Minireview

Transcriptional (dys)regulation and aging in *Caenorhabditis elegans*

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Abstract

A circuit of transcription factors has been discovered in *Caenorhabditis elegans* that could provide a link between laboratory-defined intracellular 'longevity pathways', gene dysregulation and the process of normal aging.

The fact that single-gene mutations can prolong an organism's lifespan might seem unlikely, but many 'gerontogenes' have been identified in model organisms that, when knocked out or over- or underexpressed, increase or decrease lifespan in the laboratory environment. These genes largely assort into several now-familiar pathways [1,2], many of which converge on the insulin/insulin-like growth factor I (IGF-I) signaling pathway, which in *Caenorhabditis elegans* includes *daf-2*, an insulin/IGF-I receptor homolog; *age-1*, which encodes a phosphatidylinositol 3-OH kinase (PI3K) at the top of the DAF-2-activated signaling cascade; and *daf-16*, a forkhead-family transcription factor that is inactivated by this cascade. Nevertheless, it is unclear whether the activities of these 'longevity pathways' are modulated during normal aging, and as such, their role in the process of senescent decline in wild-type individuals is uncertain. Several pathways with longevity phenotypes in knockout animals may not be relevant to normal aging; these include the insulin/IGF-1 pathway, the endoplasmic reticulum stress response mediated by the sirtuin SIR-2.1, and mitochondrial electron transport [3]. Because of this, many researchers in the field suspect that aging is primarily driven by accumulation of cellular damage and not age-related gene (dys)regulation.

In a recent paper in *Cell* [3], however, Yelena Budovskaya and colleagues in the labs of Stuart Kim and Tom Johnson have identified a circuit of GATA transcription factors that alters *C. elegans* longevity when knocked out or knocked down, and which also plays a role in regulating the changes in gene expression observed during normal aging. Moreover, this circuit helps determine lifespan. One of these factors, ELT-3, is required for the pro-longevity effects of reduced insulin/IGF-I-like signaling and dietary restriction, providing at last a potential link between these longevity pathways and the normal process of aging.

Budovskaya *et al.* [3] found that expression of the GATA-family transcription factor genes *elt-5* and *elt-6*, which act during the embryonic development of the hypodermis (the nematode epidermis) [4], gradually increases during aging. The factors ELT-5 and ELT-6 act to downregulate the expression of *elt-3*, another GATA transcription factor involved in hypodermal differentiation [5,6] (Figure 1). Half of the genes found to change expression during nematode aging have conserved GATA motifs, and Budovskaya *et al.* showed that 12 out of the 14 such genes they tested are indeed under the control of *elt-3*. Moreover, RNA interference (RNAi) against *elt-5* or *elt-6* increases longevity in an *elt-3*-dependent manner, demonstrating that this pathway has causal control over at least some lifespan-determining factors. Lastly, *elt-3* RNAi largely suppresses the long-lifespan phenotype of mutations in both *daf-2* and *eat-2* animals (these mutants are deficient in feeding, and are thus a model of dietary-restriction-induced longevity), hinting that the *elt-3/elt-5/elt-6* circuit may modulate the effects of the insulin/IGF-1 pathway and calorie intake on lifespan.

Driving the aging process

To seek out potential drivers of age-related changes in gene expression, Budovskaya *et al.* [3] carried out microarray

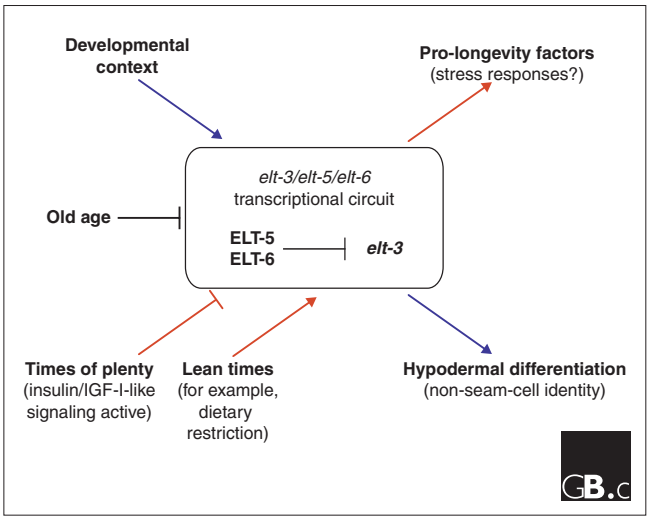

Figure 1
Crossed signals? During development, the *elt-3/elt-5/elt-6* transcriptional circuit guides cell-fate determination in the hypodermis. During adulthood, however, ELT-3 activity appears necessary for stress responses (and for the lifespan-prolonging effects of dietary restriction), and is repressed by insulin/IFG-I-like signaling. As individual *C. elegans* age, however, expression of *elt-5* and *elt-6* drifts upward. This drift reduces lifespan by decreasing the levels of ELT-3.

studies in *C. elegans* at 4, 7, 10, and 14 days of adulthood, finding 1,254 genes that change expression during aging. (By and large, gene expression decreases in older animals - a general pattern similar to that seen in human subjects [7].) Microarray studies of aging nematodes have been carried out previously (for reviews, see [8,9]), with results largely consistent with the current study. However, instead of simply categorizing the genes identified, Budovskaya *et al.* then looked for common patterns of transcriptional control. As in other work (for example, in *C. elegans* [10] and yeast [11]), computational techniques were brought to bear on the identification of DNA sequence motifs enriched in the regions upstream of genes suspected to be co-regulated. Using the CompareProspector tool, which minimizes false positives by looking for motifs with evolutionary conservation [12], the investigators found conserved GATA motifs upstream of approximately half of the genes that change expression with age.

The authors also found that GATA motifs were enriched in genes identified by previous aging microarray studies [13] and in genes identified by microarray analyses of mutations in the insulin/IGF-I pathway. Somewhat surprisingly, the overall trend that emerged from this analysis is that long-lived insulin/IGF-I pathway mutants (which have a salubrious increase in DAF-16 activity) have gene-expression patterns similar to those of old animals, whereas gene-expression patterns in *daf-16* mutants tend towards those in young animals. We hope that this, perhaps counterintuitive, result, which has now been replicated by two aging time-courses [13], will provoke future studies.

Budovskaya *et al.* [3] then selected 10 of the 14 known *C. elegans* GATA transcription factors to examine for potential longevity phenotypes. RNAi knockdowns of these yielded no longevity phenotype; however, three factors - ELT-3, EGR-1 and EGL-27 - were shown to suppress the phenotype of a *daf-2* mutant, indicating that, although their loss does not impair lifespan in lab conditions, they are required for the lifespan-prolonging effects of decreased insulin/IGF-I signaling. Furthermore, knockdown of ELT-3 also suppresses the longevity of the feeding-deficient *eat-2* mutant, suggesting a second longevity pathway that requires ELT-3. (The dietary-restriction-induced longevity of *eat-2* does not require active DAF-16 and is additive with *daf-2* mutations, indicating that these pathways are independent [14].)

The authors then examined several of the 602 age-regulated, conserved-GATA-bearing genes they had identified and confirmed that the majority of those examined were downstream targets of *elt-3*; furthermore, all genes so examined were regulated via the GATA sites in their promoter regions. Together, these data indicate that *elt-3* is indeed a regulator of several of age-related genes; further studies to establish whether *elt-3* plays a role in the regulation of the remaining age-modulated genes may prove enlightening.

Regulating the regulators

Although *elt-3* was not among the age-regulated genes identified by Budovsakaya *et al.* [3] in their microarray experiment, they found that an *elt-3::GFP* reporter shows an age-related decline in expression, indicating that perhaps the decline in *elt-3* expression is responsible for driving some portion of the age-related decline in overall gene expression - overexpression of *elt-3* may thus provide several interesting phenotypes. As a next step, the authors sought to identify the upstream regulator of *elt-3* responsible for its age-related decline. RNAi experiments showed that *age-1* (and by implication, the insulin/IGF-I pathway) exerts a negative control over the expression of *elt-3*; this regulation was independent of age, however. One theory of aging holds that senescent decline is driven by accumulation of damage from a lifetime of endogenous and environmental stressors (see, for example [15-18]); perhaps, therefore, stressors such as heat shock, oxidation, DNA damage or bacterial infection might downregulate *elt-3*? As it happens, the answer is no: Budovskaya *et al.* [3] did not find evidence for this hypothesis.

Another theory of aging notes that as organisms age toward a post-reproductive phase, the force of natural selection weakens. Thus, alleles that have beneficial early-life but detrimental late-life phenotypes can be evolutionarily advantageous, and genes that are silenced after a certain

developmental stage may become derepressed later in life [19]. During development, *elt-3*, which itself has GATA motifs in its promoter region, is regulated by several other GATA factors: *elt-1*, a positive regulator of *elt-3* [5]; and *elt-5* and *elt-6*, which are negative regulators [4]. As such, antagonistic pleiotropy theories of aging suggest that this transcriptional circuit may become dysregulated in aging animals in a way that is immune to the effects of natural selection. Indeed, Budovskaya *et al.* [3] found tantalizing evidence for age-related dysregulation of the *elt* circuit. For one thing, the expression of *elt-5* and *elt-6*, which are downregulated at the beginning of adulthood, drifts upward with time. Moreover, RNAi against these factors in adult *C. elegans* prevents much of the age-related decline in *elt-3* expression. Another piece of evidence is the fact that such RNAi substantially prolongs lifespan compared with wild-type animals, and does so in an *elt-3*-dependent manner. As expected by antagonistic pleiotropy theories of aging, RNAi against *elt-5* and *elt-6*, while lifespan-extending in older animals, is detrimental in younger ones.

Old and out of control?

What, then, drives senescence in elderly animals? Do the parts simply wear out, or is gene dysregulation to blame? Many genes in well-known longevity pathways such as insulin/IGF-I signaling, mitochondrial oxygen transport, or the response to dietary restriction seem to be involved in modulating physiological stress responses [2,20]. Indeed, the ability to mobilize an effective heat-shock response is a potent marker for the eventual longevity of an individual *C. elegans* [21]. These and other observations [15] augur well for the damage-accumulation view of aging, in which stress-response and damage repair are key to lifespan extension. On the other hand, the lack of a well-defined role for these pathways in normal aging, despite intensive investigation, is most curious. The identification by Budovskaya *et al.* of age-related transcriptional (dys)regulation, in a protein required for various lifespan-prolonging interventions, certainly suggests that antagonistic pleiotropy may play a significant part in aging.

Several observations in this work, however, suggest that these two views on aging need not be mutually exclusive. First, the authors note that the long lifespan of *daf-2* mutants (insulin/IGF-I signaling deficient) and *eat-2* mutants (feeding deficient) both require expression of *elt-3*. These pathways are generally thought to be genetically independent [14], so any common thread must be fairly far downstream, perhaps in the stress-response pathways. This suggests that future experiments to identify more closely the positions of *elt-3*, *elt-5*, and *elt-6* in these genetic pathways will be most informative. One perspective on the insulin/IGF-I pathway is that it serves to repress the stress response in times of plenty [20]; thus, repression of *elt-3* by insulin/IGF-I signaling is consistent with a role for that gene in stress responses. The similarity of gene-expression patterns in old age (after accumulation of many cellular stressors) and those in insulin/IGF-I signaling deficient mutants (with derepressed stress responses) may also be telling. (A comparison of stress-regulated and aging-related genes would be illuminating on this point.) Finally, Budovskaya *et al.* [3] observe that *elt-3*-null *C. elegans* are more sensitive to heat shock and oxidative stress than are wild-type animals, whereas *elt-5* RNAi (which increases the level of *elt-3*) causes a slight stress resistance. If *elt-3* were involved in regulating stress responses, its downregulation later in life (through dysregulation of *elt-5* and *elt-6* levels) might lead to impairment of damage repair and decreased longevity. Although theoretical arguments have suggested that antagonistic pleiotropy is most likely to appear in regulators of stress-response and damage-repair pathways [22], this work may be the first demonstration of that principle.

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