# Reconciling the *clk-1* and aging paradox and categorizing lifespan curves by taking individual specificity into account

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## Abstract

*Clk-1* gene encodes the demethoxyubiquinone (DMQ) hydroxylase that is required for biosynthesis of ubiquinone (coenzyme Q). Deletion of *clk-1* is lethal in mice, but its mutation in *C. elegans* mildly extends lifespan, slows physiological rate and leads to sickness. We found that if growth retardation was taken into account the average lifespan of *clk-1* mutants would not be prolonged or would be shortened, suggesting the necessity to reevaluate the

role of *clk-1* in aging. In addition, recent study showed that knocking down of *clk-1* shortened lifespan. Although the extension of lifespan in *clk-1* mutants was mild and was not observed sometimes, some progenies indeed had prolonged maximum lifespan even if retardation of growth was taking into account. These paradoxes implicate the existence of individual specificity in the aging process even in the same cohort, just like a drug is beneficial for some people while for others it is detrimental. We also categorized the lifespan curves into five patterns to reflect the kinds of lifespan alternations observed in organisms: N (normal); L (long-lived); S (short-lived); F (flattened); ST (steepened), and found that the curve of *clk-1* mutants fit into the F pattern. The mechanism behind the individual specificity in aging is yet to be revealed.

**Keywords:** *clk-1*; lifespan; individual specificity; aging; *C. elegans*

## 1. Introduction

Coenzyme Q (CoQ), also known as ubiquinone (UQ), is mainly localized in mitochondrion and functions to transport electrons in the respiratory chain (Larsen and Clarke 2002; Vajo et al., 1999). *Clk-1* encodes the *C. elegans* ortholog of COQ7/CAT5 that is necessary for biosynthesis of Coenzyme Q (Ewbank et al., 1997; Gu et al., 2017). Mutation of *clk-1* led to pleiotropic phenotypes including retardation of growth, reduced brood size, long defecation cycle, and mild extension of lifespan (Lakowski and Hekimi 1996;

Takahashi et al., 2012). *Clk-1* was considered as one of the first genes found to be correlated with aging and was widely used to explore aging and neurodegenerative disease related mechanisms (Gu et al., 2017; Lakowski and Hekimi 1996; Larsen and Clarke 2002). However, RNAi knocking down of *clk-1* shortened lifespan while growth rate was not slowed down (Ren et al., 2015; Zhang et al., 2017). In mice, deletion of *clk-1* was lethal and its reduced expression promoted neuroinflammation and subsequent death of dopaminergic cells (Gu et al., 2017; Nakai et al., 2001). These results suggest the complexity of the effects of *clk-1* deficiency on aging.

## 2. Material and methods

### 2.1. *C. elegans* strains

The wild type N2 and the *clk-1* mutant MQ130 *clk-1(qm30) III* strains were obtained from Caenorhabditis Genetics Center (University of Minnesota, USA), and were fed with E. coli OP50 as described by Brenner (Brenner 1974).

### 2.2. Lifespan tests

Lifespan was tested as described with minor modifications (Ren et al., 2015). Briefly, twenty gravid adults were put onto OP50 seeded NGM plates and were let lay eggs for eight hours. Then the adults were removed and when the F1 progenies were reached L4 stage, one hundred of them were transferred onto fresh prepared plates every day during the reproduction period, and

every two or three days after that. Worms that did not respond to gentle nose touch with the toothpick were scored as dead, and that crawled off the plate or died from egg hatching in the uterus were censored.

### 2.3. Phenotype analysis

Phenotypes including body size and brood size were analyzed as described (Ren et al., 2012).

### 2.4. Statistical analysis

The Kaplan-Meier method and log-rank test were performed for analysis of lifespan data. Paired student's-t test was used for growth and reproduction analysis. Differences in means were considered statistically significant at $p < 0.05$. Data were analyzed by using the Graphpad Prism 5.0 software.

## 3. Results and discussion

We also found phenotypes including slowing down of growth (Fig 1A, B), reduction and retardation of reproduction (Fig 1C), and mild extension of lifespan in worms with *clk-1* mutation (Zhang et al., 2017). But the increase of longevity was not reproduced quite well (Fig 1D), perhaps due to the minor effect on lifespan or aging. Consistently, the average lifespan of worms with *clk-1* mutations was even found to be shortened (Larsen and Clarke 2002), although other labs reported mild extension by around 5% to 20% percent or 1 to 3 days (Lakowski and Hekimi 1996; Takahashi et al., 2012). We noticed

that the adult or post growth span (scored from the time when rapid growth stopped) of the *clk-1* mutants was not prolonged (Zhang et al., 2017), and was even shortened as shown here (Fig. 1E), suggesting retardation of growth contributed largely if not completely to lifespan extension associated with *clk-1* mutation.

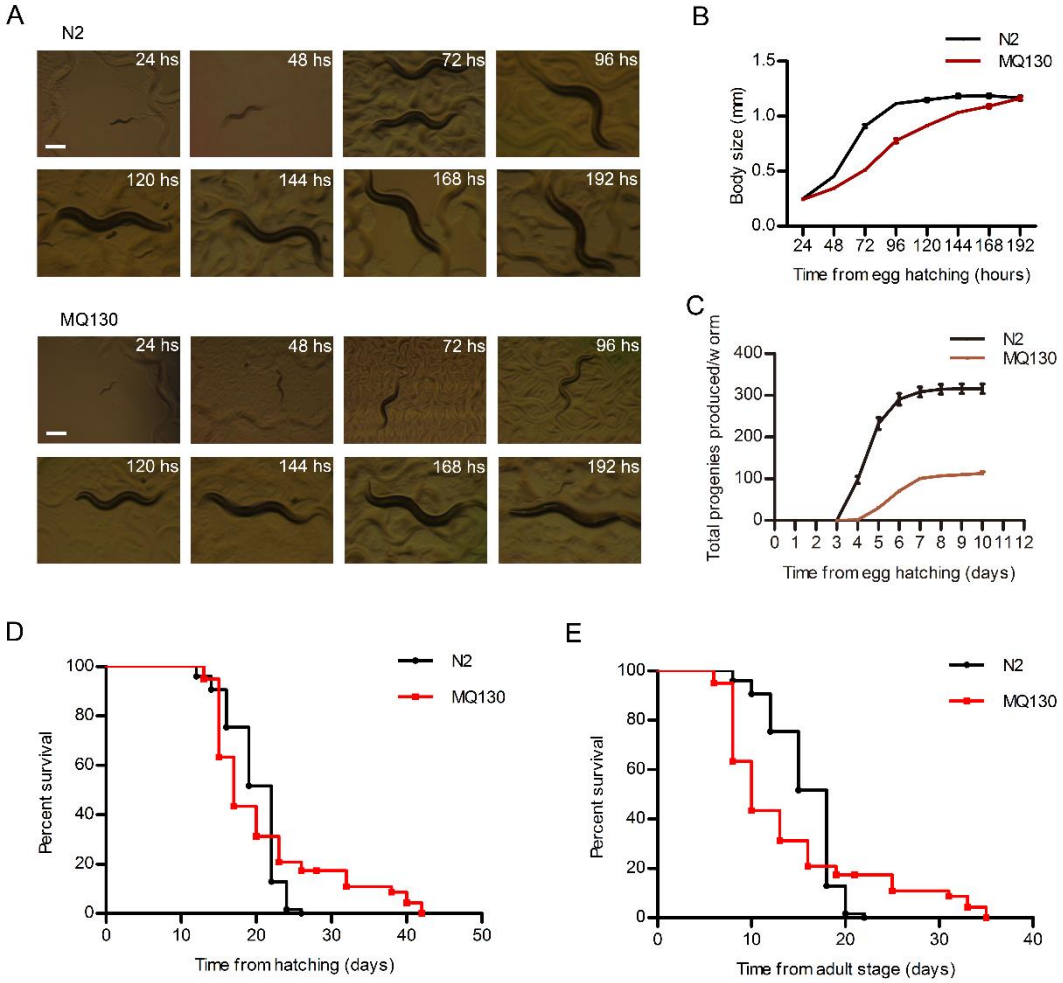


**Fig. 1. Analyzing lifespan date of the *clk-1* mutants (MQ130) by taking retardation of physiological behavior into account.** (A, B) Growth of the *clk-1* mutant strain MQ130 was retarded, compared to that of wild type N2, $p<0.05$. Scale bars= 200μm. (C) Reproduction was reduced and retarded in *clk-1* mutants, $p<0.01$. (D) Average lifespan of MQ130 (20.28±7.76 days, n=61) was similar to that of N2

(19.60±3.40 days, n=75), $p>0.05$. (E) Average adult lifespan of MQ130 (13.28±7.76, n=75) was reduced compared to that of N2 (15.60±3.40, n=61), $p<0.05$.

The physiological behavior was also slowed down in adults (Vajo et al., 1999), and might contribute to lifespan extension too. Then is it reasonable to interpret slowing down of physiological behavior as slowing down of aging? We speculate that the answer should be “not”, considering retardation of physiological behaviors including growth is quite common in *C. elegans* under detrimental conditions. For example, in large scale genomic RNAi screens growth could be retarded by knocking down hundreds of genes respectively (Simmer et al., 2003). And comparing to vertebrates, worms’ growth was much easier to be affected by environmental stresses such as perturbation of temperatures (Hirsh and Vanderslice 1976). If slowing down of growth is interpreted as slowing down of aging, the aging related factors will be too many for researchers. Thus, the extension of the post-growth span rather than the entire lifespan should be considered as evidence for slowing down of aging sometimes. Interestingly, although the average lifespan was not prolonged by *clk-1* mutation, the maximum lifespan was increased and the minimum lifespan was decreased as shown by crossing of survival curves of the *clk-1* mutant strain MQ130 and wild type N2. The results are supported by previous studies (Larsen and Clarke 2002; Zhang et al., 2017). The maximum lifespan of *clk-1* mutant was more than 40 days, which cannot be explained by retardation of growth. The combined results suggest there is individual

specificity in aging process, which may also exist in all organisms. For example, most people die at around seventy years old, while a few can live to more than one hundred years old. It is reasonable that some worms were unusually long-lived while some others were short lived under *clk-1* loss of function. Just like a drug that is beneficial for some persons but for others it might not work or is detrimental.

According to the excessive response model, when the prooxidant stress goes high the antioxidant stress will go higher and lower ROS will be observed in the long-term (Ren and Zhang 2017). This response is used to guard against more serious and unpredictable conditions forthcoming (Ren and Zhang 2017). Similar mechanisms may also exist in the context of mitochondrial dysfunctions such as that induced by deficiency of *clk-1*. Consistently, retrograde responses were indeed motivated including increased expression of antioxidant enzymes and chaperones, increased autophagy, reprogramming of energy metabolism, and increased activity of the hypoxia-inducible factor HIF-1 (Shore et al., 2012). Some of these responses were also reported to have anti-aging effects besides enhancing adaptions (Shore et al., 2012). Lifespan should be affected by both pro-aging factors such as the side effects resulted from cellular dysfunctions and the anti-aging factors such as some of the retrograde responses shown above (Fig. 2A). Due to individual specificity, in some *clk-1* mutated worms the effects of the pro-aging factors overshadowed that of the anti-aging factors

and lifespan would be shortened. But in some other worms, the effects of anti-aging factors overshadowed that of the pro-aging factors and the lifespan was prolonged. This perspective explains why the survival curve of *clk-1* mutants was flattened and crossed that of N2 (Larsen and Clarke 2002). Here, the lifespan curves were classified into five kinds of patterns (Fig. 2B).

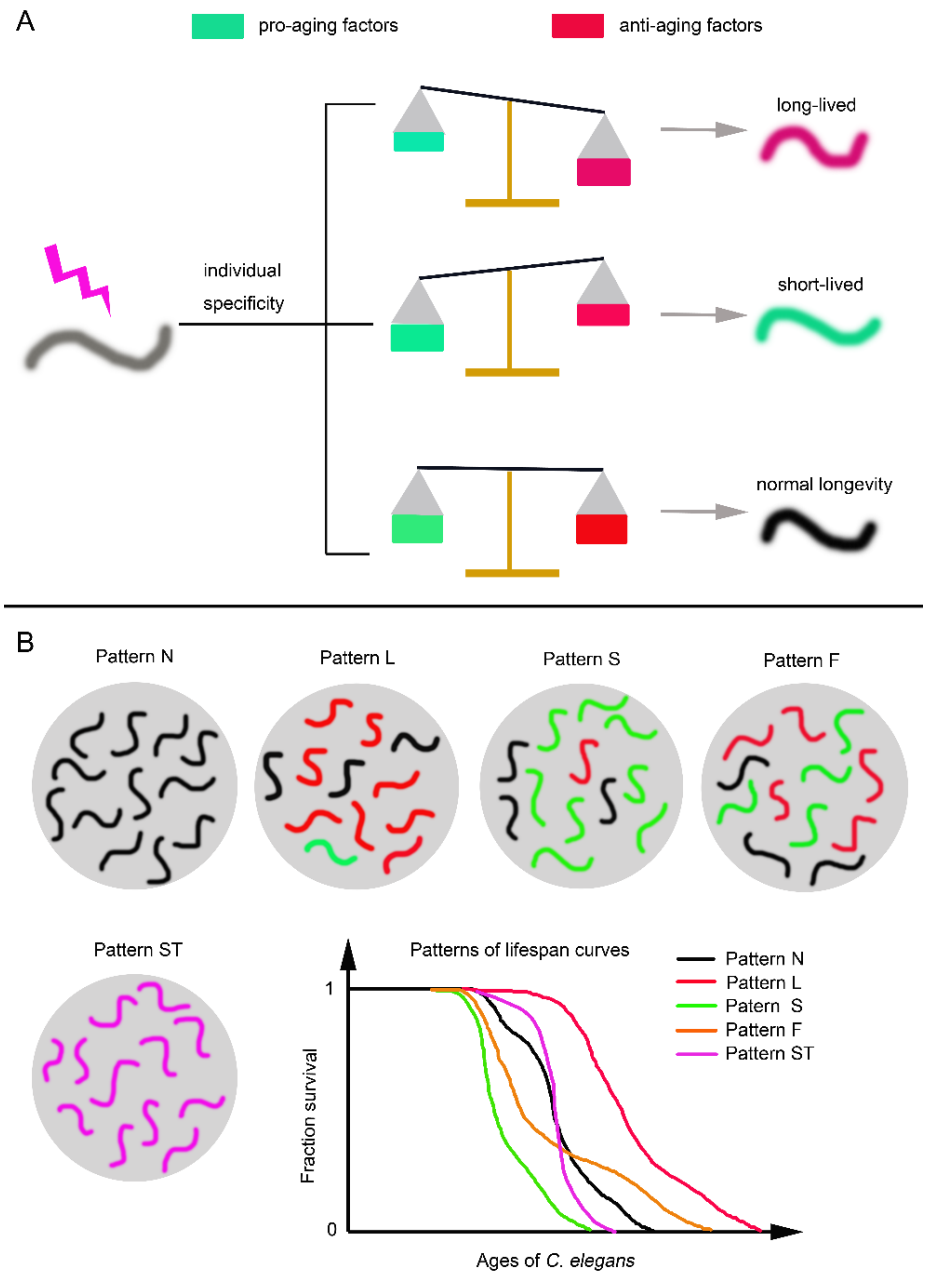


**Fig. 2. Categorization of lifespan curves by taking individual specificity**

**into account.** (A) If organisms are stressed side effects will be produced and retrograde responses will be activated. Due to differences in individual specificity, the individual's lifespan will be different. (B) Categorization of lifespan curves based on individual specificity. Black, red, green colors indicate worms that have relatively normal, prolonged, and shortened lifespan respectively. Purple color indicates cohort of worms whose variation of lifespan is smaller than that of wild type N2.

The curves similar to that of *clk-1* mutant are defined as F (flattened) patterned. In *clk-1* RNAi worms, the side effects were less severe and the retrograde responses should also be motivated to relatively lower extent than in *clk-1* mutants, because of residual levels of *clk-1* mRNA. According to the excessive response model it is very likely that in *clk-1* RNAi worms the anti-aging factors failed to compete with pro-aging factors, which explains their short average lifespan. The lifespan curves of *clk-1* RNAi worms fit into the S (short-lived) pattern. Worms with mutation in *mev-1*, encoding subunit of mitochondrial respiratory chain complex II, should also have S patterned survival curve (Ishii et al., 1990). Worms with mutations in *daf-2*, *mfn-1*, *cco-1*, *nuo-6*, or others had dramatically prolonged lifespan (Apfeld and Kenyon 1998; Ren et al., 2015), and their survival curves should be L (long-lived) pattern. Lifespan curves similar to that of wild type N2 are defined as N (normal) patterned. It is predictable that there exists a ST (steepened) curve, the average lifespan of which is similar to that of N2, but the minimum lifespan is increased and the maximum lifespan is decreased. This ST patterned

survival curve is yet to be discovered. It seems that knocking down of *Y45F10D.4*, which encodes a putative iron-sulfur cluster assembly enzyme, is likely to produce the ST patterned curve (unpublished data). Unlike worms whose body plan is simple, vertebrates like mice have much more complicated tissues and organs and deletion of *clk-1* should be much more detrimental and unbearable for them, and it is not surprising that lethality will be observed.

In conclusion, by taking into account previously unnoticed factors including individual specificity, the excessive response, and retardation of growth, we here explained why in *clk-1* RNAi worms the lifespan was shortened while in *clk-1* mutants it was only mildly prolonged or unchanged. We also categorized the lifespan curves into five patterns to reflect different kinds of lifespan alternations discovered in model organisms. The individual specificity in aging might be affected by epigenetic factors, although in-depth studies are needed in the future.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Author contributions

Y.R. conceived this work; Y.R., Congjie Z., and W.G. performed the experiments; Y.R. and Chao Z. contributed to the analysis and interpretation

of data; Y.R. and Chao Z. wrote the manuscript; Chao Z. provided reagents and instruments.

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