Current Biology 19, 1591–1598, October 13, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2009.08.016

Article

Extension of *Drosophila* Life Span by RNAi of the Mitochondrial Respiratory Chain

Jeffrey M. Copeland,[1] Jaehyoung Cho,[1] Thomas Lo, Jr.,[1] Jae H. Hur,[1] Sepehr Bahadorani,[1] Tagui Arabyan,[1] Jason Rabie,[1] Jennifer Soh,[1] and David W. Walker[1,2,*]

[1]Department of Physiological Science

[2]Molecular Biology Institute

University of California, Los Angeles, Los Angeles, CA 90095, USA

Summary

Background: Mitochondria have long been proposed to play an important role in the aging process. In the nematode *Caenorhabditis elegans*, genes important for mitochondrial electron transport chain (ETC) function stand out as a principal group of genes affecting life span. However, it has been suggested that this may be a peculiarity of nematode biology. In the present study, we have used an in vivo RNA interference (RNAi) strategy to inactivate ETC genes in *Drosophila melanogaster* and examine the impact on longevity.

Results: Here, we report that RNAi of five genes encoding components of mitochondrial respiratory complexes I, III, IV, and V leads to increased life span in flies. Long-lived flies with reduced expression of ETC genes do not consistently show reduced assembly of respiratory complexes or reduced ATP levels. In addition, extended longevity is not consistently correlated with reduced fertility or increased resistance to the free-radical generator paraquat. Targeted RNAi of two complex I genes in adult tissues or in neurons alone is sufficient to extend life span.

Conclusions: Our data suggest that the role of mitochondrial ETC function in modulating animal aging is evolutionarily conserved and might also operate in humans. Furthermore, our findings suggest that the longer life span of flies with reduced ETC gene expression cannot simply be attributed to reduced energy production leading to decreased "rate of living."

Introduction

The fruit fly *Drosophila melanogaster* provides an excellent experimental system to investigate the effects of perturbations in mitochondrial electron transport chain (ETC) function on longevity. Flies, like mammals, live in an oxidative environment, and insect flight muscle is reported to be among the most metabolically active tissues known [1]. Previously, we reported that flies carrying a defect in subunit b of respiratory complex II (*sdhB*) suffer from elevated oxidative stress and display hallmarks of rapid aging [2]. Interestingly, the biochemical and phenotypic consequences of complex II deficiency in flies are very similar to those of complex II deficiency in worms [3]. In this study, we examined the impact of inactivation of additional ETC genes on fly life span. We found that inactivation of five genes encoding components of respiratory complexes I, III, IV, and V can prolong life span in flies. This finding clearly establishes ETC gene manipulation as an evolutionarily conserved mechanism of life span extension. Interestingly, ETC-mediated longevity in the fly can be separated from major energetic and physiological tradeoffs, such as ATP production and fertility defects.

*Correspondence: davidwalker@ucla.edu

Results and Discussion

RNAi Inactivation of ETC Genes throughout Life Can Extend Life span

Recently, the generation of a genome-wide library of *Drosophila* RNA interference (RNAi) transgenes, enabling the systematic inactivation of gene functions in specific tissues of the intact organism, was reported [4]. The RNAi transgenes consist of short gene fragments cloned as inverted repeats and expressed via the binary GAL4/UAS system [5]. Taking advantage of this collection, we used a ubiquitous expression GAL4 line, *daughterless (da)*-GAL4, to systematically inactivate nuclear-encoded components of the fly ETC machinery (31 complex I subunits, three complex II subunits, five complex III subunits, six complex IV subunits, and eight complex V subunits) and studied the impact on viability and adult longevity. Many of the RNAi lines caused lethality or shortened life span (see Table S1, available online). In contrast, a number of RNAi lines were associated with increased life span (relative to the other RNAi lines and the control strain w^{1118}).

Genetic background effects often confound life span studies in *Drosophila* [6]. When working with the GAL4/UAS system, hybrid vigor effects are of notable concern. To confront this issue, we used the mifepristone (RU486) inducible-GAL4 system (annotated P[Switch] or Gene-Switch [7, 8]). This system, recently characterized in aging studies [9], eliminates genetic background effects because all flies share the same genetic background and only differ with respect to the presence of the inducing agent (RU486) or diluent (EtOH) in the food. We used the ubiquitous tubulin (tub)-Gene-Switch (GS) driver line to manipulate RNAi transgenes during both development and adulthood. We focused on three candidate longevity genes, isolated in the pilot screen (*CG9762*, *CG18809*, and *CG5389*). In addition, we examined two additional genes (*CG9172* and *CG17856*) that resulted in larval lethality with the strong ubiquitous constitutive driver *da-GAL4*. Using *tub-GS*, we observed that RNAi of each of these five genes results in prolonged life span in female flies (Figures 1A–1J) and variable effects on male life span (Table S2). Specifically, RNAi of genes encoding subunits of complex I (*CG9172*, Figures 1A and 1B and *CG9762*, Figures 1C and 1D), complex III (*CG17856*, Figures 1E and 1F), complex IV (*CG18809*, Figures 1G and 1H), and complex V (*CG5389*, Figures 1I and 1J) increased mean life span in female flies by 8% to 19%. We investigated the possibility that RU486 itself may affect longevity in our fly strains by simultaneously examining the *tub-GS* driver crossed to w^{1118}, the genetic background of the five RNAi lines, fed RU486 or diluent alone. Importantly, RU486 did not have positive effects on the longevity in control flies at any concentration tested (Figure S1 and Table S2). Other studies using the Gene-Switch system have also failed to see any significant effect of RU486 on life span [10–12]. Using quantitative real-time PCR (qRT-PCR), we confirmed transcript reduction specific for each targeted gene in the

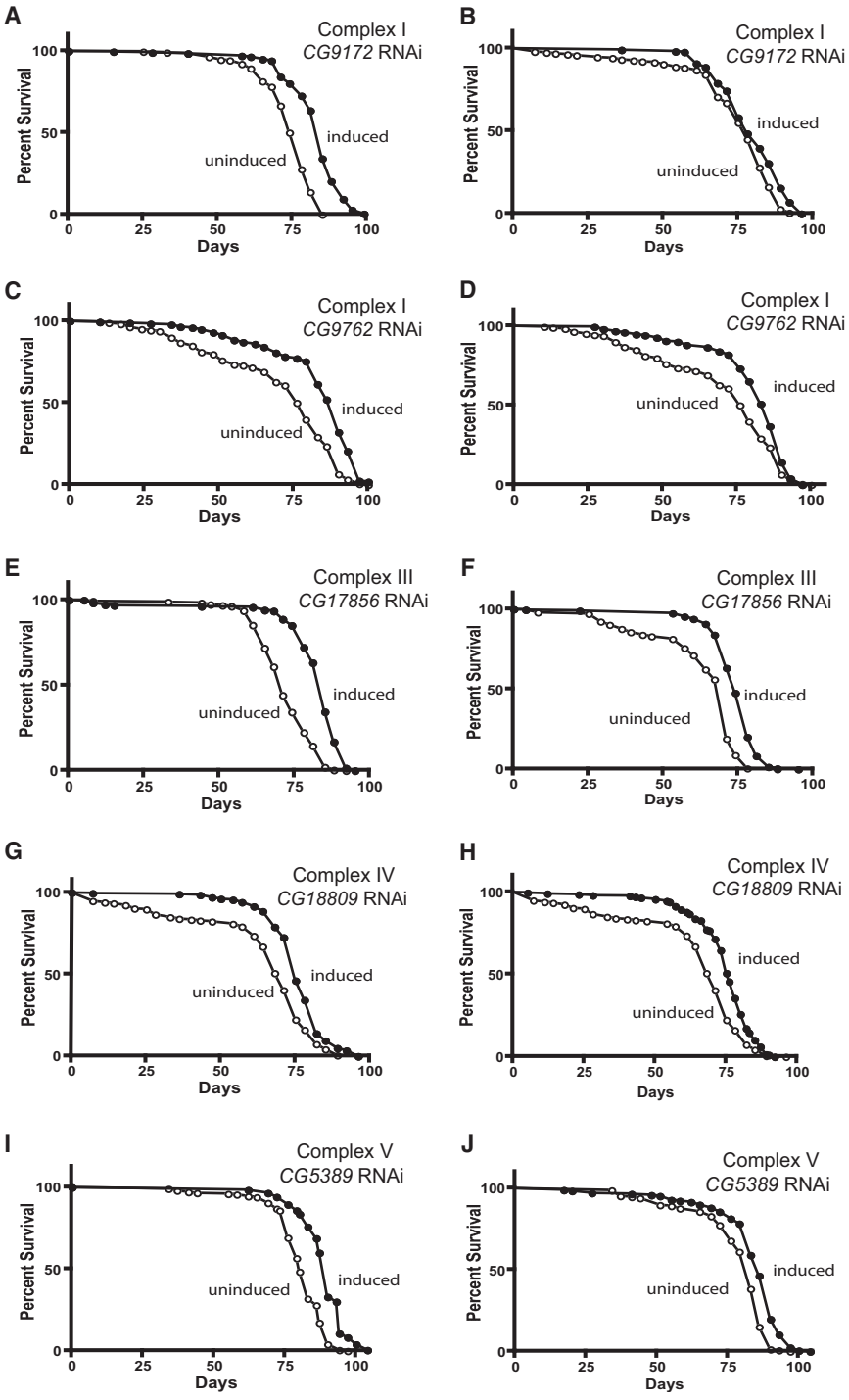

Figure 1. RNAi of Respiratory-Chain Genes Can Extend Life Span

All RNAi lines were crossed to the ubiquitous, inducible tubulin Gene-Switch driver line and are shown as induced (+RU486, solid circles) or uninduced (−RU486, open circles).

(A and B) Replicate life-span curves of RNAi knockdown of the respiratory complex I gene *CG9172*, showing an increased mean life span in females of 12% (A) and 8% (B) on 2 μg/ml RU486 during larval development and 10 μg/ml RU486 during adulthood (2/10 μg/ml RU486).

(C and D) Life-span curves of RNAi knockdown of the respiratory complex I gene *CG9762*, showing an increased mean life span in females of 18% (C) and 14% (D) on 10/50 μg/ml and 2/10 μg/ml RU486, respectively.

(E and F) Replicate life-span curves of RNAi knockdown of the respiratory complex III gene *CG17856*, showing an increased mean life span in females of 13% (E) and 18% (F) on 2/10 μg/ml RU486.

(G and H) Life-span curves of RNAi knockdown of the respiratory complex IV gene *CG18809*, showing an increased mean life span in females of 19% (G) and 16% (H) on 2/10 μg/ml and 10/50 μg/ml RU486, respectively.

(I and J) Life-span curves of RNAi knockdown of the respiratory complex V gene *CG5389*, showing an increased mean life span in females of 11% (I) and 7% (J) on 10/50 μg/ml and 2/10 μg/ml RU486, respectively.

$p < 0.0002$ for all life-span experiments.

induced RNAi lines during both development (Figure S2) and adulthood (Figure S3). Interestingly, further RNAi induction of both *CG9172* and *CG17856* by higher RU486 concentrations caused larval lethality (Table S2). This observation is consistent with the fact that *da-GAL4*-mediated silencing of these genes results in larval lethality (Table S1).

Orthologs of two of these genes have been reported to modulate nematode life span. *T02H6.11* [13], an ortholog of *CG17856* (complex III), and *atp-2* [14], an ortholog of *CG5389* (complex V), were both identified in large-scale RNAi screens to identify novel longevity genes in the worm. Long-lived ETC-deficient worms are often small and display reduced rates of behavior and development [15]. To assess whether long-lived flies display any such gross physiological trade-offs, we measured timing from egg to adulthood and climbing ability. RNAi induction of the complex I gene *CG9762* did not affect development time or ability to climb (Figures S4A and S4B). Other measurements show that long-lived flies are of the same size (Figure S4C) and mass (data not shown) as control flies.

Long-Lived Flies with Reduced ETC Gene Expression Do Not Consistently Display Reduced Assembly of Respiratory Complexes or Reduced ATP Levels

Each of the respiratory enzyme complexes comprises multiple subunits (with the exception of complex II) encoded by both the mitochondrial and nuclear genomes [16]. The coordinated assembly of these subunits is poorly understood. To determine whether RNAi of individual ETC genes affects the assembly of the respective respiratory complexes, we employed blue native polyacrylamide gel electrophoresis (BN-PAGE) (Figure 2A). We discovered that induced RNAi of the complex I subunits, *CG9172* and *CG9762*, had different effects on complex I assembly. Specifically, whereas RNAi of *CG9172* resulted in a significant decrease in fully assembled complex I, there was no detectable complex I assembly defect in flies with reduced expression of *CG9762* (Figure 2A). Similarly, RNAi of the complex III gene *CG17856* resulted in

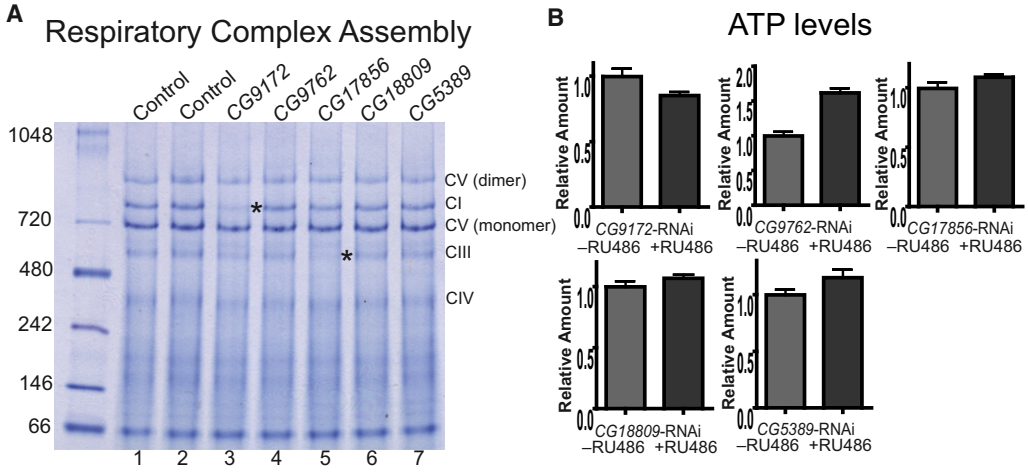

Figure 2. Long-Lived Flies with Reduced Respiratory-Chain Gene Expression Do Not Show Consistent Respiratory-Complex Assembly Defects or Reduced ATP Levels

All RNAi lines were crossed to the ubiquitous, inducible tubulin Gene-Switch driver line and were fed RU486 during both development and adulthood.
(A) BN-PAGE was performed on mitochondrial proteins isolated from control flies (w^{1118}/*tub-GS*) induced with 2 μg/ml RU486 during development and 10 μg/ml RU486 during adulthood (2/10 μg/ml RU486; lane 1) and with 10/50 μg/ml RU486 (lane 2), induced RNAi of *CG9172* with 2/10 μg/ml RU486 (lane 3), induced RNAi of *CG9762* with 10/50 μg/ml RU486 (lane 4), induced RNAi of *CG17856* with 2/10 μg/ml RU486 (lane 5), induced RNAi of *CG18809* with 2/10 μg/ml (lane 6), and induced RNAi of *CG5389* with 10/50 μg/ml RU486 (lane 7). In lane 3, RNAi of the complex I subunit *CG9172* confers a complex I assembly defect. In lane 5, RNAi of the complex III subunit *CG17856* confers a complex III assembly defect.
(B) ATP levels. Induced RNAi of *CG9172* with 2/10 μg/ml RU486 does not lead to reduced ATP level ($p = 0.1049$). Induced RNAi of *CG9762* with 10/50 μg/ml RU486 leads to a 62% increase in ATP level ($p = 0.0028$). Induced RNAi of *CG17856* with 2/10 μg/ml RU486 does not lead to reduced ATP level ($p = 0.2208$). Induced RNAi of *CG18809* with 2/10 μg/ml RU486 does not lead to reduced ATP level ($p = 0.2975$). Induced RNAi of *CG5389* with 10/50 μg/ml RU486 does not lead to reduced ATP level ($p = 0.1662$).
Data represent mean ± standard error of the mean (SEM) ($n = 3$).

a decrease in fully assembled complex III, whereas RNAi of both the complex IV (*CG18809*) gene and the complex V (*CG5389*) gene did not confer assembly defects (Figure 2A). RU486 did not have any affect on the assembly of any of the respiratory complexes in control flies (w^{1118}/*tub-GS*). Our data indicate that assembly defects are not required to promote longevity.

To investigate whether the ETC gene manipulations that confer increased life span affect overall energy production, we examined ATP levels in long-lived flies (Figure 2B). In doing so, we observed no major deficit in ATP levels in any of the long-lived RNAi lines. Induced RNAi of the complex I subunit, *CG9172*, had no major impact on ATP content. In contrast, induced RNAi of the other complex I subunit, *CG9762*, resulted in increased ATP levels. RNAi of *CG17856* (complex III), *CG18809* (complex IV), or *CG5389* (complex V) had no major impact on ATP levels. RU486 had no effect on ATP levels in control flies (Figure S5).

In four of the five long-lived RNAi lines, ATP levels were not significantly reduced relative to control (uninduced) animals. Interestingly, RNAi of the complex I subunit, *CG9762*, resulted in increased ATP levels. It remains to be seen whether this is a result of compensatory changes in other components of the ETC or in alternative energy-generating pathways. In any case, our findings indicate that ETC gene manipulations that extend longevity do not necessarily impact the assembly of the respiratory complexes or overall ATP levels.

Long-Lived Flies with Reduced ETC Gene Expression Do Not Consistently Display Reduced Fertility

To assess whether ETC-mediated longevity is associated with reproductive trade-offs, we examined female fertility over the first 30 days of adulthood in each of the long-lived RNAi lines. Induced RNAi of both complex I subunits, *CG9172* and *CG9762*, resulted in reduced fertility (Figures 3A and 3B). Similarly, induced RNAi of *CG17856* (complex III) and *CG5389* (complex V) also resulted in reduced fertility (Figures 3C and 3E). However, we observe that induced RNAi of *CG18809* (complex IV) did not confer any fertility deficit (Figure 3D). RU486, at high concentrations, had a negative impact on fertility in control (w^{1118}/*tub-GS*) flies (Figure S6).

Four of the five RNAi-based ETC gene manipulations that we observe to prolong longevity also negatively impact fertility. However, the fact that even one of the ETC gene manipulations (complex IV; *CG18809*) does not impact fertility indicates that ETC-mediated longevity, in the fly, can be uncoupled from reproductive trade-offs under these conditions. It is interesting that four of the RNAi lines displayed reduced fertility despite the fact that overall ATP levels were not reduced. This finding suggests that reduced fertility in these lines may not result from reduced energy levels, but may instead be the result of alterations in cell-signaling pathways.

Long-Lived Flies with Reduced ETC Gene Expression Do Not Consistently Display Increased Resistance to Free-Radical or Starvation Stress

In *Drosophila*, long-lived mutants often display enhanced resistance to oxidative stress [10, 17]. Therefore, we examined whether long-lived flies with reduced ETC gene expression were more resistant to dietary paraquat (PQ), which has been shown to generate reactive oxygen species (ROS) in vivo [18]. Induced RNAi of both complex I subunits, *CG9172* and *CG9762*, resulted in improved tolerance to PQ poisoning (Figures 4A and 4B). Similarly, induced RNAi of *CG17856* (complex III) led to a robust increase in paraquat resistance (Figure 4C). However, induced RNAi of *CG18809*

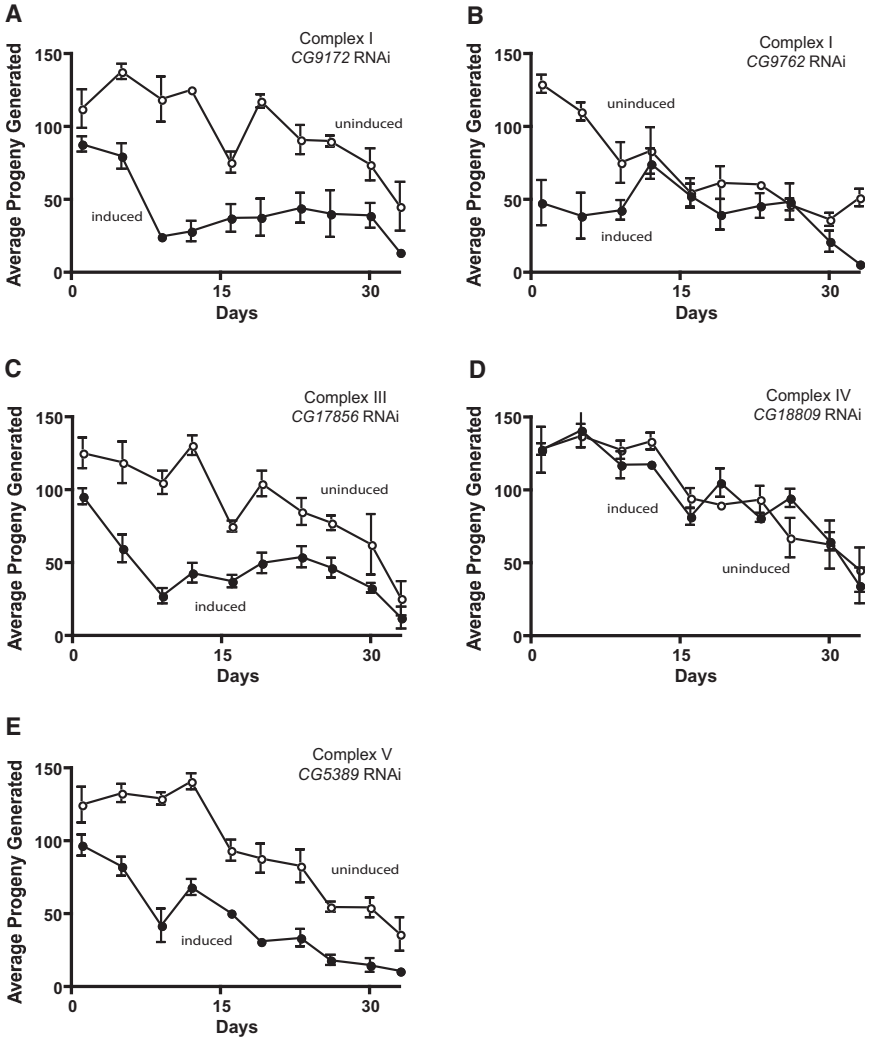

Figure 3. Reduced Fertility Is Not a Universal Feature of Long-Lived Flies with Reduced Respiratory-Chain Gene Expression

(A–E) All RNAi lines were crossed to the ubiquitous, inducible tubulin Gene-Switch driver. Females were fed RU486-spiked food during both development and adulthood; shown are curves as induced (+RU486, solid circles) or uninduced (−RU486, open circles).

(A) RNAi induction of complex I *CG9172* by 2 μg/ml RU486 during larval development and 10 μg/ml RU486 during adulthood (2/10 μg/ml RU486) lowers female fertility by 56% when compared to uninduced RNAi control lines ($p < 0.0001$).

(B) RNAi induction of complex I *CG9762* by 10/50 μg/ml RU486 lowers female fertility by 41% when compared to uninduced RNAi control lines ($p = 0.0079$).

(C) RNAi induction of complex III *CG17856* by 2/10 μg/ml RU486 lowers female fertility by 49% when compared to uninduced RNAi control lines ($p = 0.0010$).

(D) RNAi induction of complex IV *CG18809* by 2/10 μg/ml RU486 does not affect female fertility when compared to uninduced RNAi control lines ($p = 0.4620$).

(E) RNAi induction of complex V *CG5389* by 10/50 μg/ml RU486 lowers female fertility by 52% when compared to uninduced RNAi control lines ($p = 0.0022$).

Data represent mean ± SEM ($n = 4$).

(complex IV) or *CG5389* (complex V) did not confer increased resistance to PQ (Figures 4D and 4E). Administration of RU486 to the w^{1118} background conferred a slight sensitivity to PQ (Figure S7). To determine whether long-lived flies display a general resistance to extrinsic stress, we examined the resistance of the ETC-deficient flies to starvation. As opposed to the increase in resistance to paraquat, induced RNAi of the complex I subunits, *CG9172* and *CG9762*, did not improve the ability to withstand starvation (Figures S8A and S8B). Similarly, induced RNAi of *CG17856* (complex III), *CG18809* (complex IV), or *CG5389* (complex V) did not confer increased tolerance to starvation (Figures S8C–S8E).

Interestingly, we observe that increased tolerance to the ROS-generating agent paraquat is not a universal feature of long-lived ETC-deficient flies. Similar findings were reported in the worm [13], and there is no correlation between extended life span and decreased oxidative damage in ETC mutant worms [19, 20]. Our data suggest that RNAi of complex I or complex III, believed to be the major sites of ROS production [21], can protect against dietary paraquat.

Induced RNAi of Complex I Specifically in Adult Tissues Extends Life span

An advantage of the Gene-Switch system is that it can be used to determine the period(s) in life during which reduction in ETC gene expression is sufficient to promote longevity. Next, we investigated the effects of manipulating ETC gene function specifically in adult tissues. Induced RNAi of the complex I subunits, *CG9762* and *CG9172*, from the onset of adulthood, increased mean life span in female flies by 11% to 46% (Figures 5A–5D) and had variable effects in male flies (Table S3). These data indicate that selective inactivation of complex I genes in adult tissues is sufficient to extend life span. Interestingly, adult-specific RNAi of *CG17856* (complex III), *CG18809* (complex IV), or *CG5389* (complex V) did not confer robust longevity effects in male or female flies (Figures 5E–5J) and Table S3. Adult-only feeding of RU486 to control flies (w^{1118}/*tub-GS*) did not produce any positive effect on life span (Figure S1). qRT-PCR confirmed gene-specific mRNA knockdown in adult-induced RNAi flies (Figure S9).

To determine whether adult-specific ETC gene perturbations also impact reproductive output, we examined female fertility over the first 30 days of adulthood in each of the adult-induced RNAi lines (Figure S10). Adult-specific RNAi of both complex I genes, *CG9762* and *CG9172*, which increased longevity, also resulted in decreased fertility (Figures S10A and 10B). Interestingly, adult-specific RNAi of complex III (*CG17856*), which did not extend life span, did confer a fertility deficit (Figure S10C). Adult-specific RNAi of complex IV (*CG18809*) or complex V (*CG5389*) did not affect longevity or fertility (Figures S10D and S10E).

Our results highlight a potential difference between the observed life span extension in *C. elegans* and *Drosophila*: it has been reported that respiratory-chain RNAi needs to occur during the larval stages to increase longevity in worms [15, 19], whereas in *Drosophila* RNAi of two different complex I genes in

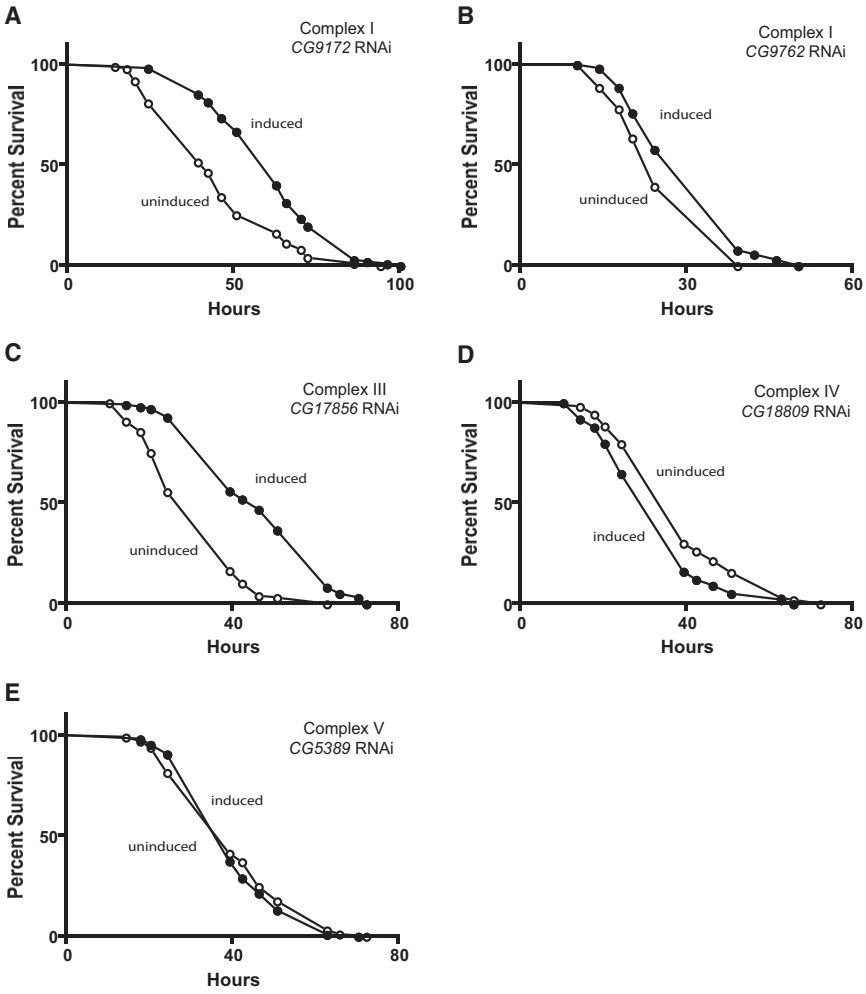

Figure 4. Increased Tolerance to the Free-Radical Generator Paraquat Is Not a Universal Feature of Long-Lived Flies with Reduced Respiratory-Chain Gene Expression

All RNAi lines were crossed to the ubiquitous, inducible tubulin Gene-Switch driver, and resistance to 30 mM paraquat was examined.

(A) RNAi knockdown of the respiratory complex I gene *CG9172* increases mean survival time by 36% ($p < 0.0001$), on 2 μg/ml RU486 during larval development and 10 μg/ml RU486 during adulthood (2/10 μg/ml RU486).

(B) RNAi knockdown of the respiratory complex I gene *CG9762* increases mean survival by 16% ($p = 0.0008$), on 10/50 μg/ml RU486.

(C) RNAi knockdown of the complex III gene *CG17856* increases mean survival by 51% ($p < 0.0001$), on 2/10 μg/ml RU486.

(D) Induced complex IV *CG18809* RNAi mutants live 15% shorter than uninduced RNAi controls ($p = 0.0017$), on 2/10 μg/ml RU486.

(E) No change in paraquat resistance was observed in induced complex V *CG5389* RNAi mutants ($p = 0.6313$), on 10/50 μg/ml RU486.

adult tissues is sufficient to extend life span. Furthermore, our observation that adult-specific inactivation of the complex III gene, *CG17856*, confers a fertility defect but does not extend longevity further supports the mechanistic uncoupling of fertility defects from ETC-mediated longevity.

RNAi of Complex I and IV in Adult Neurons Extends Life Span without Decreasing Fertility

Knowing that ubiquitous disruption of ETC gene expression increases life span, we wanted to determine whether a single tissue could mediate the effect. The nervous system has been shown to be of particular importance for life span extension in a number of organisms [22]. Therefore, we used the pan-neuronal ELAV-Gene-Switch (GS) driver line to decrease ETC gene expression specifically in neurons. Our previous results with the ubiquitous *tub-GS* driver line suggested that perturbing ETC genes in adult tissues can extend life span. Therefore, we tested the effects of both constitutive neuronal RNAi (Figures 6A, 6C, 6E, and 6G) and adult-only neuronal RNAi (Figures 6B, 6D, 6F, and 6H). Induced RNAi of the complex I genes, *CG9762* and *CG9172*, both constitutively and in adult neurons, increased mean life span in female flies by 6% to 24% (Figures 6A–6D) and had variable effects in male flies (Tables S4 and S5). In a similar fashion, neural RNAi of *CG18809* (complex IV) increased mean life span in female flies by 8% to 12% (Figures 6E and 6F) and had variable effects in male flies (Tables S4 and S5). Interestingly, RNAi of complex V (*CG5389*) in neurons did not result in robust longevity effects in male (Tables S4 and S5) or female flies (Figures 6G and 6H). Feeding of RU486 to control flies (w^{1118}/*ELAV-GS*) did not produce any positive effect on life span (Figures 6I and 6J).

Adipose tissue has been reported to play an important role in insulin/IGF-1-mediated longevity [23]. To determine whether manipulation of ETC genes in adipose tissue could extend life span, we used the Gene-Switch driver S_1106, which is predominately expressed in the fat body [9]. Interestingly, we failed to observe robust positive affects on longevity when RNAi of ETC genes was activated with this driver line (Table S6).

RNAi of the complex I genes, *CG9172* and *CG9762*, mediated by the ubiquitous *tub-GS* driver resulted in increased life span and reduced fertility. To determine whether targeted disruption of complex I in neurons also confers a reproductive trade-off, we examined female fertility when RNAi of complex I was targeted to adult neurons. Unlike ubiquitous knockdown of complex I, neural-specific knockdown of either complex I gene, *CG9172* or *CG9762*, did not significantly affect female fertility (Figures 7A and 7B). Neural-specific RNAi of complex IV (*CG18809*) also did not lead to a fertility defect (Figure 7C). RU486 had no major effect on fertility in control (w^{1118}/*ELAV-GS*) flies either (Figure 7D). This result indicates that life extension, mediated by RNAi of these complex I genes, can be separated from major reproductive trade-offs when inhibition is restricted to the nervous system.

Conclusions

In this article, we present data showing that RNAi of five ETC genes can lead to life span extension in *Drosophila*. For two of the genes, *CG9172* (complex I) and *CG17856* (complex III), we observe a threshold effect where moderate knockdown resulted in life extension and stronger inhibition was detrimental, causing developmental lethality. Recently, similar threshold

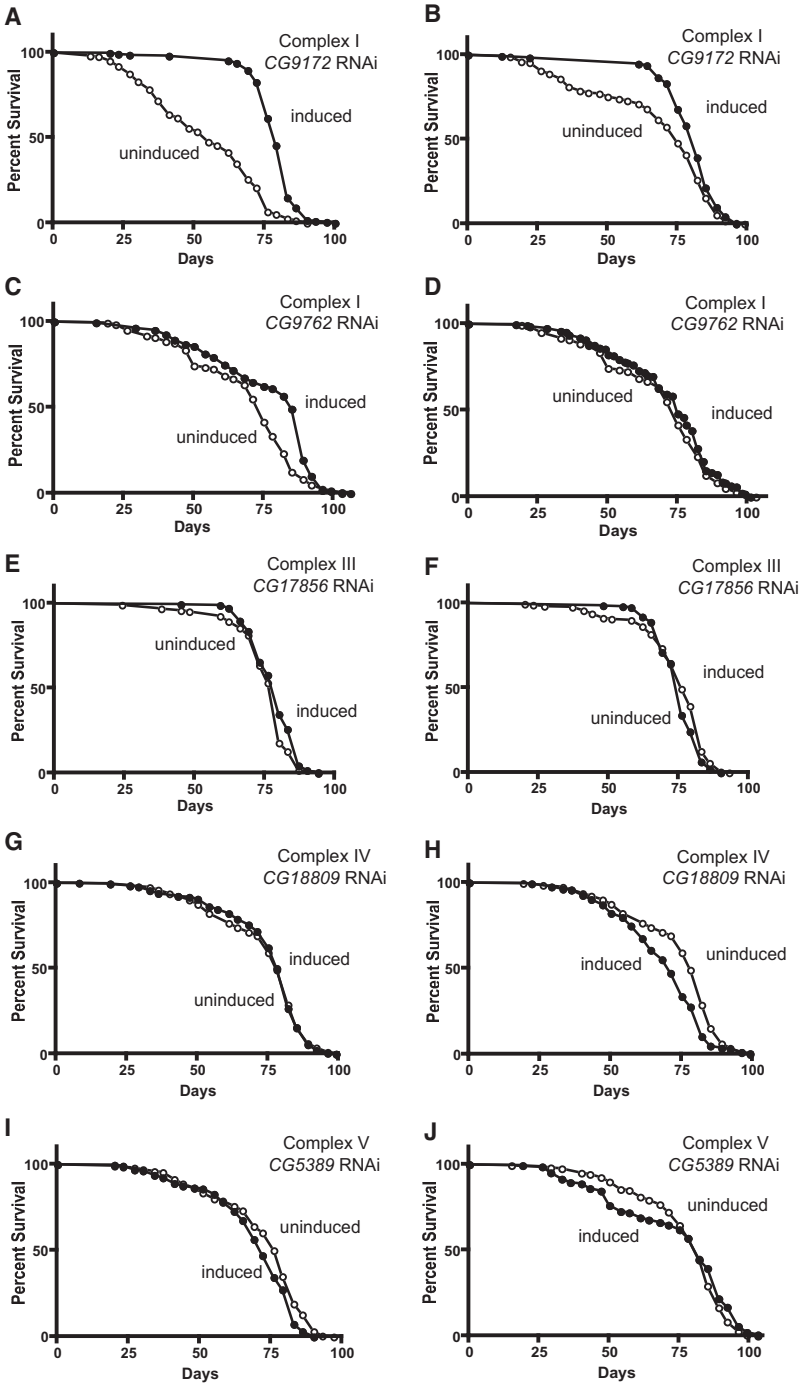

Figure 5. Longevity Effects on Targeted RNAi of Respiratory-Chain Genes in Adult Tissues

All RNAi lines were crossed to the ubiquitous, inducible tubulin Gene-Switch driver line and were fed RU486-spiked food only as adults. Induced (+RU486, solid circles) or uninduced (−RU486, open circles) curves are shown.

(A and B) Replicate life-span curves of RNAi knockdown of the respiratory complex I gene *CG9172*, showing an increased mean life span of 46% ($p < 0.0001$) (A) and 18% ($p = 0.0013$) (B), on 50 μg/ml RU486.

(C and D) Life-span curves of RNAi knockdown of the respiratory complex I gene *CG9762*, showing an increased mean life span of 11% ($p < 0.0001$) on 10 μg/ml RU486 (C) and no effect ($p = 0.5301$) on 50 μg/ml RU486 (D).

(E and F) Replicate life-span curves of RNAi knockdown of the respiratory complex III gene *CG17856*, showing a marginal increase in mean life span of 4% ($p = 0.0126$) (E) and 1% ($p = 0.0210$) (F), on 10 μg /ml RU486.

(G and H) Life-span curves of RNAi knockdown of the respiratory complex IV gene *CG18809*, showing no effect ($p = 0.9554$) on 10 μg/ml RU486 (G) and an 8% reduction in mean life span ($p < 0.0001$) on 50 μg/ml RU486 (H).

(I and J) Life-span curves of RNAi knockdown of the respiratory complex V gene *CG5389*, showing a decreased mean life span of 4% ($p = 0.0008$) on 50 μg/ml RU486 (I) and no effect ($p = 0.2360$) on 10 μg/ml RU486 (J).

effects have been reported for several ETC genes in the worm [19]. Taking into account the evolutionary distance between arthropods and nematodes, our data provide a compelling argument that *moderate* ETC gene inactivation may be an evolutionarily conserved mode of life extension. In support of this argument, mice carrying a disruption in SURF1, a putative complex IV assembly factor, are long lived [24]. In addition, reduced activity of murine CLK1, a mitochondrial enzyme necessary for ubiquinone biosynthesis, leads to a severe reduction of ETC function and a substantial increase in life span [25, 26].

The molecular and cellular mechanisms by which a reduction in ETC gene expression extends life span are not yet known in any species. Our studies in *Drosophila* indicate that life extension can be separated from energetic and physiological trade-offs. This result implies that extended longevity is not the result of a general slowing of metabolism, but may instead result from alterations in cell-signaling pathways. In *C. elegans*, *clk-1* mutants, which share many phenotypes with animals with reduced expression of ETC genes including extended longevity, have also been reported to display no major energetic defects [27]. Furthermore, as is the case in worms [13], resistance to free-radical-generating agents is not a universal feature of ETC-mediated longevity in the fly. So, what is the underlying mechanism behind ETC-mediated longevity? One possibility is that ETC gene perturbations trigger a transcriptional response that alters the animal's physiology and longevity. This response, termed the "retrograde response," is known to occur in response to ETC dysfunction in both yeast and mammalian cells [28]. A recent study in worms reported that ETC gene perturbations that extend life span result in a similar retrograde response that includes the upregulation of a number of cellular-defense and metabolic genes [29]. Moreover, retrograde-response genes have been reported to be required for mitochondrial-mediated longevity in both yeast [30] and worms [29]. It will be interesting to determine whether a similar phenomenon is taking place in the fly.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, ten figures, and six tables and can be found with this article online at http://www.cell.com/current-biology/supplemental/S0960-9822(09)01586-3.

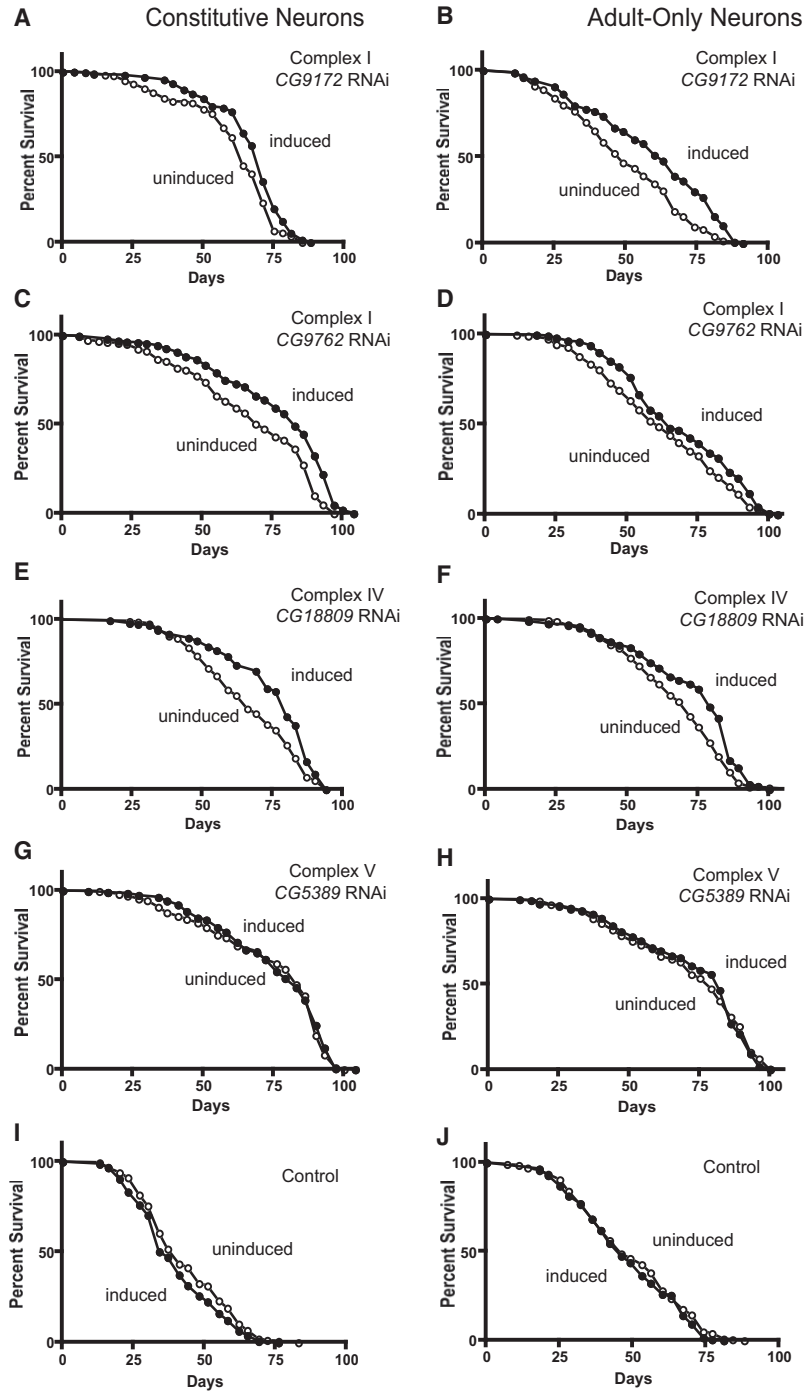

Figure 6. Longevity Effects upon Targeted RNAi of Respiratory-Chain Genes in Neurons

(A–J) All RNAi lines were crossed to the neuron-specific, inducible ELAV-Gene-Switch driver line and were fed RU486-spiked food either during development and adulthood (A, C, E, G, and I) or only during adulthood (B, D, F, H, and J). Induced (10 μg/ml during development and 50 μg/ml RU486 [for constitutive] or 50 μg/ml RU486 [for adult only], solid circles) or uninduced (0 μg/ml RU486, open circles) curves are shown.

(A and B) Life-span curves of RNAi knockdown of the respiratory complex I gene *CG9172*, showing an increased mean life span of 11% ($p = 0.0001$) upon constitutive neuronal expression (A) and 18% ($p < 0.0001$) upon targeted expression in adult neurons (B).

(C and D) Life-span curves of RNAi knockdown of the respiratory complex I gene *CG9762*, showing an increased mean life span of 14% ($p < 0.0001$) upon constitutive neuronal expression (C) and 10% ($p = 0.0114$) upon targeted expression in adult neurons (D).

(E and F) Life-span curves of RNAi knockdown of the respiratory complex IV gene *CG18809*, showing an increased mean life span of 12% ($p < 0.0001$) upon constitutive neuronal expression (E) and 8% ($p < 0.0001$) upon targeted expression in adult neurons (F).

(G and H) Life-span curves of RNAi knockdown of the respiratory complex V gene *CG5389*, showing no increase in life span ($p = 0.4979$) upon constitutive neuronal expression (G), or ($p = 0.7540$) upon targeted expression in adult neurons (H).

(I and J) Life spans of w^{1118}/ELAV-Gene-Switch control, showing no increase in life span ($p = 0.0539$) upon feeding RU486 during development and adulthood (I) or ($p = 0.1890$) during adulthood alone (J).

Acknowledgments

The authors would like to thank H. Keshishian, S. Pletcher, L. Seroude, the Vienna *Drosophila* RNAi Center [4], and the *Drosophila* Stock Center (Bloomington) for fly stocks. We thank Catherine Lindsay and Emily Elftman for help with fly work. We are grateful to the Phelps, Lin, and Jacobsen labs for generously allowing us to use their equipment. This research is supported by awards to D.W.W. from the Ellison Medical Foundation, the UCLA Center for gene environment in Parkinson's Disease, and the Muscular Dystrophy Association. D.W.W is an Ellison Medical Foundation New Scholar in Aging.

Received: March 24, 2009
Revised: August 1, 2009
Accepted: August 4, 2009
Published online: September 10, 2009

References

1. Sacktor, B. (1970). Regulation of intermediatary metabolism, with special reference to the control mechanisms in insect flight muscle. Adv. In Insect. Physiol. *7*, 267–347.
2. Walker, D.W., Hajek, P., Muffat, J., Knoepfle, D., Cornelison, S., Attardi, G., and Benzer, S. (2006). Hypersensitivity to oxygen and shortened lifespan in a Drosophila mitochondrial complex II mutant. Proc. Natl. Acad. Sci. USA *103*, 16382–16387.
3. Ishii, N., Fujii, M., Hartman, P.S., Tsuda, M., Yasuda, K., Senoo-Matsuda, N., Yanase, S., Ayusawa, D., and Suzuki, K. (1998). A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature *394*, 694–697.
4. Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., et al. (2007). A

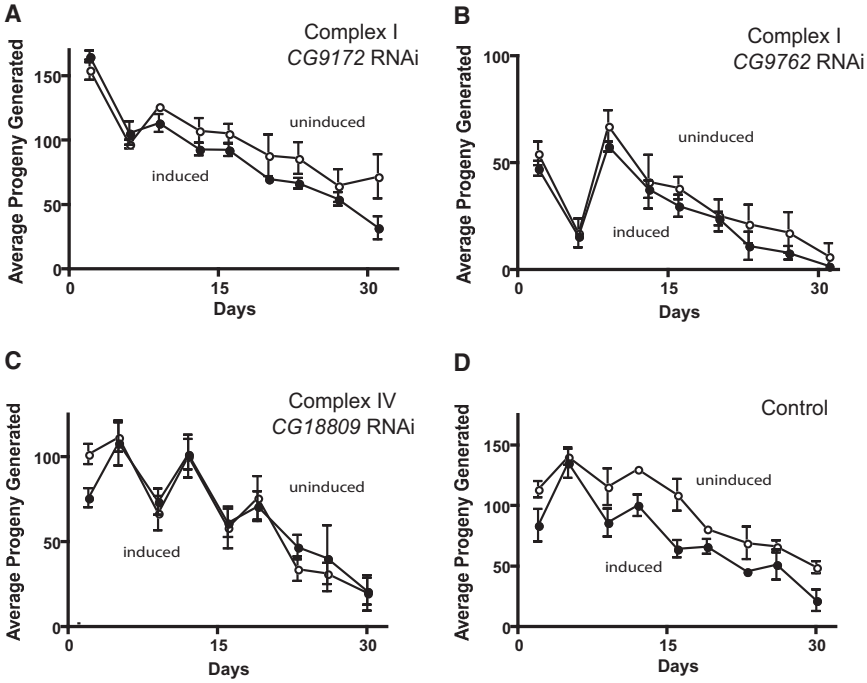

Figure 7. Long-Lived Flies with Targeted RNAi of Respiratory-Chain Genes in Adult Neurons Do Not Display Fertility Defects

(A–D) The neuron-specific, inducible ELAV-GeneSwitch driver line was crossed to the respiratory-chain RNAi lines or the control line w^{1118}. Females were fed RU486-spiked food only as adults; shown are curves as induced (50 μg/ml RU486, solid circles) or uninduced (0 μg/ml RU486, open circles). Female fertility was not changed in RNAi mutants of the respiratory complex I gene *CG9172* ($p = 0.2276$) (A), of the respiratory complex I gene *CG9762* ($p = 0.2541$) (B), of the respiratory complex IV gene *CG18809* ($p = 0.4978$) (C), or in the control line ($p = 0.0656$) (D). Data represent mean ± SEM ($n = 4$).

genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature *448*, 151–156.

5. Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.
6. Partridge, L., and Gems, D. (2007). Benchmarks for ageing studies. Nature *450*, 165–167.
7. Osterwalder, T., Yoon, K.S., White, B.H., and Keshishian, H. (2001). A conditional tissue-specific transgene expression system using inducible GAL4. Proc. Natl. Acad. Sci. USA *98*, 12596–12601.
8. Roman, G., Endo, K., Zong, L., and Davis, R.L. (2001). P[Switch], a system for spatial and temporal control of gene expression in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *98*, 12602–12607.
9. Poirier, L., Shane, A., Zheng, J., and Seroude, L. (2008). Characterization of the Drosophila Gene-Switch system in aging studies: A cautionary tale. Aging Cell *7*, 758–770.
10. Fridell, Y.W., Sanchez-Blanco, A., Silvia, B.A., and Helfand, S.L. (2005). Targeted expression of the human uncoupling protein 2 (hUCP2) to adult neurons extends life span in the fly. Cell Metab. *1*, 145–152.
11. Giannakou, M.E., Goss, M., Junger, M.A., Hafen, E., Leevers, S.J., and Partridge, L. (2004). Long-lived Drosophila with overexpressed dFOXO in adult fat body. Science *305*, 361.
12. Hwangbo, D.S., Gershman, B., Tu, M.P., Palmer, M., and Tatar, M. (2004). Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature *429*, 562–566.
13. Lee, S.S., Lee, R.Y., Fraser, A.G., Kamath, R.S., Ahringer, J., and Ruvkun, G. (2003). A systematic RNAi screen identifies a critical role for mitochondria in C. elegans longevity. Nat. Genet. *33*, 40–48.
14. Curran, S.P., and Ruvkun, G. (2007). Lifespan regulation by evolutionarily conserved genes essential for viability. PLoS Genet. *3*, e56.
15. Dillin, A., Hsu, A.L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A.G., Kamath, R.S., Ahringer, J., and Kenyon, C. (2002). Rates of behavior and aging specified by mitochondrial function during development. Science *298*, 2398–2401.
16. Saraste, M. (1999). Oxidative phosphorylation at the fin de siecle. Science *283*, 1488–1493.
17. Walker, D.W., Muffat, J., Rundel, C., and Benzer, S. (2006). Overexpression of a Drosophila homolog of apolipoprotein D leads to increased stress resistance and extended lifespan. Curr. Biol. *16*, 674–679.
18. Hassan, H.M., and Fridovich, I. (1979). Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. Arch. Biochem. Biophys. *196*, 385–395.
19. Rea, S.L., Ventura, N., and Johnson, T.E. (2007). Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in Caenorhabditis elegans. PLoS Biol. *5*, e259.
20. Yang, W., Li, J., and Hekimi, S. (2007). A Measurable increase in oxidative damage due to reduction in superoxide detoxification fails to shorten the life span of long-lived mitochondrial mutants of Caenorhabditis elegans. Genetics *177*, 2063–2074.
21. Balaban, R.S., Nemoto, S., and Finkel, T. (2005). Mitochondria, oxidants, and aging. Cell *120*, 483–495.
22. Broughton, S., and Partridge, L. (2009). Insulin/IGF-like signalling, the central nervous system and aging. Biochem. J. *418*, 1–12.
23. Russell, S.J., and Kahn, C.R. (2007). Endocrine regulation of ageing. Nat. Rev. Mol. Cell Biol. *8*, 681–691.
24. Dell'agnello, C., Leo, S., Agostino, A., Szabadkai, G., Tiveron, C., Zulian, A., Prelle, A., Roubertoux, P., Rizzuto, R., and Zeviani, M. (2007). Increased longevity and refractoriness to Ca(2+)-dependent neurodegeneration in Surf1 knockout mice. Hum. Mol. Genet. *16*, 431–444.
25. Lapointe, J., and Hekimi, S. (2008). Early mitochondrial dysfunction in long-lived Mclk1+/− mice. J. Biol. Chem. *283*, 26217–26227.
26. Liu, X., Jiang, N., Hughes, B., Bigras, E., Shoubridge, E., and Hekimi, S. (2005). Evolutionary conservation of the clk-1-dependent mechanism of longevity: Loss of mclk1 increases cellular fitness and lifespan in mice. Genes Dev. *19*, 2424–2434.
27. Braeckman, B.P., Houthoofd, K., Brys, K., Lenaerts, I., De Vreese, A., Van Eygen, S., Raes, H., and Vanfleteren, J.R. (2002). No reduction of energy metabolism in Clk mutants. Mech. Ageing Dev. *123*, 1447–1456.
28. Butow, R.A., and Avadhani, N.G. (2004). Mitochondrial signaling: The retrograde response. Mol. Cell *14*, 1–15.
29. Cristina, D., Cary, M., Lunceford, A., Clarke, C., and Kenyon, C. (2009). A regulated response to impaired respiration slows behavioral rates and increases lifespan in Caenorhabditis elegans. PLoS Genet. *5*, e1000450.
30. Kirchman, P.A., Kim, S., Lai, C.Y., and Jazwinski, S.M. (1999). Interorganelle signaling is a determinant of longevity in Saccharomyces cerevisiae. Genetics *152*, 179–190.