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Cynthia Kenyon's Lecture Part 1:
Genes that Control Aging

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Contents

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1. Keywords and Terms

Aging; longevity; *C. elegans*; DAF-2; insulin/IGF-1 receptor; DAF-16/FOXO; caloric restriction; dauer; stress resistance; antioxidants; chaperones; antimicrobials; regulatory pathways

2. Lecture Notes

Introduction

For many years, aging was thought to be a random, entropic process. Biologists hypothesized that damage accumulated in an unregulated manner in the cells of an organism, leading to its eventual death. But if this is the case, why is it that animals

such as mice, bats, and squirrels, which are of similar size and can live in similar environments, have very different lifespans? While mice rarely live longer than four years even under protected laboratory conditions, the Eastern Grey Squirrel can live up to 24 years and the longest lived bat species, *Myotis brandtii* can survive up to 41 years in the wild. Within the past two decades, research from many labs has led to a new understanding of aging. This research utilized model organisms such as the nematode *Caenorhabditis elegans,*the yeast *Saccharomyces cerevisiae*, and the fruit fly *Drosophila melanogaster* to show that even single gene mutations can dramatically affect lifespan. Our lab has used *C. elegans* to study how genes regulate aging. Many biological mechanisms controlling other aspects of biology are conserved from *C. elegans* to mammals and thus our discoveries may be extrapolated to other organisms as well.

The DAF-2/insulin/IGF-1 receptor

We found that mutation of a single gene, called *daf-2*, can double the lifespan of *C. elegans*. *daf-2*mutants appear healthy and normal when young, just like wild-type animals. However, at day 13 of adulthood (very old for *C. elegans*), *daf-2* mutants are still healthy and still look young! This is like a 90-year-old human looking like a 45-year-old.

The *daf-2* gene was cloned in Gary Ruvkun's laboratory and found to encode a hormone receptor. Since a mutation in *daf-2* leads to increased longevity, we know that at least one of the normal functions of *daf-2* is to promote or speed up aging. The DAF-2 receptor is similar to two known hormone receptors found in humans and other mammals:

1. Insulin receptor, which binds to insulin and promotes nutrient uptake into tissues after a meal.
2. IGF-1 receptor, which promotes growth.

Now the big question was, do these receptors and their hormones speed up aging in other organisms besides *C. elegans*? The short answer: yes!

1. Fruit flies (*Drosophila melanogaster*): mutations in the insulin/IGF-1 receptor as well as other proteins in the same molecular pathway can extend lifespan.
2. Mice (*Mus musculus*): IGF-1 receptor heterozygotes (i.e. they have only one good gene copy) live 20% longer than wild-type mice; Mice missing the insulin receptor in their fat tissue are also long-lived (and don't get fat on a high-fat diet).
3. Dogs: small dogs have a mutation in IGF-1 and live longer than large dogs that don't have this polymorphism.
4. Humans: Very recently, DNA variants in a human *daf-16*-like gene, called FOXO3A, have been associated with exceptional longevity in human cohorts all around the world.

Can longevity and size be uncoupled? Does an animal have to be small to be long-lived? No, it appears that a slight reduction in insulin/IGF-1 signaling can increase lifespan without decreasing size. *C. elegans* with mutations in *daf-2* are not small and neither are long-lived flies with modest reduction in insulin/IGF-1 signaling activity. Mice with insulin receptor mutations aren't small and the long-lived IGF-1 heterozygote mice are only slightly smaller than wild-type.

Since its effects on longevity and size can be uncoupled, perhaps the insulin/IGF-1 receptor works at different times during the life of an animal to control or regulate different processes. We wanted to find out when the *daf-2* gene is needed to control aging in *C. elegans*. We did this by turning down the activity of the gene with RNAi treatment. If *C. elegans* are exposed to RNAi for their whole lives, they live long. If *C. elegans* are subjected to RNAi only during adulthood, they still live long. However, loss of *daf-2* function during development only, does not increase lifespan. The conclusion: *daf-2* acts during adulthood to regulate aging.

How does insulin/IGF-1 affect aging?

When a hormone is bound to the DAF-2 receptor, it activates a kinase cascade, resulting in the phosphorylation of the DAF-16 transcription factor. When this happens, DAF-16, which is a transcription factor required for *daf-2* mutants to live long, is excluded from the nucleus. However, if the DAF-2 receptor or any of the components of the kinase cascade are mutated, DAF-16 is no longer phosphorylated and can thus

accumulate in the nucleus. Here, it regulates the transcription of many genes including antioxidant, chaperone, antimicrobial and metabolic genes.

To find out if these genes are important for lifespan, we used RNAi treatment. In this way, we could turn down the activity of individual genes and assess their impact on lifespan. We found that inhibiting the activity of genes positively regulated by DAF-16 shortens the lifespan of long-lived *daf-2* mutants. Likewise, turning down activity of negatively regulated genes lengthens lifespan in wild-type animals. These effects seem to be additive or cumulative. For example, RNAi of a gene positively regulated by DAF-16 shortens lifespan in *daf-2* mutants, but does not shorten it as much as RNAi of *daf-16* itself. This pathway can be likened to an orchestra. The individual genes represent the instruments while DAF-16 is like the conductor. DAF-16 orchestrates the activity of all these individual genes, which then work in concert to regulate aging.

What does it all mean?

Why should inhibiting genes necessary for growth and food storage extend lifespan? After all, failure to produce insulin can lead to diabetes in humans while mice (and flies and worms) without any insulin receptors die before birth. We hypothesize that when you lower the level of insulin/IGF-1 signaling, the metabolism of the organism shifts from growth and food intake to cellular maintenance and stress resistance. Indeed, long-lived *daf-2* mutants are resistant to many environmental stresses like ultra-violet light, heat, oxidative stress, toxins, and pathogens.

Why should this evolve? Why aren't all animals long-lived and resistant to environmental stresses? One hypothesis is that there are advantages to getting old. For example, aging may have evolved to eliminate parent-progeny competition. Another possibility is that these pathways evolved to allow the animal to survive harsh environmental conditions. In *C. elegans* we now know that the DAF-2/insulin/IGF-1 signaling pathway not only has effects on aging in the adult, but also much earlier, before puberty, on entry into a larval stage called dauer; if *daf-2* activity is turned down slightly, *C. elegans* are long-lived, but if activity is lowered almost completely, *C. elegans* will enter the dauer stage before it reaches adulthood. This stage allows animals to survive for long periods of time in harsh environments with little food. Thus, it seems that *daf-2* has two functions: during development it regulates entry into the dauer stage while during adulthood it regulates aging. This lifespan module may have evolved not to regulate aging *per se*, but to allow animals to survive harsh conditions, and survive until conditions become better suited for development and reproduction.

Because dauer formation protects an animal from environmental stress *before* reproduction, its evolution may have been favored by natural selection.

*A note on *C. elegans* nomenclature: genes are written italicized, in lower-case letters, for example, *daf-2*. When we are talking about proteins we use capital letters, for example, DAF-2.

3. Recommended Reading

1. Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A *C. elegans* mutant that lives twice as long as wild type. **Nature** *366*, 461-464.
2. Riddle D., Blumenthal T., Meyer B., Priess, R. (1997). ***C. elegans* II**. Chapter 28: Environmental factors and gene activities that influence life span. (http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=ce2)

More advanced basic reading

1. Tatar, M., Bartke, A., and Antebi, A. (2003). The endocrine regulation of aging by insulin-like signals. **Science** *299*, 1346-1351.
2. Dillin, A., Crawford, D.K., and Kenyon, C. (2002). Timing requirements for insulin/IGF-1 signaling in C. elegans. **Science** *298*, 830-834.
3. Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., and Kenyon, C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. **Nature** *424*, 277-283.
4. Kenyon, C. (2005). The plasticity of aging: insights from long-lived mutants. **Cell** *120*, 449-460.
5. Libina, N., Berman, J.R., and Kenyon, C. (2003). Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. **Cell** *115*, 489-502.
6. Wolkow, C.A., Kimura, K.D., Lee, M.S., and Ruvkun, G. (2000). Regulation of C. elegans life-span by insulinlike signaling in the nervous system. **Science** *290*, 147-150.(Wolkow et al., 2000).

4. Review Questions

1. Why do we use *C. elegans* to study aging?
2. How can we change genes in *C. elegans*? How can we look for gene expression changes in *C. elegans*?
3. What evidence is there that aging regulation by the insulin/IGF-1 pathway is conserved across species?
4. How does the DAF-2/insulin/IGF-1 signaling pathway work?
5. What sorts of genes are regulated by the DAF-16 transcription factor?
6. When does daf-2 act to regulate aging in *C. elegans*?
7. What processes besides aging are regulated by the DAF-2 pathway in *C. elegans*?
8. What environmental signal, mentioned in the lecture, affects aging?

5. Answers to Review Questions

1. There are several reasons including two that were mentioned in the lecture. First, they are relatively short-lived, with a mean lifespan of ~19 days. Second, many genes and biological processes are conserved between *C. elegans* and "higher" organisms, so discoveries made with *C. elegans* are likely to be relevant to mammals and humans. Some reasons not mentioned are that they are very easy to grow, their biology is well characterized, and many powerful genetic techniques for gene discovery and analysis are available.

2. As mentioned in the lecture, we can use RNAi to knock down gene activity. This method is very easy in the worm as we can simply feed *C. elegans* bacteria that express dsRNA for the gene of interest. We can also introduce mutations into *C. elegans* by exposing animals to chemical mutagens. To look for gene expression changes, we can use DNA microarrays. This method was used to discover which genes are transcriptionally regulated by DAF-16.

3. daf-2 homologs in fruit flies, mice have been shown to regulate aging. Also, although not mentioned in the lecture, there are new data showing that polymorphisms of the

human daf-16 homolog (called FOXO3A) are associated with extreme longevity in people.

4. Hormones bind to the DAF-2/insulin/IGF-1 receptors and activate a kinase cascade. This results in phosphorylation of the DAF-16 transcription factor, thus excluding it from the nucleus. When the kinase cascade is less active (either because of mutation or less circulating hormones), DAF-16 is not phosphorylated and can accumulate in the nucleus to regulate gene transcription.

5. Antioxidants, chaperones, antimicrobial genes, metabolic genes (and others – see Murphy et al., 2003).

6. daf-2 is needed during adulthood to regulate aging.

7. The DAF-2 pathway also acts during development to regulate entry into the dauer stage. This is an alternative larval stage that *C. elegans* can enter to survive periods of harsh environmental stress.

8. Dietary intake – caloric restriction can extend lifespan in *C. elegans*, as well as in yeast, fruit flies, mice, rats, and many more organisms.

6. Discussion Questions

1. Why are *daf-2* mutants resistant to environmental stresses and what are the implications of this resistance on lifespan?
2. Why is it important that long-lived *daf-2* mutants are healthy at both young and old ages? If the importance of insulin signaling in aging is translated across species, what are the implications for how it might influence “healthspan” in humans? How might studying aging be important for disease treatment?
3. Two possible reasons for the evolution of aging were mentioned in the lecture. What were they and what other possible reasons can you think of?

7. Answers to Discussion Questions

1. daf-2 mutations result in a more active form of DAF-16. This transcription factor regulates the expression of many genes including a number encoding antioxidants, chaperones, and antimicrobials. These molecules are important for general cell maintenance and resistance to environmental insults such as oxidative stress and bacterial pathogens. Now, not only will daf-2 mutants be better able to cope with stresses that they encounter in their environment, but they may also be able to alleviate the effects of reactive oxygen species and other stresses produced in normal cellular reactions. Better coping mechanisms for both internal and external stressors might allow an animal to stay healthy and survive longer.

2. One could imagine that perturbation of any gene in an animal may result in deleterious effects. After all, organisms evolved "wild-type" copies of these genes for a reason. Thus, it may follow, that gene mutations that extend lifespan, may also have deleterious effects on other aspects of the organism's biology. In other words, trade-offs would exist between extreme longevity and biological function. In fact, there are instances where we do observe this; some long-lived mutants are lethargic, suggesting a trade-off between longevity and metabolic rate. Another example is that some long-lived mutants have fewer progeny, suggesting a trade-off between longevity and reproduction. But, if a mutant (such as the daf-2 mutant shown in the video) is long-lived and active throughout life and has just as many progeny as wild type, this suggests that extreme longevity and quiescence can be uncoupled. Also, just think: if life-extending mutation that occurred during evolution were associated with a trade-off, then it is hard to see how talented, long-lived organisms like ourselves could have evolved.

   The fact that long-lived mutants remain healthy even into old age may be important for the treatment of age-related disease. Age is the biggest risk factor for many diseases, including cancer, heart disease, and neurodegenerative disease. Not only does the daf-2 mutation in *C. elegans* cause extended lifespan, but it also regulates resistance to some diseases (discussed further in lecture 2). If the insulin/IGF-1 pathway influences disease risk, studying its mechanisms of action may be very important, not so much for extending lifespan, but for extending healthspan.

Investigating pathways that control aging will allow us to better understand age-related diseases as well.

3. One reason aging may have evolved is to eliminate competition between adults and their progeny. In many organisms, there is little to no parental care for progeny. In this case, having both adults and young around at the same time might lead to increased competition for limited resources. *C. elegans*, for example, have a very short lifespan and do not care for their young. Conversely, long post-reproductive lifespans (such as in humans) may evolve due to the benefits of extended parental care.

   Another reason mentioned in the lecture is that aging regulatory pathways may have evolved to regulate responses to environmental stress. A good example is the ability of the DAF-2/DAF-16 pathway to regulate entry into a diapause state during development. This stage, called dauer in *C. elegans*, allows the animal to survive harsh conditions. Like long-lived mutants, dauers are resistant to many environmental stresses. One theory of evolution posits that investments in maintenance and repair of somatic tissue will only be good enough to keep the body going and able to produce progeny through the normal expectation of life in the wild. If extrinsic mortality is high, for example as a result of predation, spare resources will go toward reproduction and not somatic maintenance. Similarly, if conditions are not optimal for reproduction, genes that extend lifespan and delay reproduction will be favored.

   A few other theories not mentioned in the lecture: 1) Aging might evolve from the inability of natural selection to maintain survival and fecundity at later ages in the face of mutation pressure. In other words, because organisms usually die at young ages from extrinsic causes, they are most likely to reproduce during youth. Thus, genes that are beneficial early in life are favored over those beneficial later in life and furthermore, there is little selection pressure against mutations that cause deleterious effects late in life. 2) Natural selection might allow for a trade-off between youthful fitness and a subsequent rate of aging. This theory postulates that genes with beneficial effects during youth will be favored even if they have deleterious effects later in life.

   These theories are possible explanations for the evolution of aging. However, none have been thoroughly tested or proven. It would be interesting to discuss the

possible value/significance of these theories and to try and find exceptions (e.g. long-lived animals that invest little parental care in their young) for each of them.

8. Explain or Teach These Concepts to a Friend

1. Explain the lifecycle of *C. elegans* and how its regulation is linked to aging.
2. Explain how reducing the activity of *daf-2* leads to increased lifespan.
3. Explain the experiments done to figure out the timing requirements for *daf-2* regulation of lifespan.

9. Research the Literature on Your Own

1. Differences in lifespan between species were alluded to at the beginning of the lecture. What can we learn from comparing species that have vastly different lifespans? (Hint: Search the literature for “naked mole rats” and “aging”)
2. The insulin/IGF-1 signaling pathway regulates aging in both *C. elegans* and mice. However, knockdown of the IGF-1 receptor in mice must be tissue-specific (adipose only) to extend lifespan. Is there any evidence for tissue-specific activities of the insulin/IGF-1-like receptor DAF-2 in *C. elegans*? Does it matter where *daf-2* is expressed? In which tissues of the animal is the insulin/IGF-1 pathway important for the regulation of aging? (Hint: look at the references listed in the advanced reading section.)