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Dissecting Age Associated Disease in *C. elegans*

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Introduction

As the life expectancy for the human race continues to grow, the topic of age related disease has garnered increased attention from the medical research community. Age-related neurodegenerative diseases including Alzheimer's Disease (AD) and Huntington's Disease (HD), which are linked to aberrant protein aggregation, are becoming more prevalent in our increasingly older population although the pathological mechanisms of theses diseases continue to remain unclear (Cohen, 2006). To better comprehend the pathology of these diseases, it is important to determine what characteristic of the aging process causes individuals to become more susceptible to these diseases over time.

Although the study of human disease patients remains an invaluable research tool, model organisms such as the nematode *Caenorhabditis elegans* (*C. elegans*) can also be used to further examine the relationship between the aging process and disease pathology. *C. elegans* is one of the most common model organisms used to study the genetic mechanisms of disease pathology. It is estimated that approximately 60-80 percent of *C. elegans* genes have a homolog in the human genome (Markaki, 2010). *C. elegans* has many attributes that make it advantageous to use as a model organism for aging studies. These include highly conserved molecular and genetic pathways, relatively short lifespan, rapid reproduction, and ease of genetic manipulation. The worm can also be easily manipulated to transgenically express human genes, such as human Amyloid-β, which is a valuable tool for studying disease.

Previous studies focused on the mechanism underlying the onset of age dependent disease developed genetic models of AD and HD in *C. elegans*. For this study, AD was

modeled using the *C. elegans* strain CL2006, which was developed in Chris Link's lab at the University of Colorado Boulder and obtained from the *Caenorhabditis* Genetics Center (CGC). These worms, have the genotype *dvIs2*[pCL12(*unc-54*/human Aβ peptide 1-42 minigene) + pRF4(*rol-6(su1066)*)], and express the human Amyloid β peptide along with a visible marker, (*rol-6*) (Link, 1995). The *rol*-6 marker causes a "roller" phenotype, which is used to indicate that the worm is expressing the Aβ transgene. The expression of the Amyloid β peptide leads to an aggregation of protein within the animal, the development of plaques, and eventually progressive movement disorders (Tissenbaum 2012 / Dostal, 2010).

HD was modeled using the *C. elegans* strain AM140, which was generated by Richard Morimoto's lab at Northwestern University and also obtained from the CGC. These worms have the genotype rmIs132 [*unc-54p*::Q35::YFP], and express a polyQ repeat of 35 glutamine residues. This results in an age dependent, progressive transition from soluble to aggregated protein that can be visualized under a fluorescent microscope using the YFP tag. The aggregation of poly Q proteins leads to toxicity for the worm and this is likely caused by global disruption of the cells proteostasis machinery (Prahlad, 2008).

Wild type worms were modeled using strain N2, which was originally found in mushroom compost near Bristol, England in the 1960's. Since the advent of *C. elegans* genetic research, this strain has been used to represent wild type worms.

A fourth strain, CB1370, a *daf-2* mutant, was used to model long lived worms. These worms have a mutation in the insulin/Igf-1 receptor gene, *daf-2*. This mutation causes decreased receptor function and leads to a decrease in insulin/IGF-1 signaling.

The insulin/IGF-1 signaling pathway is an important neuro-endocrine regulator of stress response and longevity in worms (Vaccaro, 2012 / Narasimhan, 2009). Decreasing the level of insulin/IGF-1 signaling results in a significant increase in lifespan, with some worms living twice as long as their wild type counterparts (Lin, 1997).

Worms from all four of these strains were analyzed for both lifespan and movement as the worms aged. This allowed the simultaneous study of age-associated disease onset and lifespan.

Methods

Worm Preparation

To study the onset of symptoms in the disease models, a lifespan experiment was conducted simultaneously with all four strains. These strains were age synchronized by isolating unhatched eggs. This was achieved by using a bleaching protocol whereby gravid adult hermaphrodite worms were placed in a solution of sterilized water, sodium hydroxide, and Clorox bleach. This solution is able to dissolve the bodies of the adult worms, while leaving the eggs unharmed. Once the eggs were released from the carcasses, they were washed repeatedly in M9 buffer and then left on a gentle shaker at 15 °C for 8 hours, until hatching began to occur. The worms will arrest as larvae at this stage without food. The newly hatched L1 worms were then plated on agar plates that had been seeded previously with a field of OP50 *E. coli* bacteria as their food source. The worms were allowed to grow on these plates until they reached the L4 stage, at which time they were subsequently transferred to OP50 seeded agar plates containing fluorodeoxyuridine (FUdR) for the completion of the lifespan. The use of FUdR, a

compound that inhibits the development of progeny in the worms, allowed the ability to monitor the same synchronous group of worms throughout their complete lifespan without having to pick off eggs as they were laid (Hosono 1978).

Lifespan

For each strain, three FUdR agar plates were used containing 15-20 worms each. Lifespan analysis was done daily and worms were scored either dead or alive on each day. Dead worms were removed from the plate and discarded. If the bacterial lawn became too thin, more OP50 bacteria was dropped onto the plate.

Movement

To assess the movement of the worms as they aged, animals from each strain were recorded on days 5, 8, and 15 of their lifespans. This allowed the observation of any differences in movement patterns throughout the aging process. Analysis was done using a digital video camera which was adapted for use with a light microscope. Worms were recorded shortly after being counted for the lifespan experiment.

Results

As the worms progressed throughout their lifespans, many differences between the strains were observed. These differences included variability in movement patterns as well as differences in the length of the animal's lifespan. The differences in lifespan can be seen in **Figure 1**, which depicts the percentage of worms that were alive on a given day.

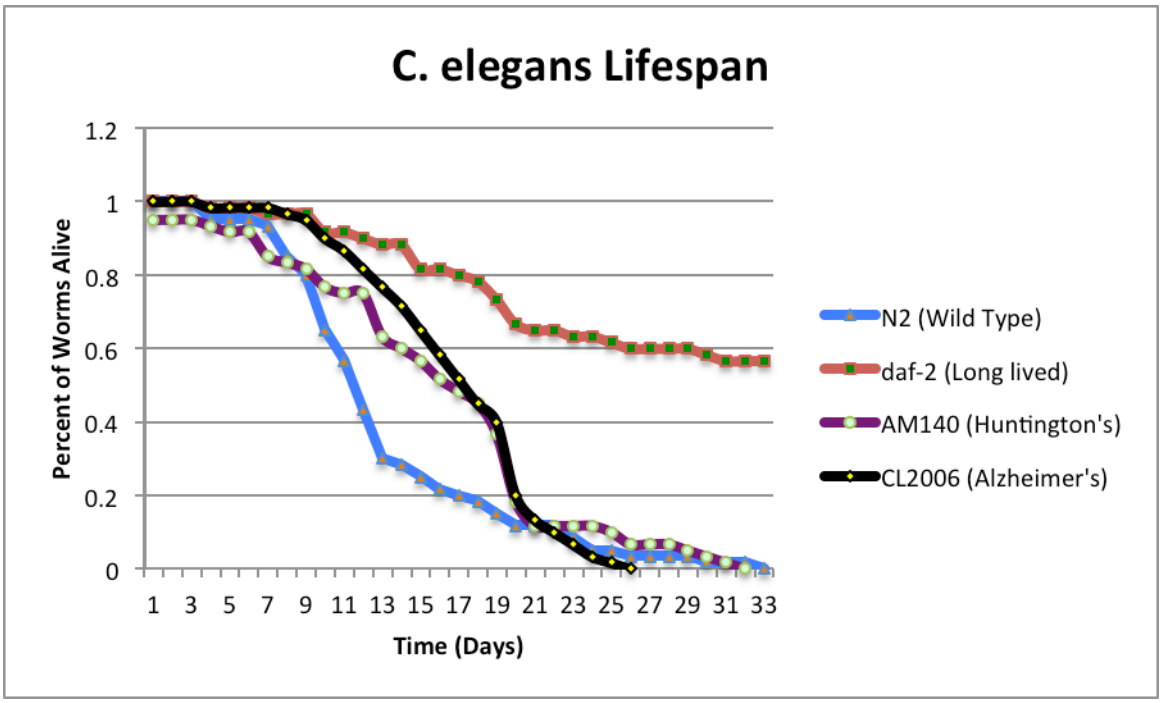

Figure 1: *C. elegans* Lifespan Assay

Figure 1 shows the day-by-day lifespan for each worm strain as a percentage of total animals alive. The shortest living worms were those of the Alzheimer's disease model with a maximum lifespan of 25 days. The wild type worms only slightly outlived the Huntington's Disease model with maximum lifespans of 32 and 31 days respectively. The *daf-2* mutant worms outlived all other strains and almost half (34/60) of the animals were still living at the time this paper was written.

As can be seen in the graph, each strain had a unique duration of lifespan with the Alzheimer's Disease model exhibiting the shortest lifespan and *daf-2* mutants showed the longest lifespan, which can be as long as 60 days. Interestingly, the disease models initially died at a slower rate than the wild type worms, although eventually, the wild type worms slightly outlived both disease models. Also the graph shows three stages that are separated by a change in mortality rate. The stages are days 1-7, days 8-20, and days 21-33. Initially, the worms died at a slow rate, with only 16 of the 237 total worms dying in the first 7 days (2.3 worms/day). Beginning at approximately day 8, animals from all of the strains begain to die at an increased rate, with 147 of the 237 total worms dying between days 8-20 (11.3 worms/day). At approximately Day 21, the survival rate of all

four strains leveled off with 27 of the total 237 worms dying between days 21 and 33. These timepoints could indicate important transcriptional or translation events such as up or down regulation of genes that are related to aging or stress resistance. For example, it has been shown that the expression of certain heat shock proteins, which play a key role in stress response, decreases with age (Lund, 2002).

The movement patterns of each strain were also analyzed throughout the lifespan assay. Beginning at the L4 stage and continuing through young adulthood, all strains exhibited a similar sinusoidal movement pattern although *daf-2* mutant animals were slightly thinner and moved marginally faster than the other strains. However, as the worms progressed throuout their lifespans, both disease models developed drastic movement defects.

The Huntington's worms developed full body paralysis that originated as early as day 8 and persisted throughout the lifespan of the worms. These worms were no longer able to travel around the plates and were only able to move their heads. Progressively, as the animals continued to age, the movement of the head also become increasingly restricted. It is possible that, due to their inability to move through the bacterial field, some of the Huntington's worms died due to starvation.

Also beginning around day 8, the Alzheimer's worms had a harder time moving. These worms had a marker that made them have a "roller" phenotype and the worms formed a circular shape and continuously twisted and rolled over. As the worms aged, the speed of their rolling movements progressively declined, possibly due to protein aggregation. This phenotype also inhibited the worms' ability to travel around the plate. Again, due to the fact that this phenotype negatively affected the worms' ability to travel

through the bacterial lawn, some of the Alzheimer's worms may have encountered starvation. Additionally, due to the fact that dietary restriction has been shown to increase lifespan in worms, this lack of consistant food may have contributed to the fact that the disease model worms initially died at a slower rate than the wild type worms (Kenyon, 2005). Therefore, the roller combined with the movement problems associated with the peptide made it progressively more difficult for these worms to move as they age.

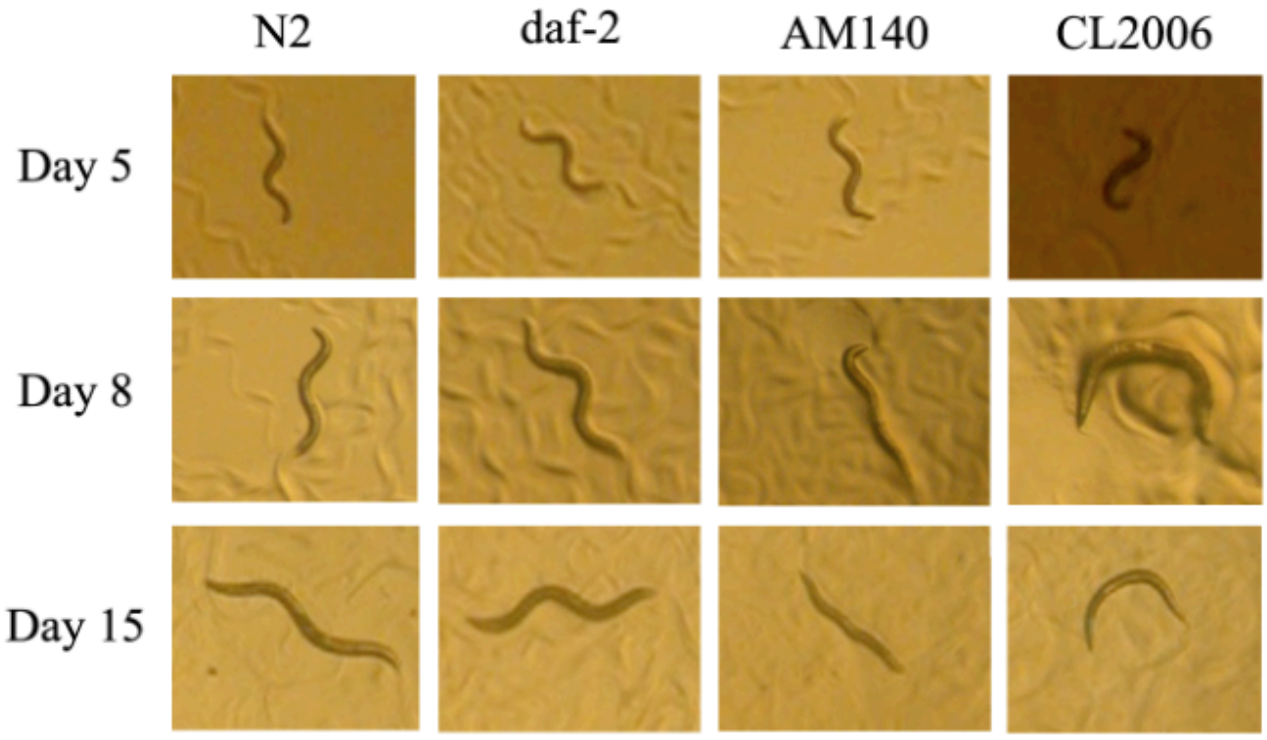

Figure 2: *C. elegans* Movement Patterns

Figure 2 shows the movement patterns of all strains throughout the aging process. Wild type (N2) and long lived (*daf-2)* worms sustained the normal sinusoidal movement pattern for the duration of their lifespans, while both the Huntington's (AM140) and Alzheimer's (CL2006) mutants developed movement defects beginning at day 8. The Huntington's model developed full body paralysis and were only able to move their heads. The Alzheimer's model developed a roller phenotype and lost the coordinated sinusoidal movement pattern.

Overall, it is interesting that both the lifespan and movement data show an interesting change at approximately day 8. At that time point, the lifespan data shows an increase in the rate of death for all strains. The movement of the disease model strains also became disrupted around day 8. These two observations in conjunction with each other could indicate an important genetic change in the worm that causes the animals to become susceptible to the symptoms of aging and age related disease.

Discussion

As our population's average lifespan continues to increase, the incidence of age related neurodegenerative disease is also increasing. As of 2005, as many as half of the population over the age of 80 and as many as 5 million total Americans suffered from Alzheimer's disease (Luo, 2005). Thusly, it is becoming progressively more important to understand the pathology of age-associated neurodegenerative diseases. Do these diseases begin developing at a young age and only show symptoms when the disease progression hits a certain threshold? Or could it be that, at some point during our lifespan, there is a genetic change that takes place in our aging bodies that causes us to become more susceptible to disease symptoms.

From the preliminary data generated through the combination of movement and lifespan experiments, it can be seen that at approximately day 8 the worms begin to show susceptibility to age related disease symptoms such as movement defects, as well as symptoms of general aging such as an increase in death rate. This time point, a little less than one third of the worm's total lifespan, also proportionally relates to early onset cases of neurodegenerative diseases in humans, which can start to show symptoms as early as the late twenties. As organisms age, changes take place in the delicately balanced

expression levels of many genes (David, 2010). In order to determine if this susceptibility to disease and aging is caused by a change in gene expression, further research could include microarray experiments at days 5 and 10 to look for any genes that may have had a shift in expression coinciding with the onset of disease symptoms. If no change in gene expression can be found, than it is likely that the onset of symptoms can be attributed to high levels of protein aggregation in the worm.

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