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Long-lived worms and aging

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Several investigators have generated long-lived nematode worms (*Caenorhabditis elegans*) in the past decade by mutation of genes in the organism in order to study the genetics of aging and longevity. Dozens of longevity assurance genes (LAG) that dramatically increase the longevity of this organism have been identified. All long-lived mutants of *C. elegans* are also resistant to environmental stress, such as high temperature, reactive oxygen species (ROS), and ultraviolet irradiation. Double mutations of some LAGs further extended life span up to 400%, providing more insight into cellular mechanisms that put limits on the life span of organisms. With the availability of the LAG mutants and the combined DNA microarray and RNAi technology, the understanding of actual biochemical processes that determine life span is within reach: the downstream signal transduction pathway may regulate life span by up-regulating pro-longevity genes such as those that encode antioxidant enzymes and/or stress-response proteins, and down-regulating specific life-shortening genes. Furthermore, longevity could be modified through chemical manipulation. Results from these studies further support the free radical theory of aging, suggest that the molecular mechanism of aging process may be shared in all organisms, and provide insight for therapeutic intervention in age-related diseases.

Keywords: Longevity assurance genes, nematode worms, aging

WHY WORMS?

The nematode worm *Caenorhabditis elegans* is a small free-living soil organism found world-wide. It feeds primarily on bacteria and reproduces with a life cycle of 3 days. There are two sexes, the hermaphrodite and the male. The hermaphrodite produces both oocyte and sperm and is capable of self-fertilization, whereas the male arises spontaneously at low frequencies and can fertilize the hermaphrodite. After hatching, the worm develops through 4 larval stages called L1–L4 within 40 h after fertilization. It then enters adulthood and will live for an additional 12–17 days depending on temperature and food supplies. If in sub-optimal conditions, the worm may enter an alternative developmental state after

the L2 larval stage, called the dauer stage. The dauer larva does not feed, and can survive up to 3 months without further development. When conditions improve, the larva resumes normal development, enters the L4 stage and continues to mature into a normal adult.¹

Because of its small size (the adult is 1 mm long), fast reproduction (it takes 3 days from fertilization to the onset of reproduction), short life span (about 20 days at 20°C), and easy storage (the worm can be frozen indefinitely without phenotypic changes), numerous mutagenesis techniques have been employed to find mutations that could increase the worm's longevity. More than 50 genes have been identified by this means, in which altered gene activity either increases longevity or accelerates aging. Thanks to the worm's capability of taking up RNAi directly from their food, the specific downstream regulators of the gerontogenes have been identified.² Although other organisms such as yeasts, *Drosophila* and mice have been used in studies of the genetics of aging, *C. elegans* has become a successful model organism for studying molecular mechanisms involved in aging processes.³

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Table 1. Some representative LAGs found in *Caenorhabditis elegans*

Strains	Increased life span	Molecular basis or gene function	References
<i>clk-1</i>	60%	CoQ7 (energy metabolism or a biological clock)	8
<i>age-1</i>	65%	PI-3 kinase (signal transduction)	6
<i>daf-2</i>	100%	Insulin receptor family, upstream of PI-3 kinase (signal transduction)	5
<i>daf-23</i>	> 100%	PI-3 kinase (signal transduction)	6
<i>old-1</i>	60%	PDK 1	41
<i>daf-16</i>	30%	Fork head transcription factor	42
<i>daf-12</i>	100%	Ste receptor (transcription factor)	43
<i>eat-1</i>	33%	Unc-1, feeding defect	11
<i>eat-2</i>	36–57%	Unc-7, slow pumping rate	44
<i>daf-2 + age-1</i>	65–100%	Same pathway	7
<i>daf-2 + clk-1</i>	500%	Insulin signal transduction and slow living	10
<i>daf-2 + eat-2</i>	200%	Insulin signal transduction and feeding defect	45

LONGEVITY ASSURANCE GENES (LAGS)

Single-gene mutations with forward genetics of more than a dozen different genes have been described that can prolong the worm's life span (Table 1).⁴ These strains include *age-1*, *daf-2*, *clk-1*, *eat-2*, *old-1*, and a combined double mutant *daf-2/clk-1*. The *age-1* and *daf-1* mutations, both discovered by Ruvkun's research group in Boston, cause a constitutive dauer phenotype.^{5,6} Thus, greater longevity is due to delay in developmental stages or a decrease in metabolism. *Daf-2* encodes an insulin receptor-like gene,⁵ and functions in a common pathway with *age-1*.⁷ *Clk-1*, named after 'biological clock', was discovered by Hekimi and colleagues in Montreal from a screen for maternal-effect mutations affecting worm development.⁸ This mutant has a 'slow rate of living' with the lengthened early embryonic cell cycle, embryonic and post-embryonic development as well as the period of rhythmic adult behaviors.⁹ These researchers further demonstrated that two genes (*daf-2/clk-1*) in combination allow the nematodes to live 5 times their normal life span,¹⁰ which is equivalent to people capable of living more than 400 years. The explanation is that the *clk-1* mutant acts on a completely separate pathway from the *daf-2* and *age-1* mutants. The *clk-1* mutation appears to affect energy metabolism or a biological clock, whereas the *daf-2* and *age-1* mutations affect an insulin-like growth factor (IGF) receptor signaling cascade that controls entrance into an alternative developmental pathway. Thus, the most dramatic increase in life span was observed in the double mutation of *clk-1* and *daf-2*. In contrast, the *age-1/daf-2* double mutant did not live longer than mutants that carry only a *daf-2* or *age-1* mutation, confirming that these two genes do function in the same signal transduction pathway.⁷ Among 50 LAGs identified, the insulin/IGF-like signaling pathway (involving *daf-2*, *daf-16* and *age-1*) is the best characterized.

Mutations in many *eat* genes were studied under the assumption that a decrease in food intake would extend the worm's life span. It is a well-known fact that reducing the caloric intake of rodents can significantly extend their life span. It turned out that all alleles of *eat-2* significantly increased life span. These *eat-2* mutants have a phenotype similar to that of *unc* genes, which cause feeding defects.¹¹ In the worms, the insulin-signaling pathway comes into play when organisms are threatened with starvation. They would become dauer, which is a dormant stage where they can survive for much longer than their normal life span. It was calculated that the average life span of humans could be boosted from 76 to 120 years if people adopted an extremely low calorie diet,¹² but this approach does not seem to be realistic.

The silenced information regulator gene (*SIR2*) provided another link between metabolism and aging. Guarente of Massachusetts Institute of Technology studied *SIR2* and its ability to slow the aging process in yeast. His group found that the enzyme co-factor NAD and the *SIR2* gene are required for slowing down aging by reduction in calories.¹³ They then inserted extra copy of the *SIR2* gene into the nematode larvae, and the worms lived 75% longer than their wild-type partners.¹⁴ Sir2 protein appears to couple longevity to nutrient availability, or energy status of the cells in a variety of organisms.¹⁵ This provided a stimulus for seeking a genetic manipulation that would increase the activity of Sir2-like protein without caloric restriction in humans, *i.e.* to trick the cells of the body into thinking that they are caloric restricted while allowing individuals to eat all the food they want. This approach showed promise recently in yeast and in *C. elegans*.¹⁶

Among all the LAGs found in the nematode mutants, there are three groups that appear to be helpful in understanding the biological determination in aging: (i) LAGs that involve the signal transduction pathway which regulates the worms development; (ii) LAGs that slow the

organism's metabolic rate; and (iii) LAGs which prevent the worm from normal feeding. These accomplishments, as well as the successes to extend the life span of variety of fruit flies and mice, have provided biologists with valuable insights into the mysteries of aging.

FREE RADICAL THEORY OF AGING

Increasingly, the toxic effects of reactive oxygen species (ROS) have been implicated in aging and degenerative diseases.¹⁷ The free radical theory of aging states that ROS, by-products of cellular respiration metabolizing oxygen, can react with and damage physiologically important macromolecules. All organisms have evolved mechanisms to prevent accumulation of the oxidative damage. When these anti-damage defenses lose efficiency due to aging, cells are chronically injured, and the accumulated damage is manifested as generalized alterations observed in aging.¹⁸

The involvement of ROS in shortening of the life span has been suggested by analyses of *C. elegans* mutants. The *age-1* mutant, which lives over 50% longer than those of the wild-type animal N2, exhibits elevated catalase and superoxide dismutase (SOD) activities,¹⁹ while SOD activity of the *mev-1* mutant, which has about 30% shorter life-span than the wild-type, is half that of the wild-type animal.²⁰ It is, therefore, conceivable that the levels of anti-oxidative defense are responsible for the difference in the life span of the mutant and the wild-type animals. Adachi found an excellent correlation between the protein carbonyl content, a measurement for oxidatively damaged protein, of whole body extract and the survival rate during the life of *age-1*, wild-type N2 and *mev-1* animals: the higher the increase in carbonyl content, the shorter the life span.²¹ Consistent with their observations, we²² recently demonstrated an increased H₂O₂-related ROS levels in a transgenic *C. elegans* model of Alzheimer's disease.²³

It has been found that all long-lived mutants of *C. elegans* are also resistant to some variety of environmental stress, such as high temperature, ROS, and UV irradiation.²⁴ Johnson and colleagues have identified genes that not only double the life span of the worm but also provide increased resistance to the damaging effect of high temperature, UV radiation and oxygen metabolism.²⁵ Several of the genes appear to accomplish this by lowering the worm's metabolic rate. The lowered metabolism may reduce aging via reducing cellular damage from oxygen metabolism. Austad and colleagues believe that extension of life could be a general by-product of an organism's response to environmental stress.²⁶ It is well-known that reducing the caloric intake of rodent can lengthen their life span up to 50%²⁷ which was then reproduced in monkeys: caloric restricted monkeys appeared healthier and less likely to develop age-related diseases.²⁸

The positive correlation between stress resistance and life span of *C. elegans*²⁹ prompted many research groups to explore the possibility of extending life span through prevention of oxidative damage. Successful approaches include manipulation of genes that increase an organism's resistance to environmental stresses, particularly the free radicals of oxygen, molecules containing unpaired electrons that cause irreparable damage to cells.

Using chemical mimetics of superoxide dismutase and catalase, which work together to break down free radicals, Lothgow and co-workers have been able to produce worms that live longer.³⁰ Ishii and colleagues directly fed the worms with the known antioxidant vitamin E and observed positive effects on extension of the life span of *C. elegans*.³¹ Larsen and Clarke found extension of life span in *C. elegans* by a diet lacking coenzyme Q, a redox-active lipid that generates free radicals.³² Administration of a radical-trapping agent also prevented age-related oxidative damage and impaired short-term memory in mammals.³³ A recent report indicates that resveratrol, a polyphenol abundant in red wine, increased life span in yeast and *C. elegans* via direct activation of *Sir2*.¹⁶

My laboratory has been interested in the neuroprotective mechanism of EGb 761, a standardized extract from *Ginkgo biloba* leaves. The *Ginkgo biloba* tree has a long life span of more than 4000 years and its leaves are known for their resistance to infection and diseases.³⁴ EGb 761 has been shown to have beneficial effects on age-related brain function, presumably due to its anti-oxidative properties. Using the *C. elegans* model, we have demonstrated that EGb 761 increases the worm's resistance to heat, or a pro-oxidant-induced stress, and extend their mean life span,³⁵ consistent with the effect of EGb 761 on cognitive behavior and longevity observed in rats.³⁶

We further demonstrated the molecular mechanism of EGb 761 on alleviating effects of oxidative stress by using transgenic *C. elegans* expressing jellyfish green fluorescent protein (GFP)-tagged inducible small heat shock protein gene (*hsp-16-2*). *hsp-16-2* belongs to a family of low molecular weight polypeptides (12–43 kDa) that have been highly conserved from yeasts through to humans. In *C. elegans*, *hsp 16-2* is only expressed under stress conditions.³⁷ We found that the expression of *hsp-16-2* induced by a pro-oxidant, juglone, and by heat shock was significantly suppressed in the nematodes fed with EGb 761.³⁸ We speculate that in the presence of EGb 761 fewer radicals are around, and fewer proteins are damaged, which makes expression of *hsp-16-2* unnecessary.

The role of amyloid- β peptide in the free-radical oxidative-stress model of neurotoxicity in Alzheimer's

disease has received much attention recently. We employed a transgenic *C. elegans* constitutively expressing human amyloid- β peptide to test the hypothesis of the involvement of amyloid- β peptide and ROS in association with Alzheimer's disease. A rise in levels of hydrogen peroxide (H_2O_2) was observed in Alzheimer's disease-associated transgenic worms compared with the wild-type controls. Feeding the *C. elegans* with EGb 761 significantly attenuated the basal as well as the induced levels of H_2O_2 -related reactive oxygen species (ROS). Among individual EGb 761 components tested, kaempferol and quercetin provided maximum attenuation in this model.²²

Taken together, at a molecular level, almost all the fundamental components involved in neurodegeneration and aging are present in the nematode, including proteins and enzymes of the endogenous antioxidant system. The genetic and cellular features of apoptotic cell death have been well described in *C. elegans*,^{39,40} thus providing a good system to evaluate pharmacological intervention and the drugs' mode of action.³⁰ The ease of culturing *C. elegans* and the availability of transgenic strains makes this nematode an ideal model for evaluation of LAGs and potential drugs affecting aging the degenerative diseases.

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