# Research on Ageing Based on D2030.4 by RNA Interference Impairs Lifespan in Caenorhabditis Elegans

Qinya Liu and Yang Li*

***Abstract*—Ageing has been a mystery to humanity for millennia. It has been a burden for numerous people, from the minority that suffer from progeria to the plethora of people who, due to ageing, are more susceptible to diseases such as Alzheimer's disease and coronary artery disease. The aim of this study was to identify genes that regulate aging and elucidate their mechanisms. A Caenorhabditis elegans RNAi screen was performed to assess the role of these genes in the regulation of aging. For the first time, D2030.4 deficiency was found to accelerate senescence at 20 °C, comparable to DAF-16, a putative regulator of senescence. The results showed that D2030.4 deletion could down-regulate the expression level of GAS-1 mRNA and actively participate in the regulation of ROS in mitochondria. Therefore, D2030.4 may inhibit senescence by regulating ROS production. The Caenorhabditis elegans gene D2030.4 is conserved in many species, including humans, making it a potential target for anti-aging drugs.**

***Index Terms*—Ageing, mitochondrial respiratory chain, caenorhabditis elegans**

## I. Introduction

Ageing has been a mystery to humanity for millennia. It has been a burden for numerous people, from the minority that suffer from progeria to the plethora of people who, due to ageing, are more susceptible to diseases such as Alzheimer's disease and coronary artery disease [1]. Through the analysis of data of 250 death causes from 195 countries, in 2040, the mean lifespan of 46 countries was predicted to rise by over 10 years [2]. Research has found that an ageing population implicates a decrease in real per capita GDP growth, while the decline is moderated if the older age groups are in good health [3]. There are already several drugs targeting ageing in clinical trials [4]. Nevertheless, the ultimate causes of ageing remain unclear. Several theories explaining the mechanisms of ageing have been proposed, among which genetics is an essential one [5]. However, genetics in ageing and the drug research are still in the primary stage. Thus, more ageing genes need to be identified.

C. elegans is an excellent established model organism used for both ageing and genetics research. It has a short generation time, which allows longevity research to be time-efficient. Moreover, there is thorough understanding of the anatomy of this organism: it is the first multicellular organism with a complete genome sequence and cell lineage map [6]. This aids the molecular identification of many genes in biological processes, including genes responsible for ageing. In C. elegans, genes have been discovered to participate in ageing. For instance, the inhibition of daf-2 and age-1 genes in the Insulin/IGF-1 signaling pathway (IIS pathway) resulted in activation of daf-16, which elongated the lifespan and the expression of longevity genes [7]. Other genes linked to lifespan have also been clarified. For example, a diet restriction-related gene eat-2, mutants of which displayed decreased pumping rate by 40% and an increased median lifespan of 25% compared to wild-type worms [8]. Taking into consideration that 60-80% of human genes have an ortholog in the C. elegans genome, these studies made in C. elegans can easily progress to be applied to the study of human health and disease related to ageing [6].

Therefore, this study aimed to explore genes responsible for ageing and its underlying molecular mechanisms. To do so, RNA interference (RNAi) was performed in C. elegans. RNAi is an inexpensive, sequence specific and time-efficient gene silencing tool that allows the downregulation of specific genes. Additionally, it has a fairly high efficiency rate: on average, 60-70% of siRNA can downregulate 50–70% of mRNA [9]. Using this tool, many genes that regulate lifespan have already been uncovered, such as pld-1, encoding a phospholipase protein. RNAi-mediated knockdown of pld-1 lead to decreased lifespan and motility [10]. However, more genes controlling ageing remain unknown.

In this research, an RNAi screen targeting seven intestinal expressed genes was performed to assess the effect of several genes on ageing. Two representative phenotypes of ageing were observed in this research. Among the tested genes, the deficiency of mitochondrial respiratory chain protein D2030.4 caused a significant decrease in survival rate and pharyngeal pumping rate compared to the control group. D2030.4 is a constitutor of complex I and conserved with human NUDFB7. To confirm the mechanisms of D2030.4 in ageing regulation, the mRNA expression level of the interactors of D2030.4 were tested through RT-qPCR. Among the interactors, another mitochondrial respiratory chain complex I protein encoding gene gas-1 was downregulated in D2030.4 deficient worms. The gas-1 gene is a known lifespan regulator that inhibits ageing processes through the increase of mitochondrial protein damage by the elevation of Reactive Oxygen Species (ROS) [11, 12]. Therefore, D2030.4 may participate in this pathway. In addition, other genes that are conserved among many species, such as cox-4 and cox-5B, were also observed to elongate lifespan through the RNAi screen. These genes are potential targets of future agents that decelerate ageing.

## II. Materials and Method

### A. Materials

#### 1) LB culture medium

LB culture medium substances needed are shown in Table I as below:

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TABLE I: LB CULTURE MEDIUM SUBSTANCES NEEDED

| Substance | Quantity |
|---|---|
| Tryptone | 20 g |
| Yeast Extract | 10 g |
| NaCl | 20 g |
| 1M NaOH | 4.2 ml |
| $ddH_2O$ | 2 L |
| Agar | 34 g |

The prepared LB culture was autoclaved for 20 min at 121 ℃, then cooled down to 50 ℃ and poured to a recipient. It was stored at 4 ℃ when arrived to room temperature.

*2) Standard NGM plates*

Standard NGM plates substances are needed as shown in Table II as below:

TABLE II: STANDARD NGM PLATES SUBSTANCE NEEDED

| Substance | Quantity |
|---|---|
| NaCl | 0.75 g |
| Peptone | 0.625 g |
| Agar | 5 g |
| $KH_2PO_4$ | 6.25 ml |
| $ddH_2O$ | 250 ml |

TABLE III: THE MIXTURE WAS STERILIZED AND AUTOCLAVED AT 121 ℃, FOR 20 MINS AND COOLED TO 60 ℃. THEN, UNDER STERILE CONDITIONS, THE FOLLOWING WAS ADDED TO THE MIXTURE

| Substance | Quantity |
|---|---|
| 1 M $CaCl_2$ | 0.25 ml |
| 1 M $MgSO_4$ | 0.25 ml |
| 10 mg/ml Cholesterol | 0.125 ml |

The plates were seeded with 50 μl OP50 liquid culture and kept at −20 ℃ for long term storage.

*3) RNAi plates*

The preparation of RNAi plates was based on that of NGM plates. The IPTG (5 mM) and carbenicillin (50 μg/ml) were added to the mixture when the liquid arrived to 50 ℃.

*B. Method*

*1) C. elegans maintenance and synchronization*

N2 worms were cultured on NGM plates and maintained as described [13]. All animals were grown at 20 ℃ as noted. The C. elegans for the RNAi screen were transferred every 2 days. To ensure the worms in the RNAi plates were the same age, forty L4 worms were cultured in each group (Fig. 1).

*2) RNAi screen and phenotype observation*

The feeding RNAi was performed as described [14]. The genes selected for the RNAi screen satisfied three requirements. Firstly, they are expressed in the intestine since it is where RNAi performs effectively (unlike in the neurons) and alterations to it are more likely to be age-related (as opposed to alterations in the muscles) [15]. Secondly, they are homologous to humans, so that the results discovered in C. elegans are comparable to humans. Lastly, the siRNA-containing bacteria exists in RNAi library. After preliminary screening, seven genes that were reported to affect lifespan of N2 worms or daf-2 mutants and conserved in humans were selected [16, 17]. Different from the previous screens mentioned, the RNAi screen in this study was performed on wild-type worms at 20 ˚C rather than at 25 ˚C, to eliminate the effect of mild thermal stress on the ageing process, which was reported in both worms and humans [18].

The known lifespan regulators daf-2 and daf-16 were set as positive control groups, and the OP50 fed worms were blank control. To ensure RNAi was induced in the C. elegans, the bli-3 RNAi was also performed, which resulted in numerous obvious blisters (Supplementary Fig. 1).

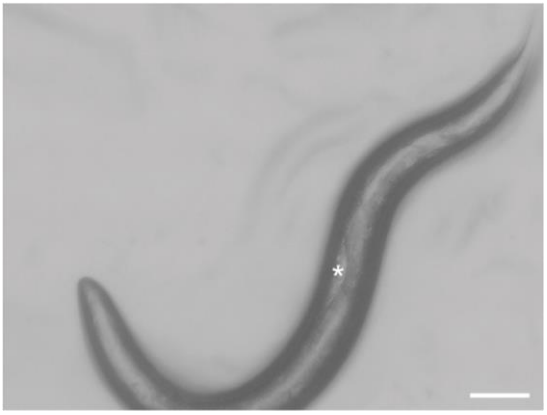

Fig. 1. C. elegans at L4 stage viewed through the dissecting microscope. The representative structure of the L4 worm is the developing vulva, a characteristic clear half circle on the ventral side (as the asterisk indicates), that allows worms from this stage to be easily identified [6]. Scale bar = 100 μm.

Two phenotypes were used to measure the ageing rate: pharyngeal pumping rate and lifespan. The survival rate was evaluated by the percentage of living worms among the total. Worms that did not react to the prodding of the worm picker were considered as dead. The pharyngeal pumping rate was calculated in ten randomly chosen worms for 30 s. A pharyngeal pump is defined as a complete contraction and relaxation of the pharynx bulbs (Fig. 2).

After one week of observation, five positive genes were selected for depth research: rab-7, idh-1, cox-5B, cox-4 and D2030.4. Ten days later, the gene D2030.4 was chosen to be studied further since its deficiency caused significant decreased lifespan and pharyngeal pumping rate.

*3) Identifying RNAi bacteria by Sanger sequencing*

The engineering bacteria recovered from RNAi library were sent for Sanger sequencing using the M13F primer, and were identified with the NCBI Blastn tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

*4) Alignment of multiple homologous genes*

To illustrate the homology of D2030.4 among multiple organisms, the phylogenetic tree of D2030.4 homologs was constructed with the Neighbor Joining method by MEGA7. For the Analysis preferences, the 'Bootstrap method' was chosen for 'Test of Phylogeny', '1000' for 'No. of Bootstrap Replications', 'Nucleotide' for 'Substitutions Type', 'p distance' for 'Model/Method', 'd: Transitions + Transversions' for 'Substitutions to Include', 'Uniform rates' for 'Rates among Sites', 'Partial deletion' for 'Gaps/Missing Data treatment', and the Site Coverage Cutoff % was set to 50. The unit in the phylogenetic tree represents the evolutionary distance, which are computed using the Maximum Composite Likelihood method.

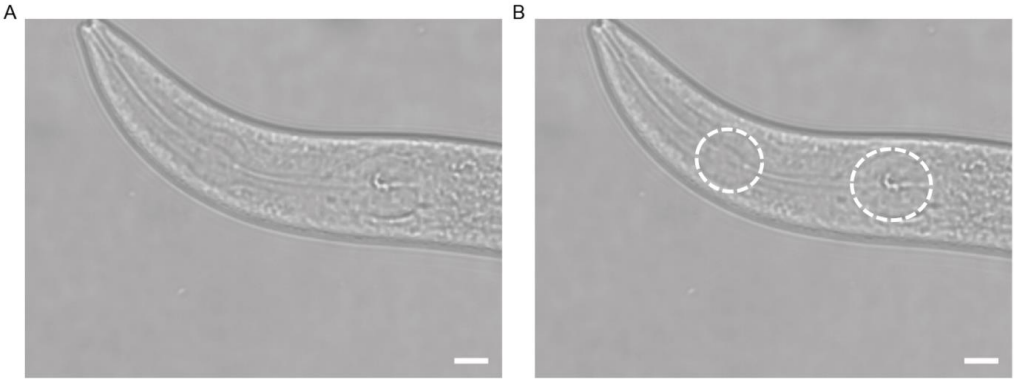


Figure 2: Pharynx bulb of C. elegans viewed through the dissecting microscope. The pharynx with its two bulbs (white circles) are located at the anterior of the worms. Food (bacteria) enters and passes through the pharynx, the neuromuscular bulbs grind the food by pumping, thus the pharyngeal pumping rate represents activity of worms [6]. Scale bar = 10 μm.

*5) RT-qPCR*

The RT-qPCR was performed to determine the regulation mechanisms of D2030.4. The interactome of D2030.4 were identified by the STRING database: nuo-1, C16A3.5, C18E9.4, C25H3.9, C33A12.1, C34B2.8, F53F4.10, nuo-4, gas-1 and R07E4.3. Worms at L4 stage were maintained with OP50 or D2030.4 RNAi bacteria for 11 days, the total RNA of which were extracted using the FastPure Cell/Tissue RNA Isolation Kit V2 (Vazyme™). It underwent reverse transcription using the HiScript 1st strand cDNA synthesis kit (Vazyme™). RT-qPCR was performed using the SYBR Premix Ex Taq Tli RNaseH kit (Takara™, code DRR420A). The experiment design is displayed in Table IV. A ubiquitously expressed gene tba-1 was set as inner control, and a group without transcriptase as blank control (Mock group). Primers were designed as presented in Table V.

TABLE IV: THE EXPERIMENT DESIGN OF RT-QPCR

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **OP50 group total RNA** | | | | | | | | | | | |
| tba-1 | Control | nuo-1 | C16A3.5 | C18E9.4 | C25H3.9 | C33A12.1 | C34B2.8 | F53F4.10 | nuo-4 | gas-1 | R07E4.3 |
| tba-1 | Control | nuo-1 | C16A3.5 | C18E9.4 | C25H3.9 | C33A12.1 | C34B2.8 | F53F4.10 | nuo-4 | gas-1 | R07E4.3 |
| tba-1 | Control | nuo-1 | C16A3.5 | C18E9.4 | C25H3.9 | C33A12.1 | C34B2.8 | F53F4.10 | nuo-4 | gas-1 | R07E4.3 |
| **D2030.4 RNAi group total RNA** | | | | | | | | | | | |
| tba-1 | Control | nuo-1 | C16A3.5 | C18E9.4 | C25H3.9 | C33A12.1 | C34B2.8 | F53F4.10 | nuo-4 | gas-1 | R07E4.3 |
| tba-1 | Control | nuo-1 | C16A3.5 | C18E9.4 | C25H3.9 | C33A12.1 | C34B2.8 | F53F4.10 | nuo-4 | gas-1 | R07E4.3 |
| tba-1 | Control | nuo-1 | C16A3.5 | C18E9.4 | C25H3.9 | C33A12.1 | C34B2.8 | F53F4.10 | nuo-4 | gas-1 | R07E4.3 |

TABLE V: PRIMERS FOR RT-QPCR

| Gene name | Forward primer | Reversed primer |
|---|---|---|
| nuo-1 | GGAGACTGGCACAAGACGAAG | CTCCTCCGATCAAGCATCCTTC |
| C16A3.5 | GCGTGGATGTTCACGAAGGC | GGTGTCAACCTCATCAGCGTTAG |
| C18E9.4 | CTACGTCTACTTGTACGATCGAAAGTATC | GTGGTTCCGTGAGTCTTGTATTGGTAAG |
| C25H3.9 | GTCTACGGAACTTGCGAATTGAAG | GTTCACCTTCAAGATGCTTAACACGTTG |
| C33A12.1 | CAACAGAGATCCATGTACGAGAACC | CTTGAACAAGAGCCAAACGCTG |
| C34B2.8 | CATTTTCGGAGCTACTGCCTACG | CTCTCCGTACCAGGTTCCAGTC |
| F53F4.10 | GTCGTTGGCATCGAACGTGC | CGCTGAGCCAAATCGAGAAGT |
| nuo-4 | CCAAACAACTTGCTGATCAACTCGG | GTTCGAGCACAACTCCTTGACC |
| gas-1 | GGAACTTGTCTCCGAGCGAAC | GATCGGAATAGGCCAACTTTTCAAG |
| R07E4.3 | GGAGAGCATAGATTTGCATTGGCTC | GTTGGCAAAATCAGCTGGATTTTTG |
| tba-1 | GAACTATGCCATCAGACCAACAAGC | GAGAACAGTGTCGATCAGTTCCTTTC |

*6) Statistical analysis*

For pharyngeal pumping rate, Student's t-test was used for comparisons between two groups in GraphPad Prism 6.0. For the RT-qPCR results, t-tests were conducted in Excel for the expression levels based on the measured fluorescent levels. $p < 0.05$ was considered as statistically significant.

## III. RESULTS

### *A. Multiple Genes were Discovered Regulating Ageing by the RNAi Screen*

To uncover the genetics in ageing processes, an RNAi screen was performed on a series of selected genes to identify the gene that caused the most prominent effect on ageing, and identify the mechanisms. The rate of ageing was evaluated by

survival rate and pharyngeal pumping rate.

As previously reported, daf-2 mutants had elongated lifespan, while daf-16 mutants had shortened lifespan, compared to wild-type worms [19]. In this screen (Supplementary Fig. 2, supplementary Table I), wild-type worms (OP50 group) reached half-death at D8-D9 stage, while that in DAF-2 deficient worms was at D9-D10 stage and in DAF-16 deficient worms were at D6-D8 stage. From D2 to D10, the survival rate of DAF-2 deficient worms was always higher by 3%–11%, while in DAF-16 deficient worms it was always lower by 5–16%, compared to the blank control OP50 group.

IDH-1 is predicted to be involved in metabolic processes (https://wormbase.org/), and its deficiency induced metabolic alterations and growth defects in GSPD-1 deficient mutants [20]. The survival rate of N2 worms with IDH-1 deficiency was initially higher but towards the latter days demonstrated no obvious differences compared to OP50 group. All the other RNAi groups, rab-7, spl-1, egl-3, cox-5B, cox-4 and D2030.4 RNAi displayed accelerated ageing compared to OP50. RAB-7 is implicated in several processes including oocyte growth and increases the susceptibility of age-related diseases through autophagy processes (https://wormbase.org/) [21]. In this screen, it reached half-death before D8. The cytochrome coxidase (COX) proteins were demonstrated to have higher expression in adult skin fibroblast lines than in fetal ones, which implied its relationship with ageing processes [22]. Among COX proteins, COX-4 and COX-5B are members of the respiratory chain complex IV, single knockdown of which caused half-death between D6 and D8. COX-5B was previously reported to be involved in the determination of adult lifespan (https://wormbase.org/). D2030.4 is part of the respiratory chain complex I, but its connection with ageing remains unclear. Here, its deficiency displayed obviously accelerated mortality rate, reaching half-death two days before OP50 group, similar to the daf-16 RNAi group (Fig. 3A).

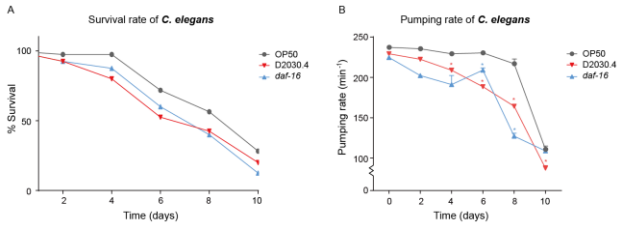


Fig. 3. Survival rate and pumping rate of C. elegans. Survival rate of C. elegans fed with RNAi bacteria (D2030.4 and daf-16). Pumping rate of C. elegans fed with RNAi bacteria (D2030.4 and daf-16). Data was represented as Mean ± SEM. *$p$ < 0.05 relative to the OP50 blank control at 20 ℃; student's t-test.

Except the daf-2 and idh-1 RNAi groups, all groups displayed lower pumping rate compared to OP50 group (Supplementary Table II). IDH-1 deficient worms exhibited significant difference in pumping rate compared to OP50 feeding worms on D4 only ($p$ = 0.008). cox-5B RNAi exhibited notable disparities on D4 and D6 ($p$ = 0.007 and $p$ < 0.001). cox-4 RNAi displayed considerable dissimilarities on D6 and D10($p$ = 0.003 and $p$ = 0.002). D2030.4 RNAi demonstrated substantial differences every day from D4 ($p$ = 0.003, $p$ < 0.001, $p$ = 0.001 and $p$ = 0.01) (Fig. 3B). The spl-1 and egl-3 RNAi groups did not display disparities with OP50 group in the first week of observation.

Taken together, D2030.4 was the most promising gene regulating ageing for its significant decrease in both lifespan and pharyngeal pumping rate.

### *B. D2030.4 is Conserved among Many Species, including Homo Sapiens*

D2030.4 is predicted to be located at the mitochondrial inner membrane, intermembrane space and respirasome. It is predicted to form part of the mitochondrial respiratory complex I, a complex responsible for the transfer of protons by employing electrons from NADH [23]. It has close homologs in over 10 species, including NDUFB7 (NADH: ubiquinone oxidoreductase subunit B7) in Homo sapiens (https://wormbase.org/). Fig. 4 illustrates the homology of D2030.4 in C. elegans, drosophila, human, mouse and rat through alignment by MEGA 7.

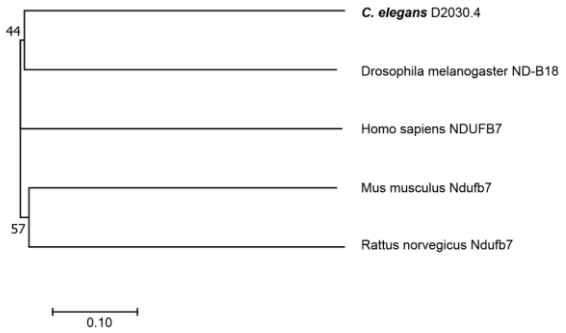


Fig. 4. The Molecular Evolutionary Genetics Analysis of C. elegans D2030.4.

The phylogenetic tree of C. elegans D2030.4 and homologs from different species. Unit, evolutionary distance. The evolutionary distances were computed using the Maximum Composite Likelihood method by MEGA7.

### *C. D2030.4 May Affect Ageing through GAS-1, a Known Ageing Regulator*

To determine how D2030.4 regulates ageing processes, the STRING database was utilized to uncover the interactome of D2030.4 (https://string-db.org/) (Supplementary Fig. 3). The mRNA expression level of the most closely linked 10 proteins were tested by RT-qPCR, including NUO-1, NUO-4, GAS-1, C16A3.5, C18E9.4, C25H3.9, C33A12.1, C34B2.8, F53F4.10 and R07E4.3 (Fig. 3, Supplementary Table III). All of them are either certain or predicted to be involved in the mitochondrial respiratory chain and among them, NUO-1, C33A12.1, F53F4.10, GAS-1, R07E4.3 are known or predicted to be part of complex I with D2030.4.

Among the 10 genes, only R07E4.3 displayed upregulation in D2030.4 deficiency background ($p$ > 0.05). The genes that exhibited statistically significant downregulation in D2030.4 deficiency background were C16A3.5 (downregulated 62.08%, $p$ = 0.006), C18E9.4, (55.98%, $p$ = 0.039), C25H3.9 (51.18%, $p$ = 0.010), C33A12.1 (48.79%, $p$ = 0.023) and gas-1 (44.18%, $p$ = 0.019) (Fig. 5). The gene gas-1 is already reported to be related to ageing. Hartman et al. demonstrated that the absence of gas-1 resulted in shortened lifespan, limited growth, reduced offspring and extremely sensitive to oxidative stress [24]. The close relationship of D2030.4 and gas-1 make it possible for D2030.4 to regulate ageing in gas-1 pathway.

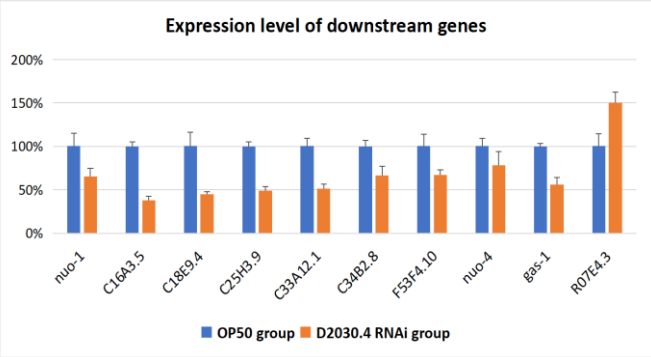


Fig. 5. mRNA expression level of D2030.4 interactors in C. elegans fed with OP50 or D2030.4 RNAi bacteria.Ten genes were examined by RT-qPCR, including nuo-1, C16A3.5, C18E9.4, C25H3.9, C33A12.1, C34B2.8, F53F4.10, nuo-4, gas-1 and R07E4.3. Two replicates were set in each group. The t-test analysis was performed in Excel: nuo-1 (0.11), C16A3.5 (0.01), C18E9.4 (0.04), C25H3.9 (0.01), C33A12.1 (0.02), C34B2.8 (0.06), F53F4.10 (0.09), nuo-4 (0.23), gas-1 (0.02) and R07E4.3 (0.06).

## IV. Discussion

To understand the mechanisms of ageing on a molecular level, the ageing genetics was studied. An RNAi screen was performed to examine the genes that were implicated in ageing researches. Deficiency of the gene D2030.4 exhibited the most determinant effects on reduction of lifespan (reaching half-death at D6, two days before OP50 group) and pharyngeal pumping rate (significantly decreased on D4, D6, D8 and D10), which represented accelerated ageing process. Subsequently, to explore the potential pathways D2030.4 undergoes, an RT-qPCR was executed. A known ageing regulator gas-1, responsible for the control of ROS production in mitochondria, was observed to be downregulated in D2030.4 deficiency background [11]. Therefore, D2030.4 may monitor ageing through the regulation of GAS-1 protein and ROS production.

Additionally, a significant proportion of the other genes from the RNAi screen were identified to be involved in ageing. The gene rab-7 and cox-5B are reported determinators of lifespan (https://wormbase.org/) [21], and exhibited shortened lifespan and decreased pharyngeal pumping rate in this screen. Some other genes were not linked to ageing, but confirmed in this study firstly, such as cox-4, which encodes a mitochondrial respiratory complex IV component (https://wormbase.org/). Therefore, this study demonstrated the importance of mitochondria and specifically the respiratory chain on ageing regulation.

The mitochondrial respiratory chain is an electron transport system located in the inner membrane of mitochondria. It utilizes the electrons to create energy to pump protons to the intermembrane space and establish a gradient of proton concentration between the intermembrane space and the matrix. This charge potential allows the energy to be focused at the ATP synthase for ATP synthesis and ultimately provide energy to the whole organism. It consists of four complexes, of which complex I is composed of D2030.4 and other NADH proteins. Complex I deliver electrons from NADH to complex III [23]. Previous studies have discovered that this chain, and specifically complex I, is associated with ageing. For instance, GAS-1 is predicted to enable NADH dehydrogenase activity and is actively employed in research, including studies of alcohol disorder and mitochondrial complex I deficiency (https://wormbase.org/). Additionally, gas-1 has been found to regulate ageing. Kondo et al. demonstrated that reactive oxygen species (ROS) production was increased in mitochondria in gas-1 deficient worms, which leads to increased oxidative stress [11]. The enhanced ROS production causes increased mitochondria DNA mutations, since the repair mechanisms in mitochondria is much more limited than that in the nucleus. Ultimately, dysfunction of mitochondria leads to ageing. Patients with chronic diseases, which are consequences of ageing, such as neurodegenerative diseases, contain higher frequency and quantity of DNA mutation in mitochondria [25]. Indeed, Hartman et al. noticed that gas-1 worms grown in 60% oxygen only had a mean lifespan of two days compared to 11 days in 21% oxygen. In contrast, wild-type lifespan was only reduced from a mean lifespan of 15 days to 11 days [24]. The mRNA expression level of gas-1 was statistically significantly downregulated in D2030.4 deficiency background. Thus, D2030.4 may regulate ageing processes through GAS-1 pathway, such as reducing ROS production.

Seen as there is plenty of evidence on the role of genetics in ageing regulation, genes should be targets for potential ageing inhibitors. There are drugs that have been reported to have anti-aging effects: for example, Dasatinib, which targets pan-receptor tyrosine kinases, is in phase I/II for Alzheimer disease [26]. Senolytics have been recently developed using both in vitro models and in vivo animal models, and the targeting ageing regulators were identified. For instance, the senolytics ABT-737, ABT-263 (navitoclax), A-1331852 and A-1155463 inhibit the activity of members of the mitochondria BCL family, which are negative modulators of apoptosis. A-1331852, A-1155463 and ABT-737 are in preclinical trials while navitoclax is in phase I/II [4, 27, 28]. A well characterized mTOR suppressor, rapamycin, which is approved for immunosuppression, decreases production of the senescence-associated secretory phenotype and has been proved to increase lifespan of laboratory mice [29].

The ageing genetics will continue to provide growing insights into ageing inhibitors development and play a major role in identifying ways in which we might slow ageing, thus helping an increasing number of people to age well.

## V. Conclusion

An RNAi screen was performed in C. elegans to identify regulators of ageing. Deficiency of the mitochondrial gene D2030.4 was proven to accelerate ageing in both lifespan and pharyngeal pumping rate aspects at 20 ℃. By employing the STRING database, the potential relationship of D2030.4 with gas-1 was illustrated. Then, it was further demonstrated that the mRNA expression level of GAS-1 was downregulated in D2030.4 deficiency background by RT-qPCR. Thus, D2030.4 is likely to inhibit ROS production in mitochondria in GAS-1 pathway. Since the C. elegans gene D2030.4 is conserved with the Homo sapiens ortholog NDUFB7, further exploration of the ageing regulation mechanisms of NDUFB7 in mammals and its potential drugs are essential in the future.

## APPENDIX

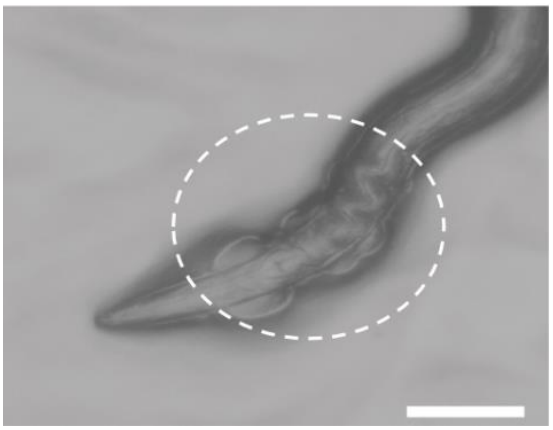

Fig. 1A. Blisters on C. elegans viewed through the dissecting microscope. C. elegans fed with bli-3 RNAi bacteria develop obvious blisters (white circle), which allows the RNAi conditions to be assessed. Scale bar = 100 μm.

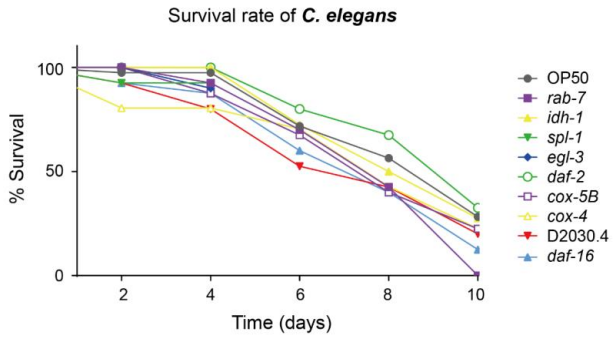


Fig. 2A. Survival rate of C. elegans fed with RNAi bacteria (D2030.4 and daf-16).

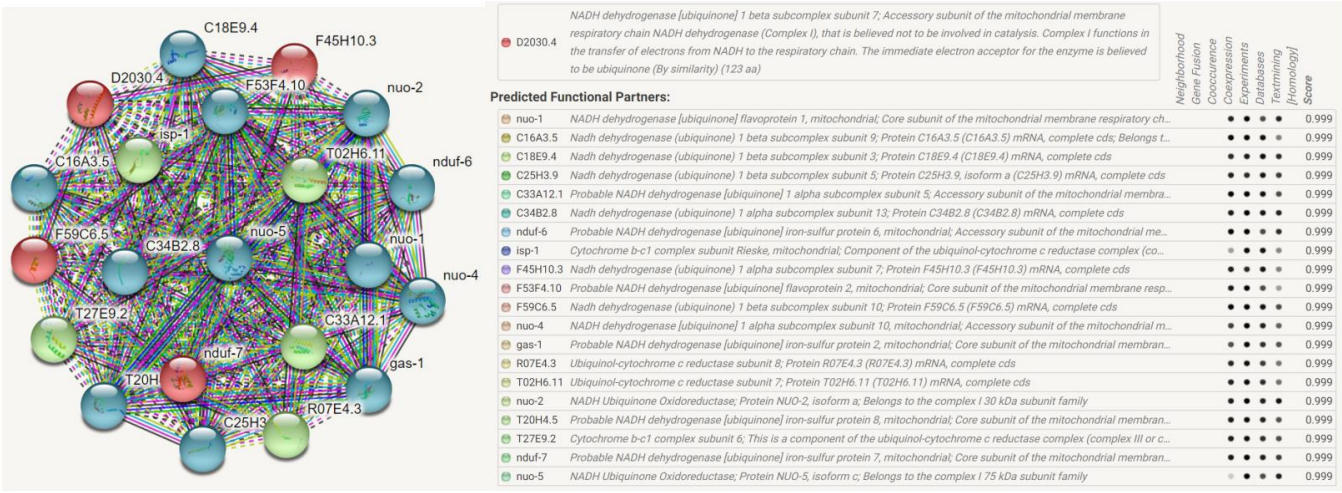


Fig. 3A. Predicted Functional Partners of D2030.4.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Qinya Liu conducted the research and analyzed the data; Qinya Liu and Yang Li wrote the paper; all authors had approved the final version.

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