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A model organism for studying aging genes under lipid peroxidation

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Abstract: *A study conducted in this paper examines the relationship between hydrogen peroxide accumulation caused by the nematode aging gene daf-16 and accelerated metabolic rate caused by the nucleolar regulatory gene ncl-1. The daf-16 mutant and wild-type nematodes (called N2) were obtained and their ncl-1 gene was blocked by RNAi technology to understand nucleolar formation, growth cycle, and longevity. Nucleus appears early if ncl-1 is blocked, regardless of whether daf-16 is present. There is an additive effect between these two genes on nematode longevity. Among the wild-type nematodes, n2 had the longest lifespan, while the double-mutant nematodes, daf-16 and ncl-1, had the shortest. A nematode's lifespan is markedly reduced when the two genes do not express at the same time. This shows that a single mutation causes the lifespan of the nematode to decrease by approximately the same amount when daf-16 or ncl-1 are not expressed.*

Keywords: *Modal organisms, Nematodes, Aging gene, Nucleolus*

1. Introduction

First described in 1900, Caenorhabditis elegans was a small, bacteriovorous nematode found in Algerian soil [1]. The first use of nematodes as model organisms in genetic research was Brenner's study of apoptosis [2]. Three Nobel Prizes have been awarded to direct application of C elegans in the 21st century: 2002 [3], 2006 [4] and 2008 [5]. About 75% of the genes in C elegans' genome have homologs in the human genome [6]. As the first multicellular organism with a complete genome sequence in 1998, C elegans has been used to identify many of the key genes involved in developmental and cellular processes [7].

C elegans and other animals, such as humans, share many molecular and cellular processes across metabolism, gene regulation, protein biology, etc., making them excellent organism models. Approximately 40% of the C elegans protein-coding genes associated with human diseases have clear orthologs in the human genome [8]. The study of human health and disease can therefore benefit from many discoveries made in C elegans. In recent years, C elegans have also been widely used to study the effects of aging-related diseases and to find drugs that regulate aging.

Many transcription factors affect the anti-oxidative stress resistance and longevity of experimental flies and mice, such as daf-2, pit-1, amp-1, clk-1, sir-2, sir-4, p66shc, lin-4, lin-14, and others [10]. As nematodes, C elegans has aging genes that affect whole organisms without affecting their normal reproduction. Nematodes can be obtained from many mutant strains. RNAi technology was used to block the nucleolar regulatory gene ncl-1 indirectly by targeting one of the aging genes, daf-16 in the mutant strain nematode. C elegans Ncl-1 gene regulates cell size and ribosomal RNA synthesis. Growth control relies heavily on the regulation of ribosome synthesis. As far as we know, little is known about the two factors that control and coordinate these two genes of double mutations, so we devised these experiments to better understand daf-16 and ncl-1 on the aging process of C elegans.

2. Materials and Methods

C elegans serves as an object in our study. Adult nematodes are about 1 mm long, and most of them are hermaphrodites. Living in soil is common, and it is best to keep the temperature at 20 °C. The nematode has only one cell at the beginning, just as humans do. Their numbers increase to as many as

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959 cells after fertilization, and they have nerves, digestive systems, and reproductive systems as do humans, as well as aging. The nematode's lifecycle is divided into several phases: embryos, L1, L2, L3, L4, and adults (see Figure 1) [7]. During the Dauer period, when he does not eat, the mouth and genital tube will be sealed to extend his life in the harsh environment. As a model organism for studying aging, the nematode is very suitable due to its small size, rapid lifecycle, transparency, well-annotated genome, and ease of observation.

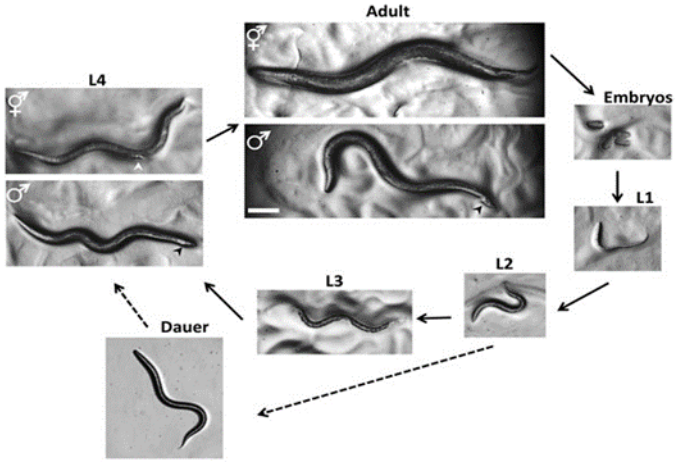

Figure 1: Lifecycle of C elegans [7]

Daf-16, an aging gene, affects both the upstream and downstream pathways of nematode insulin receptors, resulting in Dauer formation, which indirectly affects spawning and longevity. Daf-2 mutations can cause Dauer aberrations in nematodes, as they are upstream of daf-16. Daf-16's performance is suppressed. The catalase gene is located upstream of daf-16. When the catalase gene is mutated, it will not express, and the catalase that is produced cannot decompose. There was an accumulation of hydrogen peroxide in the body. Daf-16 is regulated by about 200 genes, many of which are human genes.

The nucleolar regulatory gene NCL-1 has been shown to inhibit cell growth and ribosome synthesis in nematodes in previous studies. The previous study [11] found that ncl-1 mutations caused the nucleus to appear early in the embryo, while the nematode appeared in the nucleolus when the embryo divided to 100 cells if ncl-1 did not express. It would also have larger nucleoli and larger cells when ncl-1 was not expressed, resulting in higher rDNA transcription rates and larger cells. There is an independent regulation of ncl-1 protein in different embryonic cells. In wild-type embryos, cells with enlarged nucleoli have the lowest levels of ncl-1, which represses ribosome synthesis and cell growth.

HT115, an E coli strain that produces T7, attaches to the T7 promoter junction and allows cells to begin transcription of the gene. We used RNA interference (RNAi) to block the performance of ncl-1 in this experiment. RNAi technology prevents the expression of specific genes indirectly. RNAi reactions are usually performed in the scientific community by eating nematodes into HT115. There are artificial plastids in it, which will translate the T7 protein.

PL4440 with the T7 promoter junction was also inserted into pLonc001 using genetic engineering. HT115 has the same characteristics as T7 protein, and the T7 protein is ligated to pLonc001. Translated double strand RNA (dsRNA) contains part of ncl-1. RNAi is produced after the nematode eats the dsRNA.

An enzyme called "Dicer" in the nematode attaches foreign dsRNA and cuts it into short interfering RNA (siRNA) in vivo. RNAi silencing complex (RISC) attaches siRNA to form an enzyme (Slicer) that excises mRNA explicitly. If the Slicer matches the correct mRNA, the experiment will match the mRNA of the ncl-1 that was excised. Insufficient mRNA of ncl-1 prevented translation of a sufficient amount of protein, indirectly inhibiting ncl-1 expression.

Here are the experimental procedures. To prepare a medium for nematode growth, plasmids containing ncl-1 genes (pLonc001) were implanted into HT115. Following transcription, HT115 translates the T7 protein attached to the two T7 promoter junctions on pLonc001. A nematode's dicer enzymes cut dsRNA into shorter RNA (siRNA) after it has been transcriptionally transcribed. Protein complexes (RISCs) are formed by siRNA and nematode proteins. RISC extracts the double strands, one of which forms a nucleic acid enzyme. Slicing of nematode ncl-1 mRNA is performed at random by the slicer. In the lysosome, cleaved mRNA is treated as a foreign substance, so it cannot be translated. As a final step, the slicer can be used to look for mRNA for other NCL-1 genes as well.

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Due to RNAi technology's shortcoming, nematodes destroy different proportions of mRNA, resulting in only a few nematodes producing enough protein. The embryo must show large nucleoli to confirm that the nematode ncl-1 does not perform in our experiment.

We confirmed the nucleolar formation and lifecycle of daf-16 and ncl-1 double mutant nematodes before the experiment. The emergence of nucleoli in early nematodes is known to be caused by a single ncl-1 mutation. A selection of the N2 standard wild-type strains and daf-16 mutant strains of adult nematodes was selected to the general medium and the RNAi (ncl-1) medium, and then disinfection was over-fired. After about 24 hours (adult nematodes have spawned), all five adult nematodes were picked from the medium and only the eggs were kept. Keep track of how long it takes for all eggs to hatch and grow into adult nematodes. Take the average of the above steps several times. An optical microscope was used to examine the appearance of nucleoli in daf-16 mutant strains cultured in adult medium and RNAi (ncl-1) medium. Picking N2 wild strain and daf-16 mutant nematodes in the L4 period and placing them in a standard medium and a RNAi (NCL-1) medium. Pick out any nematodes or eggs smaller than L4. New media should only be used for the target nematode. As a final step, we measured the growth life of various nematodes.

3. Results and Discussion

There were no significant differences in appearance between wild strain nematodes, each mutant nematode, and double daf-16/ncl-1 mutant nematodes in our experiments, including N2 wild-type strain nematodes, ncl-1 mutant nematodes, daf-16 mutant nematodes, and double daf-16/ncl-1 mutant nematodes.

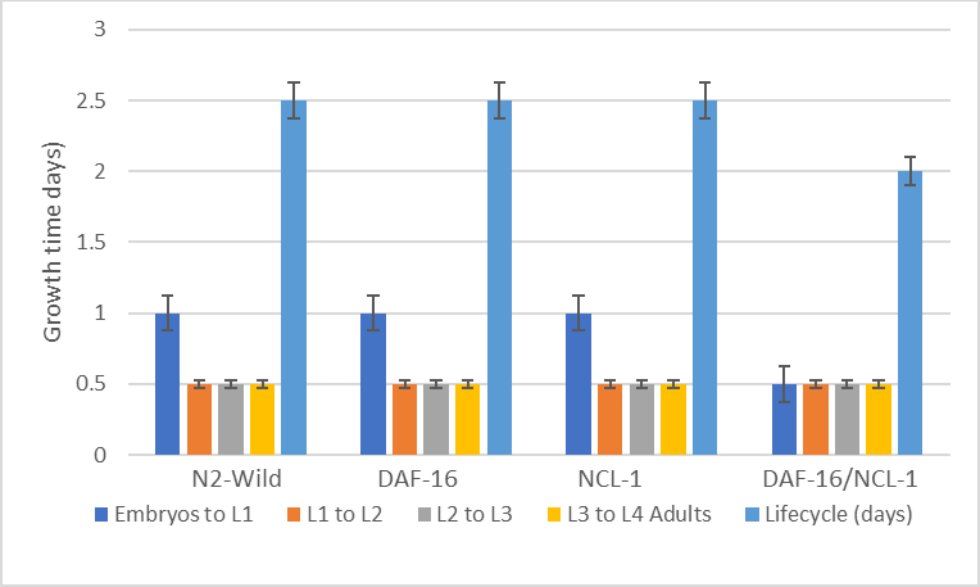

Figure 2: The hours required for different growth stages and lifecycles of n2-wild, daf-16, ncl-1, and daf-16/ncl-1 nematodes

As shown in Fig.2, except for the daf-16/ncl-1 double mutant nematode, the other three nematodes required 24 hours in the spawning (Embryos → L1) stage and 12 hours in the growth (Embryos → Adult) stage, and three larval phases (L1 to L2, L2 to L3, and L3-L4 Adult) needed 12 hours; the total growth lifecycle is about 3 days. Double mutant nematodes take only 12 hours to incubate. It has the same incubating and growth stages as the other three nematodes, which means it has a 60-hour growth cycle. From embryo to embryo-laying adult, C elegans has a life cycle of three days at 25°C [7]. Mutations that lead to developmental and behavioral defects are easily identified in genetic effects because of the invariant N2-wild type cell lineage. Based on the results, the NCL-1 nematode has accelerated its metabolism rate and shortened its growth time from 24 hours to 12 hours. Thus, ncl-1 activates the aging gene of daf-16 and shortens life by adding to the aging process.

Wild-type n2 nematodes have a lifespan of about 14 days, while daf-16 mutations and ncl-1 mutant nematodes have lifespans of about 12 days and 11 days, respectively, while daf-16/ncl-1 double mutant nematodes have a lifespan of about eight days. Based on two separate experiments, we calculated the lifespan as a reproducibility test. Each experiment's average lifespan was calculated by summing

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individual lifespans by the number of nematodes with an error within five percent of the standard deviation (see Figure 3).

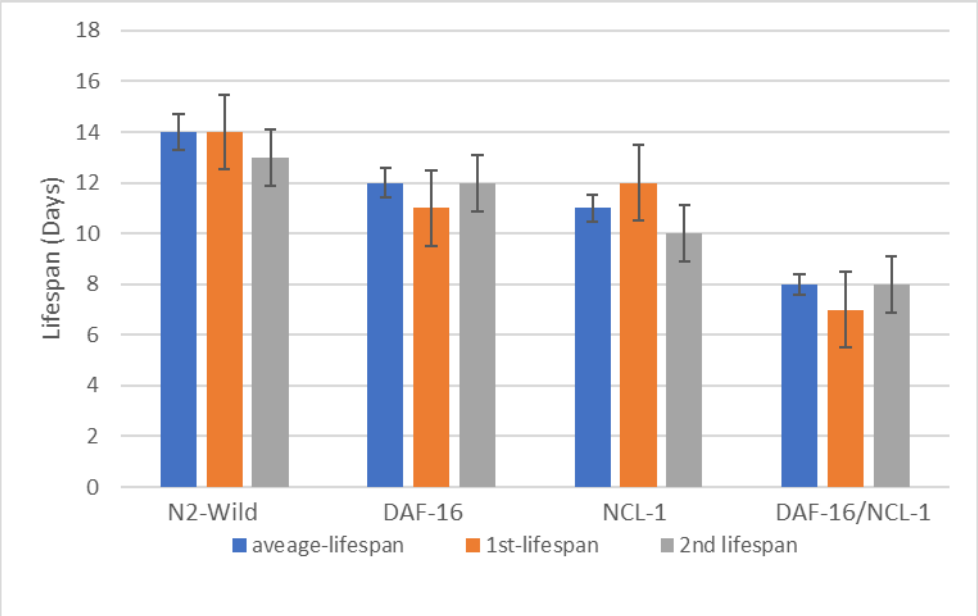

Figure 3: The lifespan for different growth stages of n2-wild, daf-16, ncl-1, and daf-16/ncl-1 nematodes

Fig. 4 shows that the survival rate curves of the daf-16/ncl-1 double mutant nematode and the n2 wild nematode did not overlap, and the daf-16/ncl-1 double mutant nematode was detected. Life expectancy does have a clear trend.

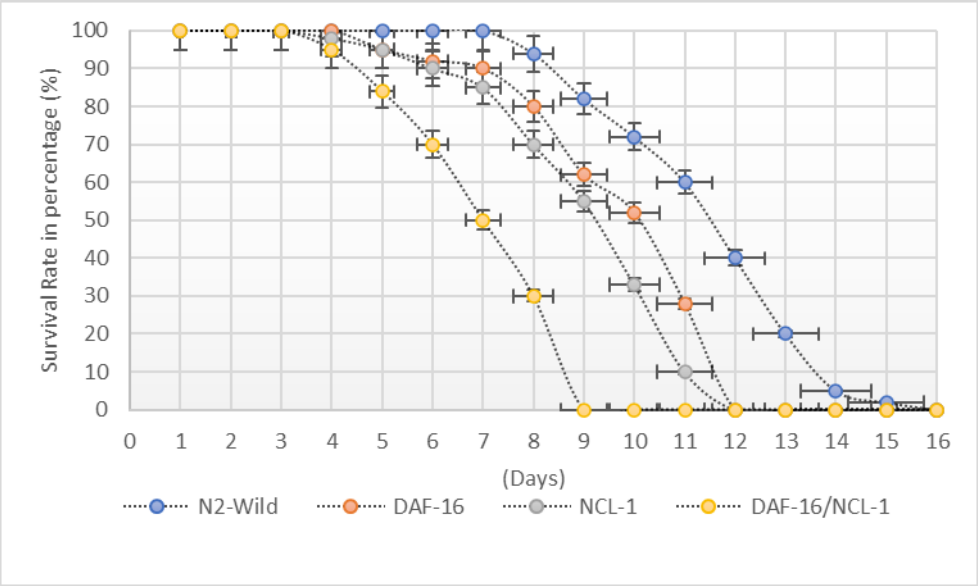

Figure 4: The survival rate curve for n2-wild, daf-16, ncl-1, and daf-16/ncl-1 nematodes.

In normal nematodes, nucleoli do not appear until about 100 cells have been formed in the egg during embryonic development. In the daf-16 mutant nematode of our experiment, we found that when the egg splits into four cells, no nucleolus appears. In addition, the daf-16/ncl-1 double mutant has shown that when nematode eggs split into two cells, the nucleolus appears earlier. A nucleolus appeared early, but only ncl-1 did not express. Daf-16 mutant nematodes did not express, and the nucleus did not appear soon. In the double mutant nematode, the nucleoli will appear early only when the daf-16/ncl-1 genes do not express, that is, regardless of whether the daf-16 gene is present or not, as long as the ncl-1 gene is absent and blocked, the nucleoli will appear early. It was concluded that daf-16 and ncl-1 would not interact.

Each nematode's growth cycle is illustrated in Figure 2. The embryos grow in four different stages:

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hatching (L1 larvae), larval growth (L1 larvae, L2 larvae, and L3 larvae, which become adults in L3 larvae). All nematodes except the daf-16/ncl-1 double mutant grow consistently. Incubation lasts about 24 hours, growth lasts about 12 hours, and growth lasts about 12 hours. It takes about 60 hours for the growth cycle to complete. In the case of the daf-16/ncl-1 double mutant nematode, each stage takes 12 hours, resulting in a 48-hour growth cycle. The growth cycle of the nematode has been significantly shortened since both daf-16 and ncl-1 genes did not express simultaneously. Further, the daf-16/ncl-1 double mutant nematode hatches within 12 hours, which is faster than the other three nematodes. According to the above, the daf-16/ncl-1 double mutant nematode gene significantly affects incubation.

Observing their growth daily from the L4 period until they died, we started recording their growth. Experiment-1 and experiment-2 yielded almost identical results. Based on the arithmetic mean, the wild-type N2 nematode had the longest life, indicating a standard life expectancy of 14 days under normal conditions after the L4 period. When the two genes aren't expressed simultaneously, the lifespan of daf-16 and ncl-1 double mutants is reduced to about 8 days. (Fig. 4) shows that the survival curves of the four nematodes do not overlap, and there is a certain drop in survival. In addition, it can show that the lifespan of the daf-16 and ncl-1 double mutants is indeed shortening. We know that the daf-16 enzyme is located upstream of the catalase enzyme. Ncl-1 can inhibit nucleolus growth if a mutation causes hydrogen peroxide enzyme deficiency; the nucleolus of normal nematodes will appear only when the egg splits into 100 cells. It causes the nucleolus to appear earlier and become larger once it is not expressed, causing metabolic rate to increase. Thus, we infer indirectly that when daf-16 and ncl-1 are not expressed in the nematode, hydrogen peroxide will accumulate, thus decreasing the life expectancy of the nematode.

Among wild-type nematodes, N2 has the longest life (14 days), with daf-16 or ncl-1 genes showing slightly shorter lifespans (12 days, 11 days), but not the shortest (8 days). It has been demonstrated that a single mutation decreases the lifespan of the nematode equally. Furthermore, the two genes appear to have additive effects on nematode lifespan, confirming that they do not belong to the same aging pathway.

4. Conclusions

The results of this experiment have led to the molecular identification of key genes of daf-16 and ncl-1 in developmental and cell biological processes. Nucleolar regulatory gene mutations, ncl-1, and aging gene mutations, daf-16, both shorten nematode lifespans. The expression of nematode aging gene daf-16 and nucleolar regulatory gene ncl-1, however, does not affect one another and is additive.

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