Tissue entrainment by feedback regulation of insulin gene expression in the endoderm of *Caenorhabditis elegans*

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How are the rates of aging of different tissues coordinated? In *Caenorhabditis elegans*, decreasing insulin/IGF-1 signaling extends lifespan by activating the transcription factor DAF-16/FOXO. If DAF-16 levels are experimentally increased in one tissue, such as the intestine, DAF-16 activity in other tissues rises. Here we test the hypothesis that this "FOXO-to-FOXO" signaling occurs via feedback regulation of *ins-7* insulin gene expression. We find that DAF-16 regulates *ins-7* expression in the intestine, and that preventing this regulation blocks FOXO-to-FOXO signaling from the intestine to other tissues. Our findings show that feedback regulation of insulin gene expression coordinates DAF-16 activity among the tissues, and they establish the intestine, which is the animal's entire endoderm, as an important insulin-signaling center.

aging | DAF-2 | DAF-16 | FOXO

In *Caenorhabditis elegans*, reducing the activity of the insulin/IGF-1-receptor DAF-2 doubles lifespan (1). A striking feature of these long-lived animals is that, throughout their lives, they closely resemble younger wild-type animals (2, 3). In other words, relative to one another, the rates of aging of the different tissues appear normal. In principle, this apparent normalcy could arise from a similar cell-autonomous reduction of insulin/IGF-1 action in all tissues. However, genetic mosaic analysis indicates that signaling between the different tissues of the animal, downstream of insulin/IGF-1 receptor activity, plays an important role (4, 31). For example, removing the *daf-(+)* gene from an early blastomere that produces only some of the tissues extends the lifespan of the entire animal, keeping wild-type tissues alive long after they would normally die (4). Thus cells that lack *daf-2* receptor activity not only change their own rates of aging, they also signal wild-type cells to change their rates of aging as well.

How might *daf-2* activity influence downstream intercellular signaling? The insulin/IGF-1 pathway affects aging, at least in part, by controlling the activity of a phosphoinositol 3-kinase/PDK/AKT-kinase cascade that phosphorylates the FOXO-family transcription factor DAF-16 and prevents DAF-16 accumulation in the nucleus (5–12). *daf-16* is required for the longevity of *daf-2* mutants. When insulin/IGF-1 signaling levels fall, DAF-16 accumulates in nuclei, where it changes the expression of downstream longevity genes (13, 32–34), including, presumably, genes encoding downstream intercellular signals. Consistent with this idea, expression of *daf-16* only in the intestine or neurons of *daf-2* mutants is sufficient to increase the lifespan of the entire animal (14).

The insulin/IGF-1 pathway influences lifespan cell nonautonomously in flies and mice as well as worms. For example, increasing dFOXO activity only in fat tissue extends the lifespan of flies (15, 16), and loss of the insulin receptor in the fat tissue, which would be predicted to increase FOXO activity, extends the lifespan of mice (17). The intestine of *C. elegans* functions as the animal's entire endoderm, including its site of fat storage. Thus identifying the downstream longevity signals regulated by insulin/IGF-1 signaling in the intestine of *C. elegans* could have general significance.

Genetic experiments indicate that signals regulated by DAF-16 in one tissue affect the activity of DAF-16 in responding cells (14). If *daf-16* expression is increased in a single tissue, such as the intestine (using a tissue-specific promoter), expression of the direct DAF-16-target gene *sod-3* (superoxide dismutase) (18, 19) is increased not only in that tissue but in other tissues as well. This increased *sod-3* expression requires DAF-16 in responding cells, because it is not observed in a *daf-16(−)* background (14). We call this communication "FOXO-to-FOXO" signaling and hypothesize that it helps to equalize the activity of DAF-16 in the different tissues of the animal, thereby helping to coordinate their rates of aging.

How might DAF-16 activity in one tissue influence DAF-16 activity elsewhere? Because DAF-16 is known to be regulated by insulin/IGF-1 signaling, an attractive model is that FOXO-to-FOXO signaling is mediated by insulin-like genes whose expression is regulated by DAF-16 (4). In this scenario, changing DAF-16 activity in one tissue would change insulin gene expression in that tissue, which in turn would alter extracellular insulin levels and ultimately the activity of DAF-16/FOXO in other tissues. Recently, we identified a number of genes encoding putative signaling molecules in microarrays of *daf-2* and *daf-16* mutants (13). Among these was an insulin-like gene, *ins-7*. In this study, we tested the hypothesis that INS-7 is a signal that is regulated by DAF-16 to influence FOXO-to-FOXO signaling. Together our findings indicate that this is the case, and in addition they establish the endoderm of *C. elegans* as an important insulin-signaling center in the animal.

Results

***ins-7* Activates the DAF-2 Pathway.** The first step in testing the hypothesis that *ins-7* influences FOXO-to-FOXO signaling was to determine the normal function of *ins-7* in the animal. *ins-7* RNAi or mutation extends the lifespan of wild-type adults and increases the penetrance of a *daf-2* loss-of-function developmental phenotype, the formation of dauer larvae, suggesting that INS-7 is a DAF-2 agonist [ref. 13 and supporting information (SI) Fig. 5]. If this is the case, then *ins-7* RNAi should increase the nuclear accumulation of DAF-16 and the expression of the DAF-16 target gene *sod-3*. We found that *ins-7* RNAi stimulated nuclear localization of a DAF-16::GFP protein fusion (Fig. 1 *a*

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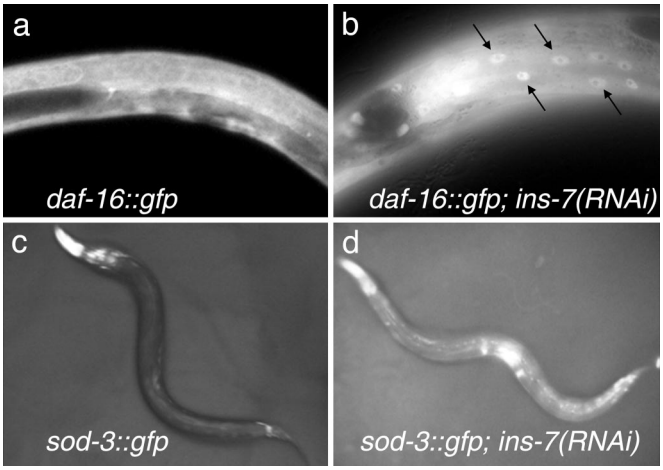

Fig. 1. *ins-7* influences targets of the DAF-2 insulin/IGF-1 pathway. (*a*) Wild-type animals expressing *Pdaf-16*::*daf-16*::*gfp* cultured on control RNAi bacteria exhibit diffuse DAF-16::GFP localization. (*b*) Subjecting these animals to *ins-7* RNAi increases DAF-16::GFP nuclear localization (arrows). (*c*) Wild-type animals expressing *Psod-3*::*gfp* and grown on control RNAi bacteria exhibit low levels of GFP in the body and higher levels in the head region (light areas). (*d*) *ins-7* RNAi increases *Psod-3*::*gfp* expression throughout the body.

and *b*) (6). In addition, *ins-7* RNAi increased *Psod-3*::*gfp* expression (Fig. 1 *c* and *d*). Thus *ins-7* RNAi likely promotes longevity and dauer formation by inhibiting DAF-2 and activating DAF-16.

DAF-2 and DAF-16 Regulate *ins-7* Expression in the Intestine. Many of the ≈38 insulin-like genes in *C. elegans* are expressed in the nervous system (20). In addition, killing specific sensory neurons or inhibiting their functions genetically triggers DAF-16 nuclear localization (9) and extends lifespan (21). Moreover, signals that regulate dauer formation change the expression of certain insulin-like genes in sensory neurons (22). For this reason, it is widely believed that the biologically relevant site of insulin production is primarily the nervous system, despite the fact that expression of insulin-like genes has been observed in other tissue types (20).

In genetic experiments, the intestine behaves as a potent source of FOXO-to-FOXO signals; in fact, DAF-16 activity in the intestine appears more effective at elevating DAF-16 activity in other tissues than is DAF-16 activity in neurons (14). Therefore, it was of great interest to learn whether *ins-7* was regulated by DAF-2 and DAF-16 in the intestine.

Previously, *Pins-7*::*gfp* was reported to be expressed exclusively in neurons (20). However, we observed that some animals carrying *Pins-7*::*gfp* (≈12%) also expressed *Pins-7*::*gfp* in intestinal cells (Fig. 2 *b* and *h*). In addition, intestinal (but not neuronal) *Pins-7*::*gfp* expression increased with age (Fig. 2*c*). On day 8 of adulthood, ≈30% of animals exhibited intestinal *Pins-7*::*gfp* expression. We note that not every intestinal cell expressed *Pins-7*::*gfp* in these animals. Instead, apparently random subsets of intestinal cells expressed the transgene in different animals. This expression pattern may reflect the endogenous expression pattern of *ins-7*; however, similar random subintestinal expression patterns have been seen with other intestinal promoter fusions, such as fusions to the *ges-1* (gut-esterase) promoter, suggesting that the random pattern may be a more general property of transgenes expressed in the intestine.

If intestinal *ins-7* mediates FOXO-to-FOXO signaling, then intestinal *ins-7* levels should respond to changes in *daf-2* or *daf-16* activity. We reduced *daf-16* activity with the *daf-16* null mutation *mu86* and observed a striking increase in intestinal *Pins-7*::*gfp* expression in young adults (to 55%; Fig. 2 *e* and *h*; $P < 0.01$). In contrast, there was no obvious effect of *daf-16(mu86)* on neuronal *Pins-7*::*gfp* expression (Fig. 2*e*). We observed a similar increase in intestinal *Pins-7*::*gfp* expression in *daf-18*/PTEN mutants, which have increased insulin/IGF-1 signaling levels because they lack a phosphatase that counteracts PI 3-kinase activity (Fig. 2*f*).

We reduced *daf-2* activity with *daf-2(e1370)* and observed a reduction *Pins-7*::*gfp* in the intestine to 2% ($P < 0.01$) but not in the nervous system (Fig. 2 *d* and *h*). Together, these findings suggested that the intestine is the primary tissue in which *ins-7* expression is regulated by insulin/IGF-1 signaling.

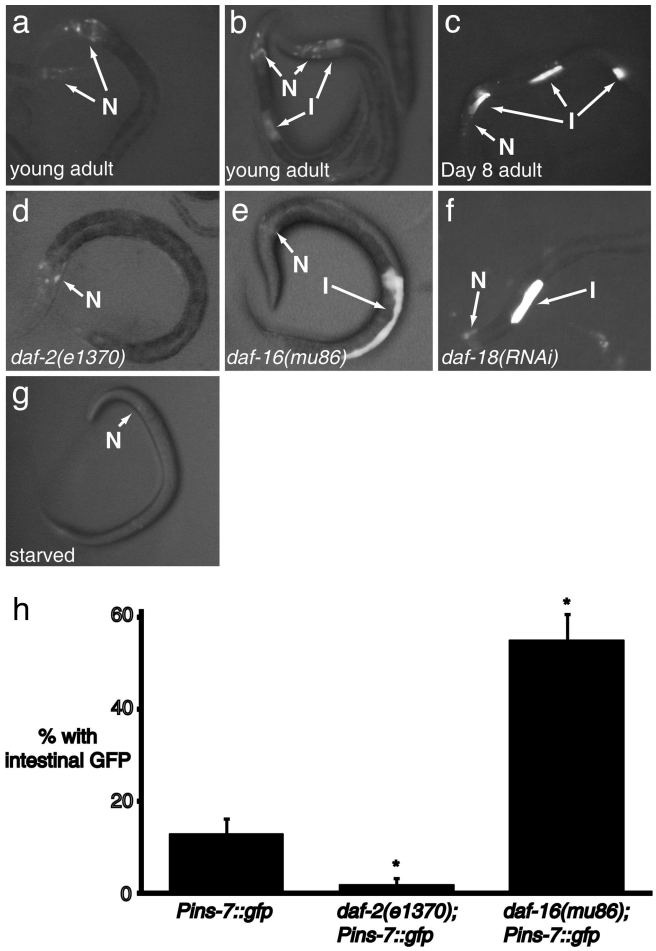

Fig. 2. Regulation of *Pins-7*::*gfp* expression. I, intestinal cell; N, neurons. (*a–g*) *Pins-7*::*gfp* expression under different conditions. (*a*) Typical young adult, exhibiting neuronal but not intestinal *gfp* expression; (*b*) young adult expressing *Pins-7*::*gfp* in intestinal cells; (*c*) typical day 8 adult, exhibiting intestinal as well as neuronal *gfp* expression (*d*) typical *Pins-7*::*gfp*; *daf-2(e1370)* mutant, with no intestinal expression (see *h*); (*e*) typical *Pins-7*::*gfp; daf-16(mu86)* mutant, with pronounced intestinal expression; (*f*) typical *Pins-7*::*gfp* animal subjected to *daf-18*/*PTEN* RNAi; and (*g*) typical starved wild-type animal, with sharply reduced *ins-7*::*gfp* expression; (*h*) percentage of wild-type ($n = 100$) and mutant *Pins-7*::*gfp* worms ($n = 84$ for *daf-16*, $n = 102$ for *daf-2*) expressing GFP fluorescence in intestinal cells. Standard error of the proportion (SEP). A *Z* test for two proportions shows that *daf-2* and *daf-16* mutants are each significantly different from the vector control at the 99% confidence level (*).

***ins-7* Likely Mediates FOXO-to-FOXO Signaling.** Because *ins-7* was regulated by DAF-2 and DAF-16 in the intestine, it was a candidate for a signal that influences FOXO-to-FOXO signaling from the intestine to other tissues. To test directly whether this

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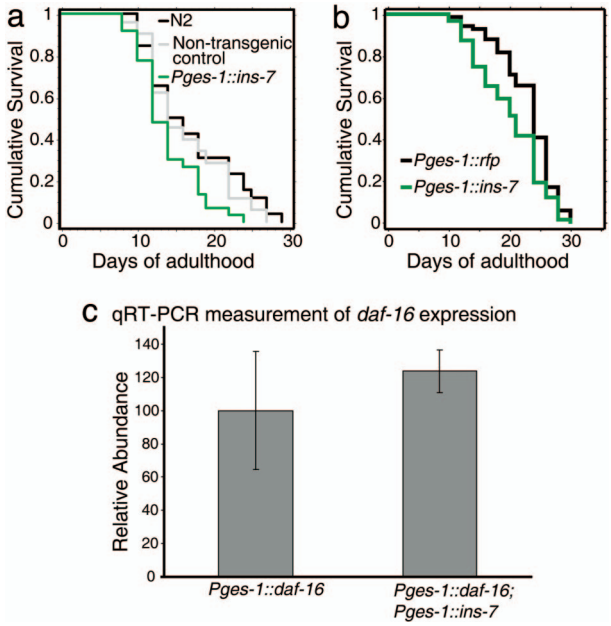

Fig. 3. Intestinally expressed *ins-7* shortens lifespan. (*a*) Mean lifespan of wild-type (N2) = 17.0 ± 1.2, non-transgenic control = 16.3 ± 1.3, and *Pges-1:ins-7* = 13.9 ± 0.7, $P = 0.0096$ (non-transgenic vs. *Pges-1*::*ins-7*). (*b*) Mean lifespan of *Pges-1*::*gfp* = 23.0 ± 0.6, *Pges-1:ins-7* = 20.1 ± 0.6, $P = 0.0007$. See SI Table 1 for additional trials and statistical analysis. (*c*) Intestinally expressed *ins-7* does not affect *daf-16* expression. Quantitative RT-PCR of intestinally expressed *daf-16* in wild-type and *Pges-1*::*ins-7* backgrounds. Results are from two biological repeats; error bars represent standard deviation.

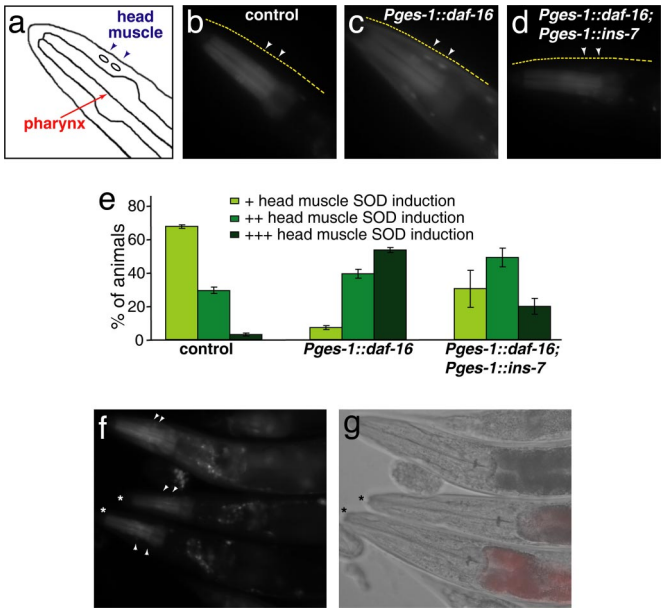

Fig. 4. Inhibiting DAF-16 regulation of *ins-7* decreases the ability of intestinal DAF-16 to affect DAF-16 activity in other tissues. All animals carry *Psod-3*::*gfp*. (*a*) Schematic illustration of the animal's head. (*b–d*) The upper edge of the animal's head is indicated by the dashed yellow line, and arrowheads point to muscle nuclei. (*b*) Control wild-type animals express *Psod-3*::*gfp* in the pharynx but not in surrounding head tissue. (*c*) Increasing intestinal DAF-16 levels by using *Pges-1*::*daf-16* increases *Psod-3*::*gfp* expression throughout the head. [This increased *Psod-3*::*gfp* expression requires increased DAF-16 activity in nonintestinal tissues, because it is not seen in a *daf-16*(−) background (14).] (*d*) Constitutive expression of *ins-7* from the *ges-1* intestinal promoter (*Pges-1*::*ins-7*) reduces the ability of intestinal *Pges-1*::*daf-16* to stimulate *Psod-3*::*gfp* expression in the head. (*e*) Semiquantification for *b–d* ($n \geq 109$). (*f* and *g*) *Pges-1*::*daf-16* animals with (*) and without *Pges-1*::*ins-7*. Animals carrying *Pges-1*::*ins-7* have red intestinal fluorescence from the coinjected *Pges-1*::*rfp* construct (see *Materials and Methods* and SI Fig. 6).

was the case, we increased DAF-16 levels in the intestine but prevented this increase from decreasing *ins-7* expression.

To insulate *ins-7* gene expression from the influence of DAF-16, we fused the *ins-7* coding region to the promoter of the intestinal gene *ges-1*. Animals expressing this transgene had a slightly shorter lifespan than did wild-type animals or control animals expressing *Pges-1*::*rfp* (Fig. 3 *a* and *b*), as predicted if the transgene increases INS-7 levels in the animal. We then increased the level of DAF-16 in the intestine using a functional *Pges-1*::*daf-16*::*gfp* fusion and assayed the level of *sod-3*::*gfp* in hypodermis and muscle. In control animals lacking the *ins-7* transgene, elevating intestinal DAF-16 levels increased *Psod-3*::*gfp* expression levels in other tissues. (Compare Fig. 4*b*, in which only the pharynx expresses *gfp*, with Fig. 4*c*, in which many additional tissues express *gfp*.) However, when intestinal DAF-16 levels were increased in animals carrying *Pges-1*:: *ins-7,* little or no increase in DAF-16::GFP was observed in other tissues (Fig. 4 *d* and *e*). (Note the similarity of the animal in Fig. 4*d* to the wild-type control in Fig. 4*b*.)

We considered the possibility that *Pges-1*::*ins-7* might reduce expression of intestinal *Pges-1*::*daf-16* by titrating factors required for *Pges-1* promoter activity, thereby decreasing FOXO-to-FOXO signaling for trivial reasons. However, quantitative RT-PCR experiments indicated this was not the case (Fig. 3*c*). Thus these findings suggest that DAF-16 activity in the intestine activates DAF-16 elsewhere in the animal, at least in part, by down-regulating intestinal expression of *ins-7*.

Discussion

In principle, a tissue's rate of aging might be specified in a purely cell-autonomous fashion, for example, by the rate of production of reactive oxygen species generated within its own mitochondria. However, the finding that expressing components of the insulin/IGF-1 longevity pathway in subsets of cells can affect the rate of aging of the entire organism implies the existence of a mechanism that actively coordinates the rates of aging between the different tissues. In this study, we have shown that in *C. elegans*, this communication between the tissues is mediated, at least in part, by insulin-like peptides. Specifically, we showed that the *ins-7* gene is regulated by DAF-16 activity in the intestine, and that this regulation, in turn, allows DAF-16 activity in the intestine to influence DAF-16 activity in other tissues.

Feedback Regulation of *ins-7* Expression. Our attention was first drawn to *ins-7* as a possible mediator of FOXO-to-FOXO signaling because its expression changed in DAF-2-pathway microarrays (13). *ins-7* is expressed in two tissues, the nervous system and the intestine; however, insulin/IGF-1 signaling appears to regulate expression of *ins-7* primarily in the intestine. Because *ins-7* functions as expected for a DAF-2 agonist, and because its expression is activated by the insulin/IGF-1-response pathway in the intestine, *ins-7* is a part of a positive-feedback (i.e., feed-forward) regulatory loop that would be predicted to amplify upward or downward fluctuations in insulin/IGF-1 signaling.

In *C. elegans*, the intestine appears to behave as the animal's entire endoderm. For example, the intestine is the animal's site of fat storage (adipose tissue) and of yolk production (liver). These studies suggest the *C. elegans* intestine may also perform functions of the mammalian pancreas, also an endodermal organ. In fact, the insulin response pathway is known to up-regulate insulin gene expression in the mammalian pancreas

(23), just as the insulin-response pathway up-regulates *ins-7* in the *C. elegans* intestine. Consistent with this, *ins-7* expression dropped sharply in the intestine (as well as the nervous system) under fasting conditions (Figs. 2*g*).

***ins-7* Behaves as Expected for a Signal That Influences FOXO-to-FOXO Signaling.** The main goal of this study was to test the hypothesis that DAF-16 activity in the intestine influences DAF-16 activity in other tissues by feedback regulation of insulin gene expression. The finding that DAF-16 regulated the expression of *ins-7* within the intestine satisfied one prediction of this hypothesis. However, it was important to test the significance of this feedback regulation directly, because the effects of changes in the expression of a small subset of insulin genes might be masked by the constitutive expression of many other insulin-like genes in the animal.

We tested our hypothesis by preventing DAF-16 from down-regulating *ins-7* gene expression. We found that constitutive intestinal *ins-7* expression prevented elevated levels of intestinal DAF-16 from triggering muscle and hypodermal expression of *Psod-3*::*gfp*. The simplest interpretation of this finding is that our model was correct: down-regulation of *ins-7* in the intestine by DAF-16 lowers INS-7 levels in the animal, which in turn triggers DAF-16 activity in muscle and hypodermis. Because we know that DAF-16 does down-regulate ins-7 expression in the intestine, and that lowering INS-7 levels in the animal reduces DAF-2 pathway activity, this interpretation is clearly the most straightforward. However, we cannot rule out the possibility that, as with any overexpression experiment, artificially high levels of a protein, in this case INS-7, bypasses the activity of the normal signaling system. Thus, we conclude that *ins-7* can function in FOXO-to-FOXO signaling and most likely does so in normal animals (Fig. 4).

INS-7 may not be the only insulin-like peptide involved in FOXO-to-FOXO signaling. In our microarray analysis and subsequent experiments (ref. 13; unpublished data), we found that the gene *ins-18* is also feedback-regulated by DAF-16 in the intestine, but in the opposite direction from *ins-7*. An RNAi clone predicted to target *ins-18* prevented DAF-16 nuclear localization, shortened lifespan and prevented dauer formation, suggesting that it targeted a DAF-2 antagonist. This RNAi clone also completely prevented FOXO-to-FOXO signaling from the intestine to other tissues (SI Fig. 7). It is possible that this clone targeted more than one insulin-like gene, because most of these phenotypes were not observed with an *ins-18* deletion mutant (data not shown). These findings raise the possibility that insulin-like genes that behave as DAF-2 antagonists rather than agonists may also contribute to FOXO-to-FOXO signaling.

Positive-Feedback Loops May Contribute to Cellular Consensus Mechanisms. Feed-forward regulatory loops should, in principle, continue to amplify small perturbations until the system has reached a state of maximal or minimal activity. This may occur during dauer formation. When exposed to mild dauer-inducing conditions, some worms in a population become dauers, whereas others become adults. Within each animal, all cells reach a consensus and adopt the same (dauer vs. adult) fate. A positive-feedback system involving intercellular signals such as the one described here is likely to facilitate this decision process. In the regulation of lifespan, there must be factors that limit the ability of this system to spiral endlessly up or down, because many insulin-pathway mutations have intermediate effects on lifespan.

Insulin-like peptides cannot be the only signals that act downstream of DAF-16 to influence lifespan. When *daf-16* is expressed only within the intestine of *daf-16*; *daf-2* double mutants, lifespan is increased ≈60% (14). In these animals, tissues that would normally die much earlier, such as muscles and neurons, remain alive, because the long-lived animals continue to move. Because these nonintestinal tissues do not contain DAF-16, another signaling pathway downstream of DAF-16 must also influence aging. The secreted peptide *scl-1* is a candidate for such a downstream signal (24), as are other predicted signaling molecules whose gene expression changes in DAF-16 in microarrays (13, 32).

In summary, we have shown that an important mechanism by which the activity of the DAF-2/DAF-16 signaling pathway is coordinated among the different tissues of the animal is through positive-feedback regulation of an insulin-like peptide. This feedback regulation has the effect of equalizing DAF-16 activity in the different tissues, which would be expected to bring the rates of aging of the different tissues into alignment. In worms as well as flies, the nervous system has been regarded as the primary site of insulin production, despite the fact that insulin-like genes can be expressed in non-neuronal tissues in both organisms (20, 35). This study shows that, at least in *C. elegans,* the intestine/endoderm is an important site of insulin production and regulation.

In *Drosophila*, overexpressing dFOXO in the pericerebral fat body decreases expression of the insulin gene *dilp2* in insulin-producing neurons (15). In this case, feedback regulation of insulin-like gene expression is taking place, but across two different cell types. The signaling pathway by which dFOXO activity in fat tissue communicates with neurons is unknown. It will be interesting to learn whether, as in worms, this signaling is mediated by insulin-like peptides produced by fat tissue. Interestingly, dFOXO may play a key role on the receiving end of this pathway, because dFOXO has been shown to act within neurons to down-regulate *dlp2* expression (15, 25). Thus this situation in flies could potentially represent another case of FOXO-to-FOXO signaling.

Materials and Methods

Mutations. Mutations were as follows: LGI, *daf-16(mu86),* a null allele (9); LGIII, *daf-2(e1370)*; LGIV, *ins-7(tm1907)*, *ins-7(tm2001)*, *ins-7(ok1573)*, *ZK1251.1(tm1849)*, and *ZK1251.3(tm1723).*

Strains. Strains were as follows: CE284, *Pins-7*::*gfp* (20); CF2362, *Pins-7*::*gfp; daf-2(e1370)*; CF2340, *Pins-7*::*gfp; daf-16(mu86)*; CF2266, *muEx340*[*Pges-1*::*rfp* + *Pges-1*::*ins-7*]; TJ356, *Pdaf-16*::*daf-16*::*gfp* (6); and CF1553, *muIs84* (*Psod-3*::*gfp*) (14); CQ1: *ins-7(tm1907)* outcrossed three times with wild type (N2); CQ4: *ins-7(tm2001)* outcrossed three times with N2; CQ3, *ins-7(ok1573)* outcrossed three times with N2; FX1849: *ZK1251.1(tm1849)*; FX1723: *ZK1251.3(tm1723).*

Molecular Biology and Generation of Transgenic Worms. To express *ins-7* specifically in the intestine (*Pges-1*::*ins-7*), we introduced Acc65I and EcoRI sites immediately 5′ of the first ATG and 3′ of the stop codon of the *ins-7* gene, respectively, by PCR amplification of *C. elegans* genomic DNA with TTTGGTAC-CATGCCACCAATAATTTTGGTTTTC and TTTGAAT-TCTTAAG GACAGCACTGTTTTCGAATG primers. The Acc65I/EcoRI fragment was inserted into the Acc65I/EcoRI sites of *Pmyo-3*::*rfp* (gift from C. I. Bargmann, Rockefeller University, New York). A 2.5-kb upstream regulatory sequence of *myo-3* was excised with XbaI and BamHI and blunt-ended, and a SnaB1 fragment from pNL213 (14) that has a 3.3-kb upstream sequence of intestinal *ges-1* gene was introduced. Our *Pges-1*::*ins-7* construct was not tagged; instead, we inferred its presence from a coinjected *Pges-1*::*rfp* construct. Red intestinal fluorescence likely indicates *ins-7* expression, because red intestinal fluorescence coincided with green intestinal fluorescence in control experiments in which the same *Pges-1*::*rfp* construct was coinjected with a *Pges-1*::*gfp* construct (SI Fig. 6).

Standard techniques were used to generate transgenic animals

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(26). *Pges-1*::*ins-7* was injected into N2 animals at 100 ng/μl with intestinal *Pges-1*::*rfp* at 100 ng/μl as a coinjection marker.

RNAi. Bacterial-feeding RNAi experiments were carried out as described (27, 28). Control vector, *daf-2*, and *daf-16* RNAi vectors were from Dillin *et al.* (28), and the *ins-7* RNAi clone was from the Ahringer library (27). Each clone was verified by PCR and sequence analysis, and isopropyl β-D-thiogalactoside was added to increase induction. We also wrote a perlscript to identify possible secondary RNAi targets of *ins-7* (see *SI Text*), but no matches met the 200-nt threshold for secondary targets, suggesting that the clone targets only *ins-7*.

Survival Analyses. The first day of adulthood was defined as $t = 0$, and the log-rank (Mantel–Cox) method was used to test the null hypothesis (StatView 5.01, SAS Software), as described (29). Lifespan experiments were carried out at 20°C, and for all experiments, $n > 60$ for each sample.

GFP Assays. Populations of live worms were scored for GFP by using a fluorescence dissecting microscope. The transgenic arrays carried by the animals we examined were extrachromosomal and therefore meiotically unstable. Because of this, a consistent fraction of the progeny of all GFP-expressing transgenic worms exhibited no GFP signal or Rol (coinjection marker) phenotype; these animals were discarded. The fraction of worms expressing intestinal GFP fluorescence and the standard error of the proportion (SEP) was determined. To avoid any artifacts due to prolonged fixation, all assays were performed on live nonanesthetized worms. $n > 75$ for all *Pins-7*::*gfp* assays.

***Psod-3*::*gfp* Assay.** The expression of *Psod-3*::*gfp* was assayed as described (14). Briefly, well fed 3-day-old adult animals grown at 20°C were mounted on 2% agarose slides (≈10 per slide). The expression of *Psod-3*::*gfp* in head muscles was assayed by using a combination of fluorescence and Nomarski microscopy.

Quantitative RT-PCR. Purification and reverse transcription of RNA were carried out as described (30). Quantitative RT-PCR was performed in a 7300 Real Time PCR System (Applied Biosystems) and analyzed by using the Ct method (Applied Biosystems Prism 7700 Users Bulletin No. 2). mRNA levels of the actin gene, *act-1*, were used as controls for normalization.

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