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Caenorhabditis elegans 2007: The Premier Model for the Study of Aging

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Abstract

This is the 25th anniversary of the discovery of extended longevity mutants in *Caenorhabditis elegans*. About one hundred papers describing results from studies on *C. elegans* in aging research appeared this year. Many themes were pursued including dietary restriction, *daf-9* action, the role of proteolysis and autophagy, and the continued search for more Age mutants. I use the word "modulate" not "regulate" so as to be consistent with the evolutionary theory of aging, which is also consistent with the empirical findings of all extended-longevity (Age) mutants. These Age mutants universally result from deficits in known physiologic systems, rather than in some process designed to kill the animal in old age.

Keywords

Genetics of aging; Aging models; Inflammation; Immune Response; Dietary Restriction; Protein Synthesis; Drugs; Longevity Mutants

This year marks the 25th anniversary of the use of extended longevity mutants of *Caenorhabditis elegans* to identify processes involved in aging of this species (Johnson and Wood, 1982). About one hundred papers appeared this year, far more than we can review in detail here. Many themes were pursued and will be highlighted below. These include dietary restriction, *daf-9* action, the role of proteolysis and autophagy, and the continued search for more Age mutants. Note that I use the word "modulate" not "regulate" so as to be consistent with the evolutionary theory of aging and the empirical findings of many extended-longevity mutants, which show that these mutants all result from deficits in known physiologic systems, rather than in some process designed to kill the animal in old age.

Dietary restriction (DR)

As in many other species, dietary restriction (in *C. elegans* this means reduced bacterial food concentration), the use of an Eat mutant which ingests less food, or the use of axenic (no living organisms) prolongs life. Several studies have examined the basis of DR. In perhaps one of the most bizarre series of studies, and in this case carried out by at least three independent labs, it was shown that even complete food deprivation (starvation) can lead to significant life extension (Cypser et al., 2006; Lee et al, 2006; Kaeberlein et al., 2007). Cypser et al reviewed a series of experiments on hormesis and its dependence on genes in the insulin-signaling pathway, which they had published over the years. Most of these hormetic experiments dealt

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with environmental stressors such as heat or reactive oxidants but Cypser et al. also found that starvation led to life extension of 30–40% that was abrogated somewhat in an *age-1* background, which they took to show that the pathways may partially overlap.

Kaeberlein et al. (2007) showed that complete starvation of worms after the eighth day of adult life resulted in about a 50% life extension and almost a doubling of thermotolerance. This was independent of DAF-16 and DAF-2, in that *daf-16* mutants shortened the DR lifespan and *daf-2* mutants showed synergistic effects. Similarly, *sir-2* deletions had no effect on longevity in either a fed or DR state. In contrast, in EAT-2, starvation led to no greater increase than N2, suggesting that the Eat-2 pathway is utilized by starvation. Lee et al (2006) found similar results for these mutants but performed a more extensive series of analyses and also studied the relationship between progeny production and DR-increased longevity, concluding that there is some trade-off.

Bishop and Guarente (2007) replicated much of the work mentioned above in their own bacteria-limiting culture conditions and went on to trace the locus of control to the SKN-1 transcription factor acting in the two ASI neuronal cells, a requirement for life extension, as mediated by reduced food concentrations. This demonstrates a central neuronal function that is somehow communicated to the periphery via a proposed neuroendocrine function. They also obtained data suggesting that the increased respiration seen in a few studies of DR, might underlie DR effects. The increased respiration was dependent on SKN-1 and was blocked by mitochondrial respiration inhibitors.

Lenaerts et al (2007) examined axenic culture, finding reduced exponential increase of age-specific mortality. Life span was largely independent of nutritional status during development. Panowski et al (2007) found that the PHA-4 transcription factor (orthologous to the mammalian family of Foxa transcription factors and essential for embryonic development of the foregut) also regulates glucagon production and glucose homeostasis in adulthood, particularly in response to fasting. They describe a role for PHA-4 in lifespan determination that is specific for dietary restriction in *C. elegans.* PHA-4 is not required for the increased longevity caused by other genetic pathways that modulate aging.

Sirtuins have been implicated in the regulation of molecular mechanisms of aging (Trapp and Young, 2006). The overexpression of sirtuins leads to an increase of lifespan in *Saccharomyces cerevisiae* and *C. elegans* that can also be reached by calorie restriction.

Autophagy

C. elegans activates its autophagic system during dauer development, presumably as a way to recycle limited components needed to allow the formation of the dauer, under conditions of limited food availability. Morck and Pilon (2007) demonstrated increased autophagic action as well as smaller cell size in feeding-defective mutants. Hars et al (2007) followed up on studies where RNAi-mediated knock-down of *bec-1*, a key gene in autophagy, shortened the long life of *daf-2* mutants. Here they knocked down two other autophagy genes, *atg-7* and *atg-12* and showed that RNAi shortened the lifespan of both wild type and *daf-2*, although not very much. Although they claim strong support for autophagy in the “regulation” of aging, it seems possible or even probable that inhibiting autophagy shortens life span via mechanisms not necessarily involved in aging itself. This is a problem inherent in looking at shorter lifespan without other end points also being examined simultaneously.

The regulation of protein aggregation

Several labs have followed up on the seminal studies of protein aggregation by Morimoto and colleagues and human Abeta (1-42) by the Link lab (Brignull et al., 2007; Link 2006). Most

important is work by Cohen et al (2006) who used the worm expressing the human Abeta (1-42) amyloid peptide in its muscle cells and found that well-studied mutations (especially *daf*-2) in the insulin/IGF-1 pathway promote increased survival and decreased paralysis that is characteristic of Abeta expression in the muscle cells. These phenotypes were opposed by HSF-1 action. However, these studies involved RNAi and in some cases double RNAi treatment, which have proved to be of uncertain efficacy, making it unclear as to whether the rather confusing results with HSF-1 and DAF-16 are real; unfortunately, nuclear mutations were not used. The authors concluded that this pathway could play a role in preventing aggregation of other toxic proteins (huntingtin), as well and suggested that these protective mechanisms may link longevity to protein homeostasis.

Steroid action in specifying longevity

The Antebi lab (Gerisch et al., 2007) has been studying endogenous DAF-12 ligands (3-keto bile acid-like steroids), called dafachronic acids. These compounds rescue hormone-deficient mutants, such as *daf-9* (cytochrome P450) and *daf-36* (Rieske oxygenase), and activate DAF-12. They examined the effect of dafachronic acid on pathways modulating lifespan and found that dafachronic acid shortened the lifespan and lowered the stress resistance of long-lived *daf-9* mutants. However, this “proaging” activity in the dauer pathways is opposite to its antiaging activity in germ-line ablated *daf-9* and *daf-36* mutants. Thus, dafachronic acid modulates *C. elegans* lifespan differentially depending on the signaling system being studied.

The Baulieu lab (Broue et al., 2007) resurrected studies on steroids in the worm examining dozens of steroids some of which are also present in humans. Pregnenolone (3beta-hydroxy-pregn-5-en-20-one; PREG) and other pregnane and androstane derivatives are being studied. Germline ablation (which extends lifespan) cause PREG levels to rise in a *daf-9* -dependent fashion. They found that PREG could slightly extend the lifespan and conclude that “PREG extends the lifespan of germline-defective *daf-9* mutants dramatically, but has no effect on *daf-12* mutants. Thus, germline removal may extend lifespan, at least in part, by stimulating the synthesis of PREG.”

The search for more longevity genes

As in the past, the search for mutants and RNAi targets that can lead to a longer than normal life span has occupied many labs, utilizing a variety of new targets and models. In a few recent very comprehensive reviews (Henderson et al., 2005; Tissenbaum and Johnson, 2007), we have emphasized the use of the word “modulation” rather than “regulation” because it is value free, not carrying the same intellectual associations inherent in the word “regulation”. Similar points were made by Lithgow (2006) and were highlighted in a debate at the recent 2007 annual meeting of the American Aging Association. Despite this, a number of labs continue to use this approbation, in part because it is favored by the Editors of the major journals where these papers continue to be published and highly cited, and this year is no exception.

Two groups utilized evolutionary theory to identify new gerontogenes. Chen et al. (2007) identified 57 genes known to be essential, causing developmental arrest after RNAi. Amazingly, 24 of these extended life span when inactivated during adulthood. As stated by the authors: “Many of these genes are involved in regulation of mRNA translation and mitochondrial functions. Genetic epistasis experiments indicate that the mechanisms of lifespan extension by inactivating the identified genes may be different from those of the insulin/insulin-like growth factor 1 (IGF-1) and dietary restriction pathways. Inhibition of many of these genes also results in increased stress resistance and decreased fecundity, suggesting that they may mediate the trade-offs between somatic maintenance and reproduction.” Similarly, Curran and Ruvkun (2007) screened highly conserved genes necessary for growth and development and found 64 genes that extend lifespan when

inactivated post-developmentally. These genes included insulin and metabolic pathways but also revealed enrichment for translation, RNA, and chromatin factors. DAF-16 seemed to be required for many of these.

Hansen et al (2007) carried out a systematic analysis of conditions that lead to inhibition of protein synthesis and found that many of these lead to life extension. These include reducing levels of specific ribosomal proteins or initiation factors and inhibition of Tor, which has previously been shown to lead to life extension. Although both DAF-16 dependent and independent pathways were identified, both seemed to involve increased resistance to stress, consistent with models suggesting that increased longevity results from increased repair and resistance to the pathologic insults associated with aging. Similarly, Pan et al (2007) showed that mRNA translation exerts pleiotropic effects on growth, reproduction, stress resistance and lifespan in *C. elegans*. The Tavernarakis lab (Syntichaki et al., 2007) showed that loss of a specific eIF4E isoform (IFE-2) functions in somatic tissues, reduces global protein synthesis, protects from oxidative stress and extends lifespan (the eukaryotic initiation factor 4E (eIF4E) is a principal regulator of protein synthesis). Lifespan extension is independent of the forkhead transcription factor DAF-16. In addition, IFE-2 deficiency further extends the lifespan of long-lived Age, Daf, Clk, and Eat mutants. Knockdown of TOR further increases the longevity of *ife-2* mutants. Thus, signaling via eIF4E in the soma is a newly discovered pathway influencing aging in *C. elegans*.

Stochastic Effects on Aging

Two papers from the Melov lab (Golden et al., 2006; Golden and Melov, 2007) continued to explore the stochastic element determining individual life span. Since isogenic populations of *C. elegans* still show a huge amount of variation in time of individual death, this remains a very interesting and largely unexplored aspect of aging research. They demonstrated both a systematic loss of nuclear DNA, as well as dramatic age-related changes in nuclear genome copy number. These changes are delayed or attenuated in long-lived *daf-2* mutants.

There are now upwards of three hundred published alterations (many of them driven by RNAi) in “The Worm” that lead to life extension and there are apparently hundreds of Age genes that have not yet been published. One of the BIG questions must be: “How can so many genes modulate aging?” It may be that the very lack of evolutionary selection for aging has made it very easy to modulate the aging processes. Moreover, the lack of one central process governing aging may mean that each organ system, perhaps even each cell establishes its own local aging identity and that many of these influence longevity. The lack of these mutants in other systems, especially Drosophila, could be primarily due to inbreeding depression which makes it very difficult to do quantitative analyses on life-history traits (Johnson and Wood, 1982). Twenty-five years into it, the 2006/2007 period remains an important time in continuing to establish *C. elegans* as the premier organism in which to do aging research.

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