaling longevity pathway^{39, 40}. A return to a replicatively competent state could also explain the observed stability of the somatic nuclear pore complex in the insulin signaling mutants if the subunits are being replaced with new copies, which does not occur in non-proliferative cell types⁴¹. This idea fits with the observation that RNAi toward the nuclear pore component *dct-3/hTpr* increases lifespan in wild type animals but decreases the fitness of *daf-2/InsR* mutants⁴², similar to our analysis of the germline misexpressed genes.

The fact that the most potent transcriptional regulator of the lifespan extension phenotypes in insulin-like signaling mutants, *daf-16/FoxO*, mediates the soma-to-germline transformation points to the importance of this phenotype in the regulation of lifespan and stress resistance^{14, 15}. We demonstrate here by EMSA and ChIP that DAF-16/FoxO can bind to the *pie-1* promoter in somatic cells. The *in vivo* binding is weaker than previously described target promoters of *sod-3* and *mtl-1*, however, this level of binding correlates with a lower induction of *pie-1* mRNA compared to the canonical targets (Fig. S5). A similar level of enrichment of DAF-16 on the *pie-1* promoter in a strain expressing DAF-16::mCHERRY in the hypodermis, muscle, and neurons but not intestine points to these cell types as the key regulatory tissues (Table S3)^{43–45}. In conjunction with our qRT-PCR and genetic epistasis analyses we have uncovered that DAF-16/FoxO is an important direct regulator of *pie-1* expression and modulator of the soma-to-germline transformation phenotype.

Our data support a model where the somatic tissues of insulin-like signaling mutants display transcriptional dysregulation to become more germ cell-like. Unlike the *cct* RNAi treated animals, the detection of misexpressed germline proteins in the insulin-like signaling mutants has been complicated. However, germ cells are also under tight translational control, which may explain the lowered level of germline proteins⁴⁶. This added layer of expression control might be important corollary for the longevity phenotype as canonical synMuvB mutants such as *mep-1*, *lin-15b* and *lin-35* strongly misexpress germline proteins in the soma⁵ and have general sickness and a short lifespan (data not shown, Fig. S11).

Similar to the longevity and soma-to-germline transformation, the enhanced RNAi phenotype of the insulin-like signaling mutants is dependent on DAF-16/FoxO³. In addition, loss of the DAF-16 transcriptional target gene *zfp-1/AF10 22* can suppress the multivulval

phenotype of synMuv mutants and partially suppress the lethality of *mep-1* mutants³⁴. Moreover, synMuvB mutants transcriptionally dysregulate the gonad distal tip cell (DTC) marker *lag-2* in other somatic tissues, which is partially dependent upon *zfp-1/AF10*³⁴. Finally, *zfp-1* has been shown to be required for the ability of *C. elegans* to perform RNAi⁴⁷. Taken together these data suggest that soma-to-germline transformation, enhanced RNAi, and longevity share common regulatory mechanisms.

The idea that somatic tissues have the potential of adopting a more germ cell-like character provides a means of dissecting the dichotomy between the instability of somatic cells and the immortality of germline stem cells. Uncovering the mechanisms that mediate this transformation phenotype will provide insight into the pathways that maintain germ cell viability, cell fate specificity and increased survival stemming from the acquisition of germline quality by somatic cells.

Given that protection of the germline is an evolutionarily shared trait across species, it will be interesting to investigate if this is a broadly conserved mechanism of modulating lifespan. The idea that somatic cells maintain the potential to reacquire pathways lost during differentiation is tantalizing and may facilitate the elucidation of therapeutics to assist in cellular repair and possibly regeneration.

Methods summary

C. elegans was cultured using standard techniques⁴⁸ and fed on *Escherichia coli* OP50 or HT115 harboring a dsRNA expressing plasmid⁴⁹.

Microscopy

Worms were paralyzed with 2,3-Butanedione monoxime, mounted on agarose pads, and imaged on a Zeiss Axioplan microscope. Live worms were imaged with a 10X objective under a Zeiss Discovery Microscope.

Quantitative RT-PCR

Synchronized animals were removed from growth plates and thoroughly washed in M9 buffer. RNA was isolated by Trizol (Invitrogen) and potential DNA contamination removed by TurboDNA-Free (Ambion). The purity/stability of the RNA was tested by gel electrophoresis and UV spectroscopy. cDNA was synthesized from 2ug of RNA with SuperscriptIII reverse transcriptase (Invitrogen) and quantitative PCR performed with SYBR Green (BioRad) on a BioRad iCycler. Three biological replicate samples were tested in triplicate. Samples were normalized to *rpl-32* and/or *snb-1* transcript levels.

Lifespan Assays

Post-developmental RNAi lifespan analysis was performed as previously described in Curran et al (2007)¹. In brief, synchronized worms fed on vector control bacteria were transferred to RNAi bacteria on day 1 of adulthood and traditional lifespan analysis performed.

Statistical analysis

Statistical analyses were performed using the software SPSS (SPSS Inc.). The survival rate of each RNAi-treated population is compared with that of the population treated with control RNAi using the log rank test. A p -value < 0.05 was considered as significantly different from control.

Immunostaining

Animals were permeabilized using a freeze-crack method by freezing between a polylysine-coated slide and a coverslip followed by rapid removal of the coverslip. Slides were immediately immersed in ice-cold 100% methanol for 120 min followed by ice-cold 100% acetone for 15 min. Larvae were stained using monoclonal (1:40 dilution in PBSTB) anti-PGL-1 (K76) antibodies (gift from C. Mello) overnight at 4°C followed by Alexa Fluor-conjugated goat anti-Mouse IgM (1:100 dilution in PBST; Invitrogen).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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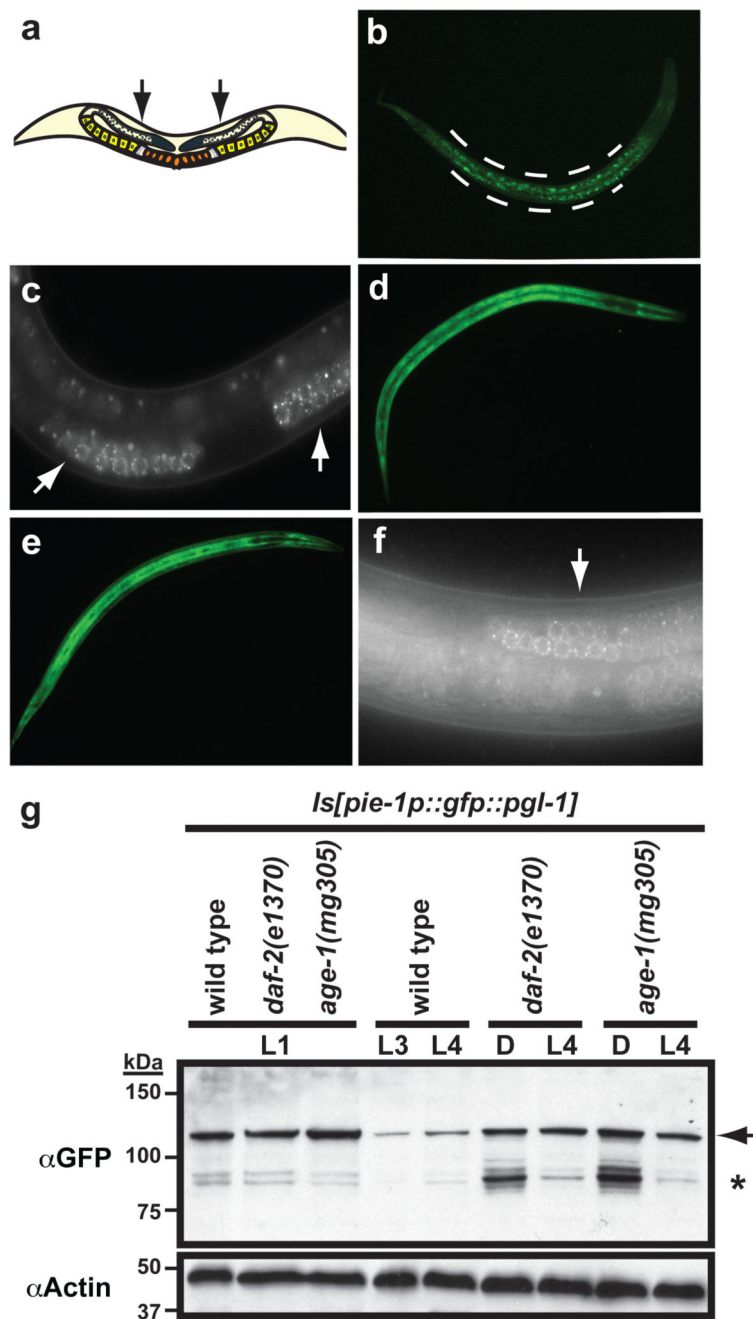


Fig. 1. Mutations in the insulin/IGF-like signaling pathway cause soma-to-germline transformation

(a) Schematic of the *C. elegans* germline (b) wild type L2/L3 larvae express *pie-1p::gfp::pgl-1* only in the germline (region between dashed lines marks background autofluorescence), (c) in perinuclear structures (arrows). (d) *daf-2(e1370)* and (e) *age-1(mg305)* mutations cause somatic expression of *pie-1p::gfp::pgl-1* in the intestine and hypodermis in dauers and (f) *daf-2(e1370)* L3/L4 larvae. (g) SDS-PAGE resolved whole worm lysates of wild type, *daf-2(e1370)*, and *age-1(mg305)* animals harboring the

pie-1p::gfp::pgl-1 reporter. GFP::PGL-1 (Arrow). *, unknown crossreacting protein. L1, Larval stage 1, L3, Larval stage 3, D, dauer, L4, Larval stage 4.

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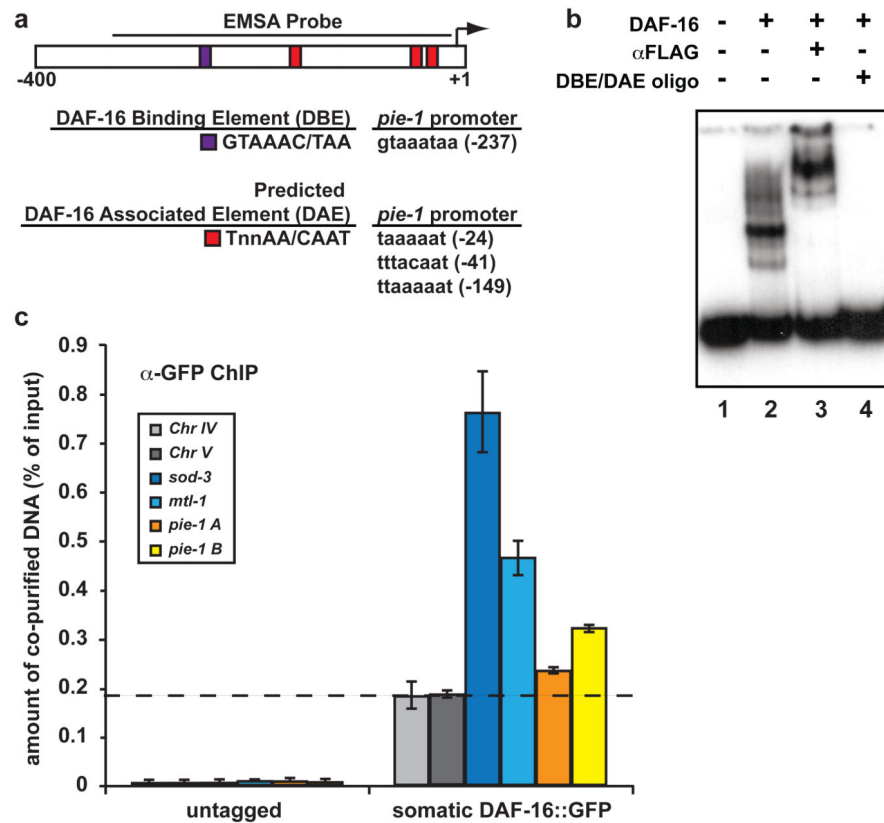


Fig. 2. DAF-16 regulates the expression of *pie-1*

(a) Schematic of the *pie-1* promoter. (b) A ^{32}P labeled region of the *pie-1* promoter (EMSA probe, lane 1) can recruit purified recombinant DAF-16-FLAG (Lane 2). The bound DAF-16 protein can be supershifted with αFLAG antibodies (lane 3). Binding is competed away with a cold dsDNA oligo containing DAF-16 binding/associated elements (DBE/DAE, lane 4). (c) DAF-16 is enriched on the promoter of *pie-1* in *daf-2* RNAi treated worms. Chromatin IP (ChIP) was performed using α-GFP antibodies in wild type (untagged) and a somatic exclusive *daf-16::gfp* strain (somatic DAF-16::GFP). Binding of two non-DAF-16-associated control regions (ChrIV, ChrV), two DAF-16-associated promoter regions (*sod-3*, *mtl-1*), and two regions in the *pie-1* promoter (*pie-1* A, *pie-1* B) was measured from the IP. Error bars represent S.E.M.

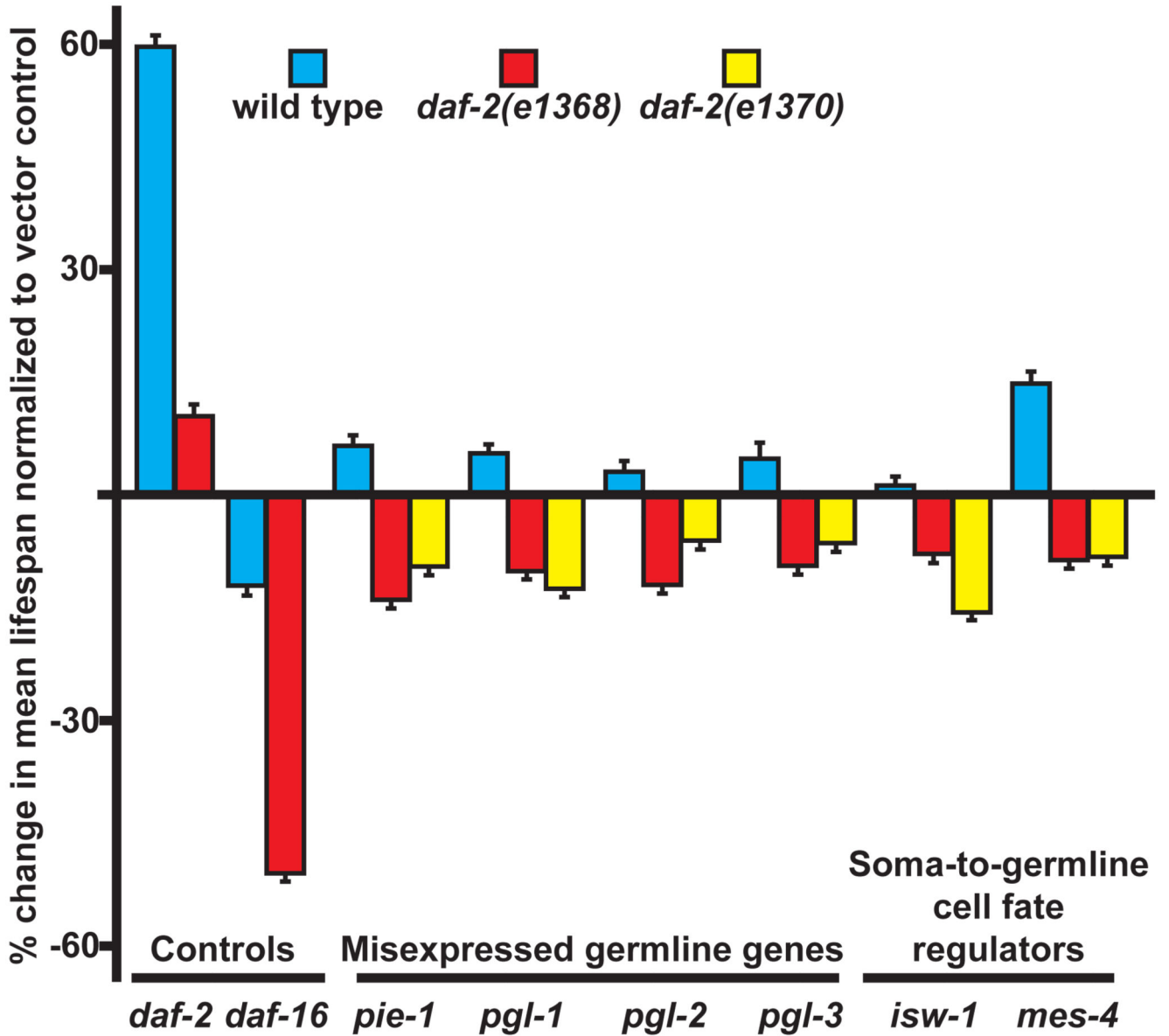


Fig. 3. The somatic misexpression of germline-specific genes in insulin-like signaling mutants contributes to their increased longevity

Wild type (blue) or two hypomorphic insulin-like receptor mutants *daf-2(e1368)* (red) and *daf-2(e1370)* (yellow) animals were fed RNAi clones targeting germline-specific genes that are misexpressed in somatic cells and two known soma-germline specificity regulators only in adulthood. RNAi depletion of germline-restricted genes in wild type animals increases lifespan compared to vector control while inactivation in *daf-2* mutants shortens the lifespan of these normally long-lived animals. RNAi targeting *daf-2* (increased lifespan) or *daf-16* (decreased lifespan) were used as controls and lifespan was normalized to animals fed vector control RNAi.

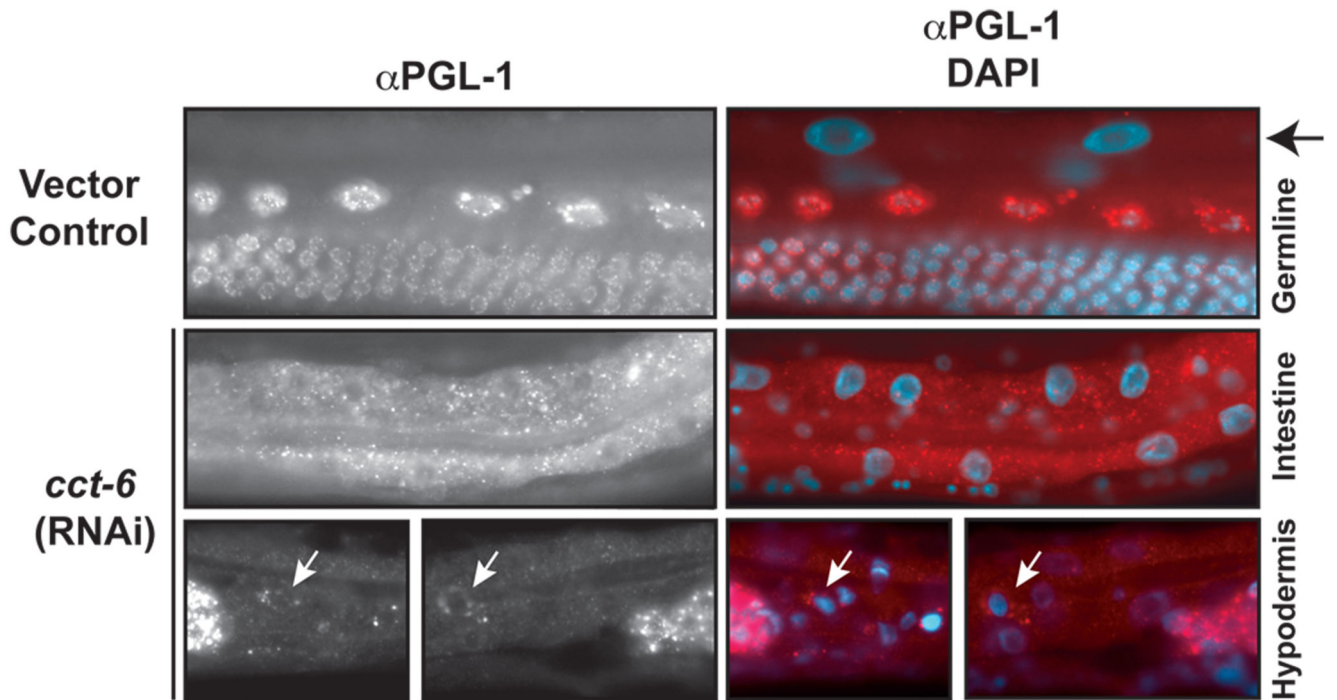


Fig. 4. The cytosolic chaperonin complex regulates the expression of PGL-1 in somatic cells
PGL-1 immunostaining is restricted to perinuclear structures of meiotic and mitotic germ cells in animals fed vector control (top panel). Black arrow points to four intestinal nuclei lacking PGL-1 expression. RNAi targeting *cct-6* causes somatic PGL-1 expression in intestinal (middle panel) and hypodermal cells (bottom panel). White arrows indicate perinuclear PGL-1 localization.

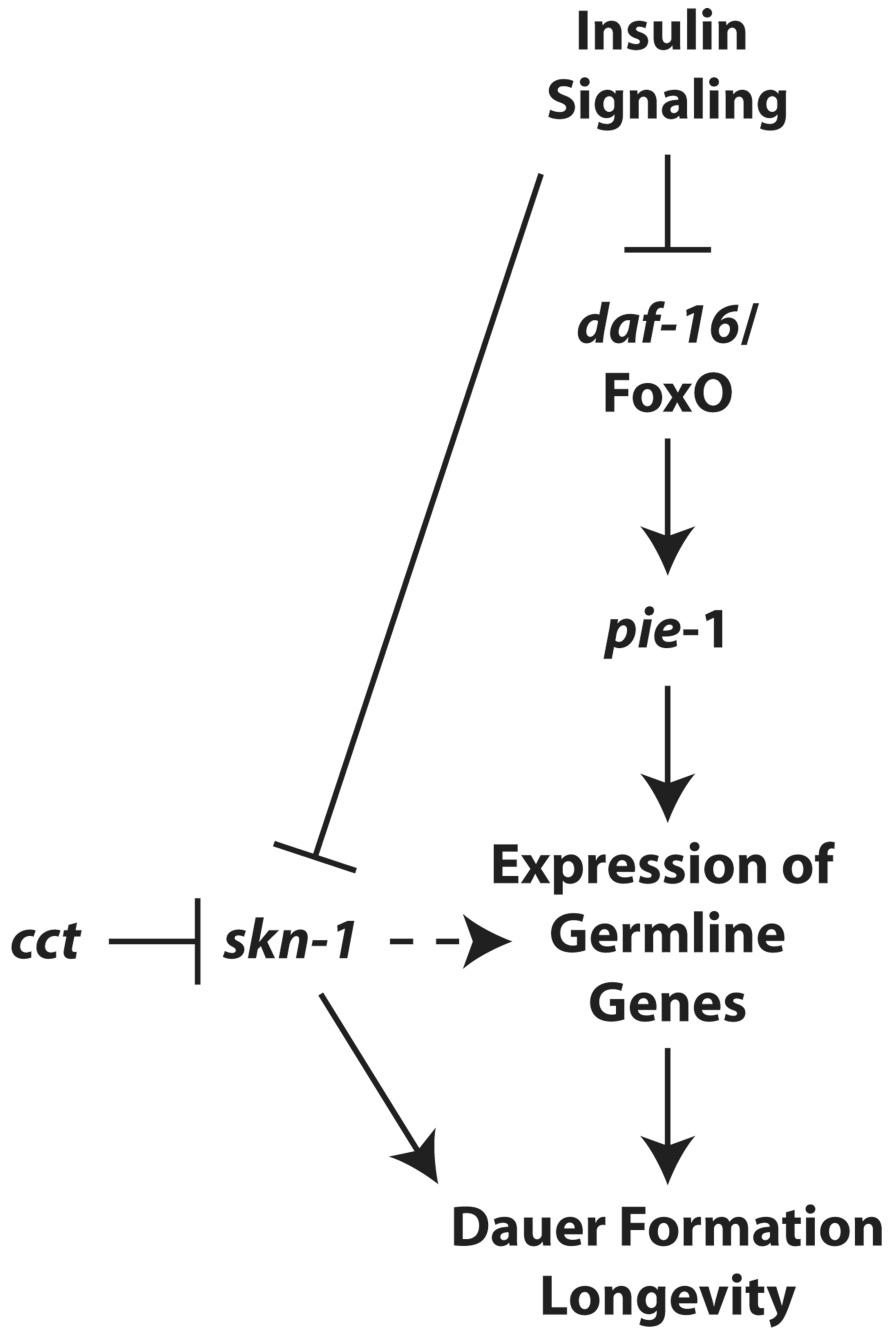


Fig. 5. Model for the regulation of germline gene expression in the soma by *C. elegans* longevity regulators

Insulin-like signaling negatively regulates the FoxO transcription factor DAF-16. DAF-16 positively regulates the expression of *pie-1* and germline specific genes in somatic tissues. The cytosolic chaperonin complex (*cct*) negatively regulates the activity of SKN-1 and the somatic expression of PGL-1. Solid lines represent experimentally observed regulation. Dashed lines indicate potential regulation

Table 1

Insulin-like signaling regulates the expression of germline genes in somatic cells

	pie-1	pgl-1	pgl-2	pgl-3
<i>daf-2(e1368)^a</i>	2.1±0.1 [#]	1.0±0.1	4.6±0.9 [*]	1.3±0.2 [*]
<i>daf-2(e1370)^a</i>	4.5±1.0 [*]	1.40±0.2	3.3±0.2 [#]	1.9±0.3 [#]
<i>daf-16(mgDf47); daf-2(e1370)^a</i>	0.8±0.1 [*]	0.4±0 [#]	0.9±0.1	0.6±0.1 [#]
<i>glp-4(bn2)a</i>	0.007±0 [#]	0.007±0 [#]	0.005±0 [#]	0.005±0 [#]
<i>glp-4(bn2); daf-2(e1370)^b</i>	3.7 ± 1.0 [#]	3.9±1.5 [#]	2.8±0.1 [#]	2.3±0.8 [#]

^a qRT-PCR analysis is represented as fold change relative to wild type samples normalized to *rpl-32* and *snb-1* expression.

^b Same as (a) except samples are compared to *glp-4(bn2)* mRNA levels normalized to *rpl-32* and *snb-1* expression.

Student's t-Test

* p-value <0.05

p-value <0.01