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Longevity determined by developmental arrest genes in *Caenorhabditis elegans*

Di Chen, **Kally Z. Pan**, **Julia E. Palter**, and **Pankaj Kapahi**
Buck Institute for Age Research, 8001 Redwood Boulevard, Novato, CA, USA

Summary

The antagonistic pleiotropy theory of aging proposes that aging takes place because natural selection favors genes that confer benefit early on life at the cost of deterioration later in life. This theory predicts that genes that impact development would play a key role in shaping adult lifespan. To better understand the link between development and adult lifespan, we examined the genes previously known to be essential for development. From a pool of 57 genes that cause developmental arrest after inhibition using RNA interference, we have identified 24 genes that extend lifespan in *Caenorhabditis elegans* when inactivated during adulthood. Many of these genes are involved in regulation of mRNA translation and mitochondrial functions. Genetic epistasis experiments indicate that the mechanisms of lifespan extension by inactivating the identified genes may be different from those of the insulin/insulin-like growth factor 1 (IGF-1) and dietary restriction pathways. Inhibition of many of these genes also results in increased stress resistance and decreased fecundity, suggesting that they may mediate the trade-offs between somatic maintenance and reproduction. We have isolated novel lifespan-extension genes, which may help understand the intrinsic link between organism development and adult lifespan. Key words: developmental arrest; antagonistic pleiotropy; aging; mRNA translation; mitochondria; *C. elegans*.

Introduction

The use of *Caenorhabditis elegans* has led to a number of insights into the molecular mechanisms of aging. Mutations in the *daf-2* insulin/insulin-like growth factor 1 (IGF-1) receptor gene double adult lifespan (Kenyon *et al.*, 1993; Kimura *et al.*, 1997). DAF-2 functions through a kinase cascade to inhibit the DAF-16/forkhead box O (FOXO) transcription factor, which regulates the expression of many downstream genes (Lin *et al.*, 1997; Ogg *et al.*, 1997; Lee *et al.*, 2003a; Murphy *et al.*, 2003; Oh *et al.*, 2006). Steroid hormone signaling from the reproductive system affects lifespan through DAF-16 (Arantes-Oliveira *et al.*, 2003). Genes such as *sir-2.1*, *jnk-1*, *smk-1* and *cst-1* have been shown to regulate lifespan by modulating

Correspondence: Pankaj Kapahi, Buck Institute for Age Research, 8001 Redwood Boulevard, Novato, CA 94945, USA. Tel. 415-209-2201; fax: 415-209-2232; pkapahi@buckinstitute.org.

Supplementary material
The following supplementary material is available for this article:
Fig. S1 Groups of genes.
Table S1 List of 57 genes that show larval arrest after RNAi inactivation.
Table S2 An RNAi screen identified 24 genes with functions in *C. elegans* development and aging.
Table S3 Lifespan extension in different genetic backgrounds.
Table S4 Heat stress, oxidative stress and fecundity assays.
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DAF-16 activities (Tissenbaum & Guarente, 2001; Oh *et al.*, 2005; Berdichevsky & Guarente, 2006; Lehtinen *et al.*, 2006; Wolff *et al.*, 2006). Dietary restriction extends *C. elegans* lifespan, although the molecular mechanisms remain to be defined. Down-regulation of the TOR (target of rapamycin) pathway (Jia *et al.*, 2004) and inhibition of mitochondrial respiration and adenosine triphosphate (ATP) synthesis also extend worm lifespan by DAF-16 independent mechanisms (Dillin *et al.*, 2002b; Lee *et al.*, 2003b).

To identify new genes affecting *C. elegans* lifespan, two groups have performed genome-wide RNA interference (RNAi) screens independently (Hamilton *et al.*, 2005; Hansen *et al.*, 2005). Eighty-nine and 23 genes were identified by each group, respectively. Surprisingly, only three genes were common in the two screens, suggesting the screens have not been saturated. In those screens, animals were treated with RNAi from early larval stages. Genes that play essential roles during development were likely excluded from the screens as knocking down those genes may cause larval arrest or embryonic lethality. However, evolutionary theories of aging suggest that genes that play essential roles during development are likely to be important for aging.

The underlying principle of evolutionary theories of aging is that the force of natural selection declines with age (Medawar, 1952). Two possible hypotheses have been proposed to explain the existence of aging based on this principle. The mutation accumulation theory suggests that deleterious mutations that manifest their effects late in life would accumulate in a population and lead to senescence as they are not removed due to lack of selective pressure (Medawar, 1952). The antagonistic pleiotropy theory suggests the existence of pleiotropic genes that endow benefits early in life at the cost of deleterious effects later to explain the evolution of senescence (Williams, 1957). A prediction of this theory is that genes that are essential for early life development play important roles later on in adulthood and aging. *daf-2* represents a good example of an antagonistic pleiotropic gene. Null mutations of *daf-2* cause embryonic lethality or L1 larval arrest, whereas weak alleles of *daf-2* extend adult lifespan (Kenyon *et al.*, 1993; Kimura *et al.*, 1997; Gems *et al.*, 1998). Another example is the microRNA (miRNA) gene *lin-4*, which regulates developmental timing and adult lifespan through the downstream transcription factor LIN-14 (Boehm & Slack, 2005).

In this study, we found 24 out of 57 genes that play essential roles during development also regulate adult lifespan. Our findings are consistent with the evolutionary theory of aging, and may help understand the role of developmental processes in longevity.

Results

A previous *C. elegans* genome-wide RNAi screen performed by Kamath *et al.* (2003) identified 57 genes that lead to larval arrest phenotypes consequent on RNAi inactivation (Supplementary Table S1 and Fig. S1). We treated animals with these RNAi clones only during adulthood, and measured the adult lifespan. Surprisingly, 24 out of 57 RNAi treatments caused lifespan extension. One gene, *atp-3*, has been previously reported to extend lifespan (Dillin *et al.*, 2002b; Lee *et al.*, 2003b; Hamilton *et al.*, 2005; Curran & Ruvkun, 2007). Thus, we have identified 23 new genes that affect adult lifespan in *C. elegans* (Table 1, Fig. 1 and Supplementary Fig. S1). We classified these genes into multiple functional groups. BLAST (basic local alignment search tool) search indicates that most of the identified genes have strong orthologs in other organisms (Supplementary Table S2), suggesting their roles in aging may be conserved.

Among the 24 genes identified, most of them are involved in regulation of mRNA translation (42%, 10/24) and mitochondrial functions (38%, 9/24). Previous studies have shown that RNAi inactivation of various components of the eukaryotic initiation factor 4F (eIF4F) complex,

which is composed of eIF4E (a 5′-cap binding protein), eIF4A (an ATP-dependent helicase) and eIF4G (a scaffold protein), decreases cap-dependent translation and extends lifespan in *C. elegans* (Henderson *et al.*, 2006; Curran & Ruvkun, 2007; Hansen *et al.*, 2007; Pan *et al.*, 2007; Syntichaki *et al.*, 2007). In this study, we identified eIFs that have not been previously linked with determining adult lifespan. Genes Y39G10AR.8, D2085.3 and D2013.7 encode eIF-2γ, eIF-2Bε and eIF-3f, respectively. RNAi inactivation of these genes results in most robust lifespan extension in wild-type animals (Table 1 and Supplementary Table S3). The initiation of mRNA translation in eukaryotic cells requires coordinated activities of multiple eIFs. A ternary complex composed of eIF2, guanosine triphosphate (GTP) and methionine transfer RNA (Met-tRNA) associates with the 40S ribosome to form a 43S pre-initiation complex (Hershey & Merrick, 2000). eIF2, composed of eIF2α, β, γ, delivers Met-tRNA to the 40S ribosome. The eIF2γ subunit contains a consensus GTP-binding domain. The 43S pre-initiation complex binds to an mRNA near the 5′-cap facilitated by eIF4 factors, and it scans the mRNA for the AUG start codon. Once the start codon is found, GTP hydrolysis makes the guanosine diphosphate (GDP)-binding eIF2 detach from the ribosome. The guanine nucleotide exchange factor (GEF) eIF2B exchanges GTP for GDP on eIF2 and allows eIF2 to participate in subsequent rounds of translation initiation (Hinnebusch, 2000). The eIF2B∈ subunit has GEF activity and nteracts directly with the eIF2γ subunit (Alone & Dever, 2006). The binding of the ternary complex to the 40S ribosome is promoted by eIF3 (Majumdar *et al.*, 2003). eIF3 consists of an active core and subunits that serve to modulate eIF3 activity. eIF3f, one of these subunits, is a member of the Mov34 family, which is involved in translation initiation, regulation of the proteasome and transcription (Aravind & Ponting, 1998). eIF3f is down-regulated in several human tumors. It is a negative regulator of translation and cell growth, and it is also involved in apoptosis. eIF3f might play important roles in the regulation of eIF3 activity for translation of specific mRNAs (Shi *et al.*, 2006).

We also identified other components of the translation machinery, including genes encoding a ribosomal subunit (C09D4.5), and genes involved in ribosomal biogenesis (W01B11.3, F59C6.4 and Y48G1A.4) and tRNA synthesis (C47D12.6 and F22D6.3). It has been shown by Hansen *et al.* (2007) that RNAi inactivation of C09D4.5 extends lifespan. Two genes (F14B4.3 and C48E7.2) encoding subunits of RNA polymerase I and III were also identified. As the major transcriptional products of RNA polymerase I and III are precursors of rRNA and tRNA, respectively, these genes are also likely to play essential roles in regulation of mRNA translation. Taken together, various genes involved in post-transcriptional regulation of gene expression are important regulators of *C. elegans* lifespan.

Among the identified genes that regulate mitochondrial function, four genes are involved in mitochondrial protein synthesis. Three of these genes encode mitochondrial ribosomal proteins and the fourth gene Y47G6A.10 (*spg-7*) encodes an AAA-type ATPase. Mutations in the human homolog of *spg-7* cause hereditary spastic paraplegia. The yeast homolog of *spg-7* encodes a mitochondrial inner membrane protein that controls mitochondrial ribosome assembly (Nolden *et al.*, 2005). We also identified other genes that are involved in mitochondrial respiration and ATP synthesis from our screen (Table 1), which is consistent with previous studies showing that inactivation of the genes involved in these processes extends lifespan (Feng *et al.*, 2001; Dillin *et al.*, 2002a; Lee *et al.*, 2003b).

We set out to characterize the genetic mechanisms of lifespan extension using epistasis experiments. Mutations in the *daf-2* insulin-like/IGF-1 receptor double lifespan (Kenyon *et al.*, 1993; Kimura *et al.*, 1997). We treated the *daf-2(e1370)* animals with RNAi during adulthood. Surprisingly, all of the RNAi treatments can further extend *daf-2* lifespan (Table 1, Fig. 2 and Supplementary Table S3). However, it is possible that the maximal lifespan extension due to perturbing the insulin-like/IGF pathway may have not been achieved in this long-lived mutant. For example, *daf-2* RNAi has been shown to further extend the lifespan of

a *daf-2* mutant (Arantes-Oliveira *et al.*, 2003). Mutations in DAF-16/FOXO transcription factor functions can fully suppress lifespan extension by *daf-2* mutations (Lin *et al.*, 1997; Ogg *et al.*, 1997). Therefore, we examined lifespan phenotypes in the *daf-2; daf-16* background to test whether the additive lifespan extensions in the *daf-2* background were independent of the insulin-like/IGF signaling pathway. We treated the *daf-2; daf-16* double mutant with RNAi against the genes identified from the targeted screen. Lifespan extension was observed in every RNAi treatment (Table 1, Fig. 3 and Supplementary Table S3). Therefore, the genes that we identified are likely to affect lifespan through mechanisms different from the insulin-like/IGF signaling.

The *eat-2* gene encodes a subunit of nicotinic acetylcholine receptor. Mutations in *eat-2* result in slow pharyngeal pumping and extended lifespan (Lakowski & Hekimi, 1998). *eat-2* mutants have been suggested to be genetic mimics of dietary restriction in previous studies (Lakowski & Hekimi, 1998). We treated the *eat-2(ad465)* mutant with RNAi. Inactivation of all these genes by RNAi during adulthood further extends *eat-2* lifespan (Table 1, Fig. 4 and Supplementary Table S3), suggesting genetic mechanisms of lifespan extension distinct from those regulated by dietary restriction.

The disposable soma theory suggests that aging takes place due to trade-offs between resources spent on somatic maintenance, which ensures prolonged survival, vs. resources for growth and reproduction, which enhance the fitness of the individual (Martin *et al.*, 1996). To test whether the identified genes modulate lifespan by mediating such a trade-off, we examined survival under stressful conditions as well as fecundity. Animals at the fourth larval stage were treated with RNAi for 2 days. Adult animals were incubated at 35 °C for heat stress assays, or transferred to nematode growth medium (NGM) agar plates containing 360 mM juglone for oxidative stress assays. Various RNAi treatments increased tolerance to heat or oxidative stress (Table 2 and Supplementary Table S4) supporting the idea that common mechanisms may be shared between regulation of lifespan and stress tolerance (Johnson *et al.*, 1996; Martin *et al.*, 1996). We also measured the brood size of animals treated with different RNAi. Interestingly, all RNAi inactivation except C48E7.2, which encodes a subunit of RNA polymerase III, reduced fecundity (Table 2, Fig. 5 and Supplementary Table S4). Taken together these data support the idea that lifespan is determined by trade-offs between somatic maintenance vs. growth and reproduction.

Discussion

In his classic paper, Williams postulated the existence of pleiotropic genes that endow benefits early in life at the cost of deleterious effects later to explain the evolution of senescence (1957). Williams predicted ‘It would be expected that if development could be completely arrested there would be no senescence…’ Results of our study lend support to the idea that there is an intrinsic link between cellular processes that determine development and lifespan. Antagonistic pleiotropic genes have been proposed to function in two distinct ways (Partridge & Gems, 2006). The first type is when lifespan extension is observed by a gene's action during development. For example, inhibition of a gene involved in ATP synthesis during development is sufficient for adult lifespan extension (Dillin *et al.*, 2002a). The second form of antagonistic pleiotropy is where the gene is essential for developmental processes (i.e. inhibition is harmful), but if its action is inhibited during adulthood it leads to lifespan extension. This study describes genes that belong to the latter category. Our data lend support to the evolutionary theories of aging that propose that the rate of aging is caused by the force of natural selection acting to optimize fitness early in life.

From a pool of 57 genes that showed developmental arrest when inactivated during development, we have identified 23 novel genes that extend lifespan in *C. elegans* when

inhibited using RNAi only during adulthood. Remarkably, a number of these genes possess very close orthologs in flies and mammals including humans. These results uncover the identity of a number of genes that are consistent with the evolutionary theory of aging, and are likely to extend lifespan by conserved mechanisms across species.

Our results and previous studies indicate that down-regulation of key components of mRNA translation machinery extends adult lifespan in *C. elegans* (Curran & Ruvkun, 2007; Hansen *et al.*, 2007; Henderson *et al.*, 2006; Pan *et al.*, 2007; Syntichaki *et al.*, 2007), but the molecular mechanisms of lifespan extension remain unclear. It is possible that animals can sense reduced mRNA translation states and shift metabolism from growth to somatic maintenance. It is also possible that lifespan extension is caused by key factors that are regulated at the mRNA translation level. Previous studies showed that upon inhibition of translation initiation factors eIF4E and eIF4γ, the synthesis of most proteins is decreased. However, mRNAs encoding for some heat shock proteins are preferentially translated (Joshi-Barve *et al.*, 1992). Therefore, identification of genes that are differentially translated upon inhibition of genes involved in protein synthesis may help explain the roles of mRNA translation in aging.

The disposable soma theory suggests that longevity is determined through the trade-offs between resources spent on somatic maintenance and reproduction (Martin *et al.*, 1996). It has been estimated that the fraction of genes devoted to translation may be as high as 35–45% (Hershey & Merrick, 2000). We reason that inhibition of this costly process is likely to shift investment towards somatic maintenance and away from development, growth and reproduction. Our results are consistent with this notion, and we observed that inhibition of genes that regulate protein synthesis lead to increased stress resistance and a reduction in fecundity.

Previous studies showed that reduced activities of the electron transport chain and ATP synthase by RNAi during development but not adulthood lead to lifespan extension in *C. elegans* (Dillin *et al.*, 2002b). However, we found that knocking-down genes involved in mitochondrial functions solely during adulthood can extend lifespan. One possible reason for this discrepancy could be the variable effectiveness of RNAi. Knocking down the genes identified in this study results in larval arrest, which was not the case in the previous study. Another possibility is that these genes may be involved in different aspects of mitochondrial functions. Further analysis on how mitochondria are involved in aging may help explain this discrepancy.

While our manuscript was under review, Curran & Ruvkun (2007) reported an RNAi screen for longevity genes from 2700 genes that play essential roles during development. They identified 64 genes that extend lifespan after inactivation by RNAi during adulthood. Four genes were common to both studies. Interestingly genes identified by Curran and Ruvkun are also enriched with those involved in protein synthesis and mitochondrial functions, suggesting these key processes link development of the organism and adult longevity.

Experimental procedures

Strains

Caenorhabditis elegans strains were cultured as described (Brenner, 1974). Genotypes of animals used were: *daf-16(mu86) I, eat-2(ad465) II* and *daf-2(el370) III*.

RNAi experiments

RNAi bacteria strains were cultured as previously described (Kamath *et al.*, 2001). Worms were staged at old L4s and fed with *Escherichia coli* expressing different double-stranded RNAs. In all RNAi assays, *E. coli* carrying the empty RNAi vector L4440 was fed to animals

as controls. Clone identity of all RNAi bacteria was verified by polymerase chain reaction (PCR) and DNA sequencing.

Lifespan assays

Lifespan assays were performed as previously described (Lithgow *et al.*, 1995), except that 80 μL of 0.2 mM (+)-5-fluorodeoxyuridine (FUdR) was added onto plates during the reproductive phase to eliminate progeny. Worms were transferred onto fresh plates every 3–6 days. In all experiments, RNAi was introduced to old L4 larvae. All lifespan assays were performed at 20 °C. The first day of adulthood is Day 1 in the survival curves. Animals were scored as alive, dead or lost every other day. Animals that did not move in response to touching were scored as dead. Animals that died from causes other than aging, such as sticking to the plate walls, internal hatching or bursting in the vulval region, were scored as lost.

Statistical analysis

Statistical analyses were performed using the Prism 4 software (Graphpad Software, Inc., San Diego, CA, USA). Kaplan–Meier survival curves were plotted for each lifespan and compared using the log-rank test.

Fecundity analysis

Wild-type N2 L4 larvae growing at 20 °C were transferred to RNAi plates. Those animals were transferred every day to fresh RNAi plates and progeny produced during that 24-h period were counted.

Stress-resistance assays

Wild-type N2 L4 larvae were treated with RNAi and FUdR at 20 °C for 48 h. Adult animals were then collected for stress-resistance assays.

Thermotolerance—Thermotolerance assays were performed as previously described (Lithgow *et al.*, 1995). Synchronized 2-day-old adults were shifted from 20 to 35 °C and survival scored by means of touch-provoked movement. Worms not responding to touch were scored as dead.

Oxidative stress—Juglone resistance was performed as previously described (de Castro *et al.*, 2004). Ethanol-dissolved juglone solution was added to liquid NGM to a final concentration of 360 μM and poured immediately onto plates to dry. One hundred microliters of *E. coli* OP50 was spotted on the plates and dried. About 50 2-day-old adults were transferred to each plate 3 h after the plates were made. Worms were incubated at 20 °C and scored hourly until death.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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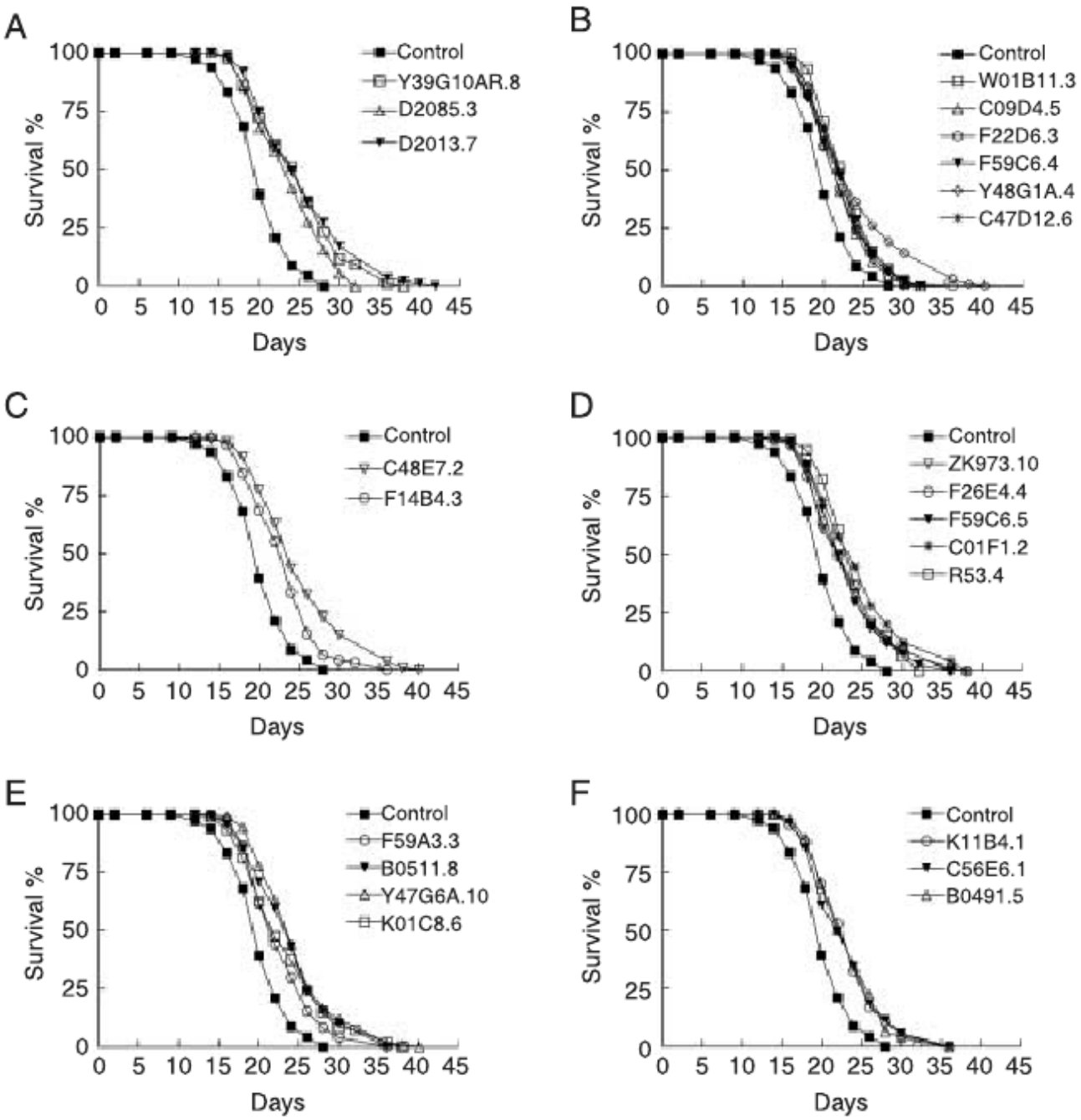

Fig. 1.

RNAi inactivation of 23 genes extends N2 lifespan. Lifespan of animals treated with RNAi against genes involved in translation initiation (A); ribosome and tRNA biogenesis (B); transcription (C); mitochondrial respiration and adenosine triphosphate (ATP) synthesis (D); mitochondrial translation (E); and signaling and unknown functions (F).

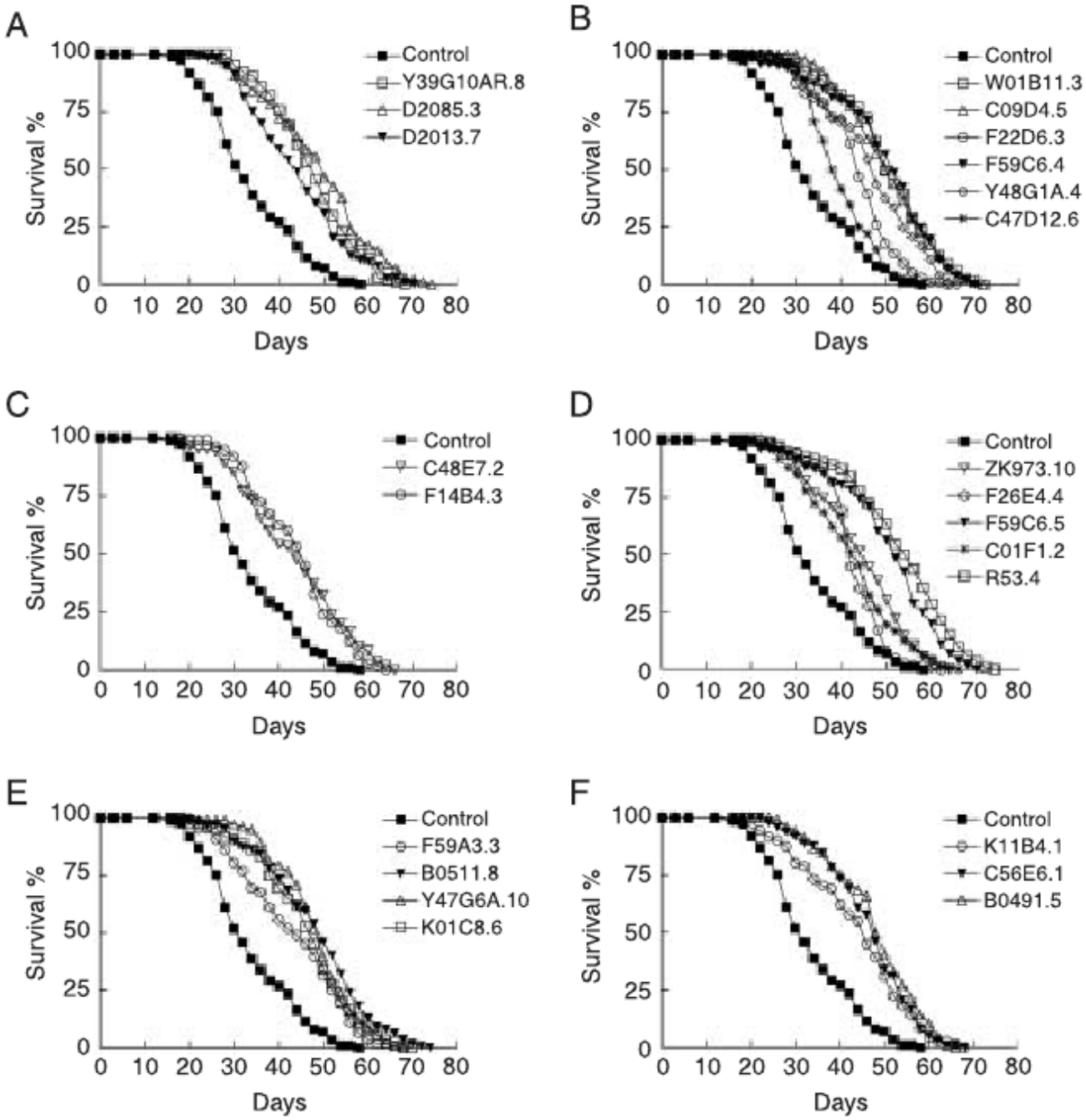

Fig. 2.

RNAi inactivation of 23 genes further extends *daf-2(e1370)* lifespan. Lifespan of animals treated with RNAi against genes involved in translation initiation (A); ribosome and tRNA biogenesis (B); transcription (C); mitochondrial respiration and adenosine triphosphate (ATP) synthesis (D); mitochondrial translation (E); and signaling and unknown functions (F).

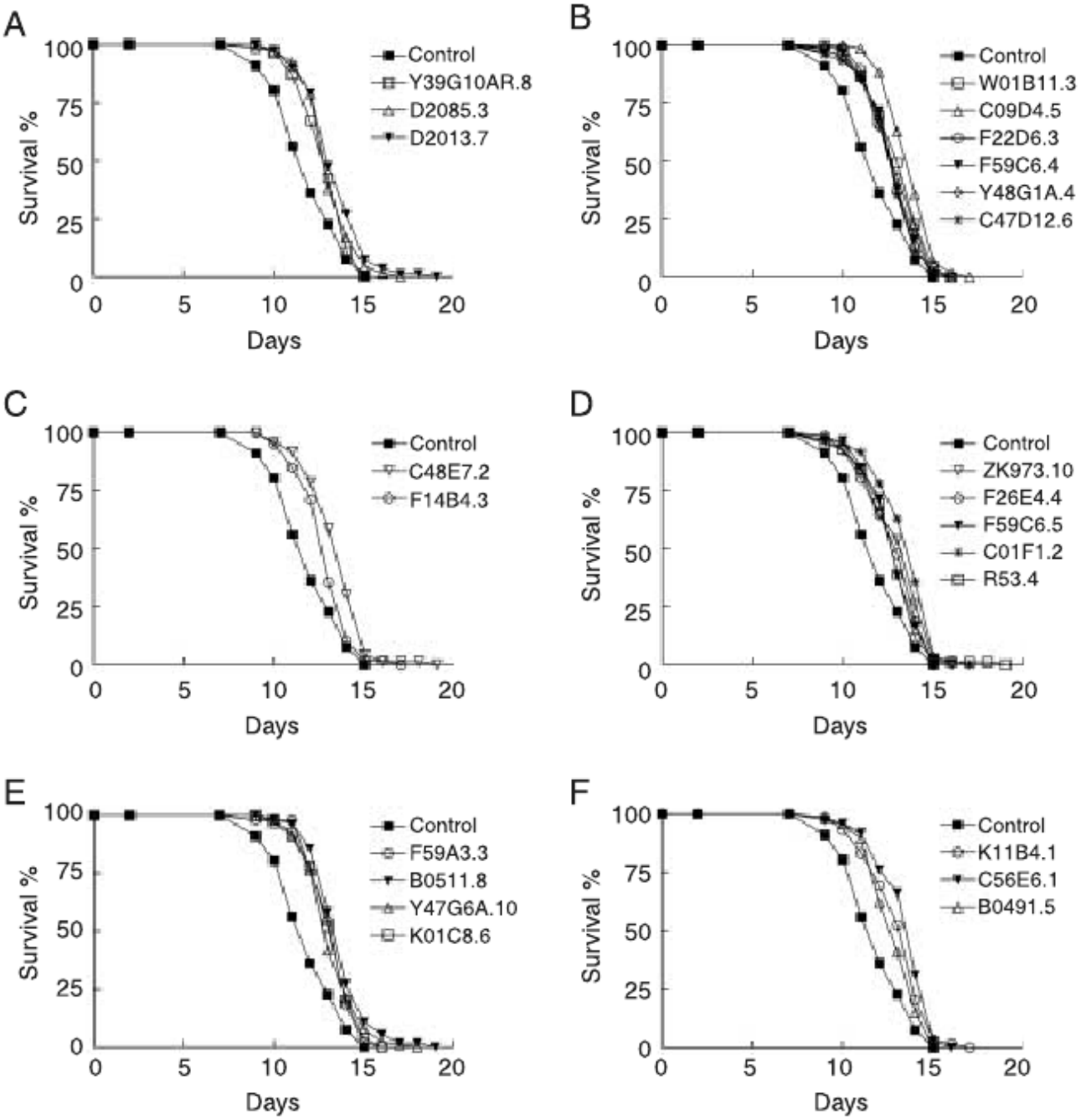

Fig. 3.

RNAi inactivation of 23 genes extends *daf-16(mu86); daf-2(e1370)* lifespan. Lifespan of animals treated with RNAi against genes involved in translation initiation (A); ribosome and tRNA biogenesis (B); transcription (C); mitochondrial respiration and adenosine triphosphate (ATP) synthesis (D); mitochondrial translation (E); and signaling and unknown functions (F).

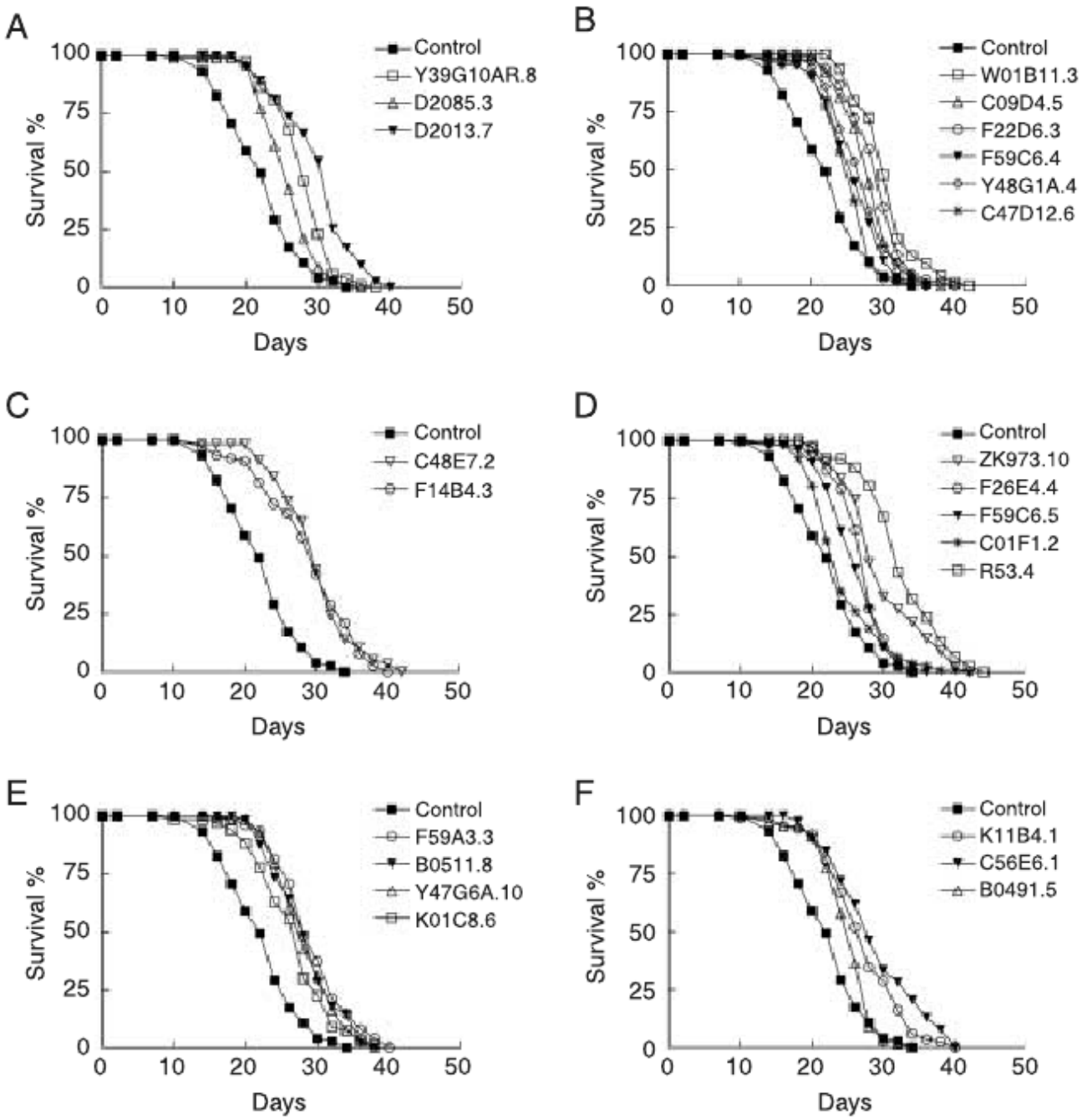

Fig. 4.

RNAi inactivation of 23 genes further extends *eat-2(ad465)* lifespan. Lifespan of animals treated with RNAi against genes involved in translation initiation (A); ribosome and tRNA biogenesis (B); transcription (C); mitochondrial respiration and adenosine triphosphate (ATP) synthesis (D); mitochondrial translation (E); and signaling and unknown functions (F).

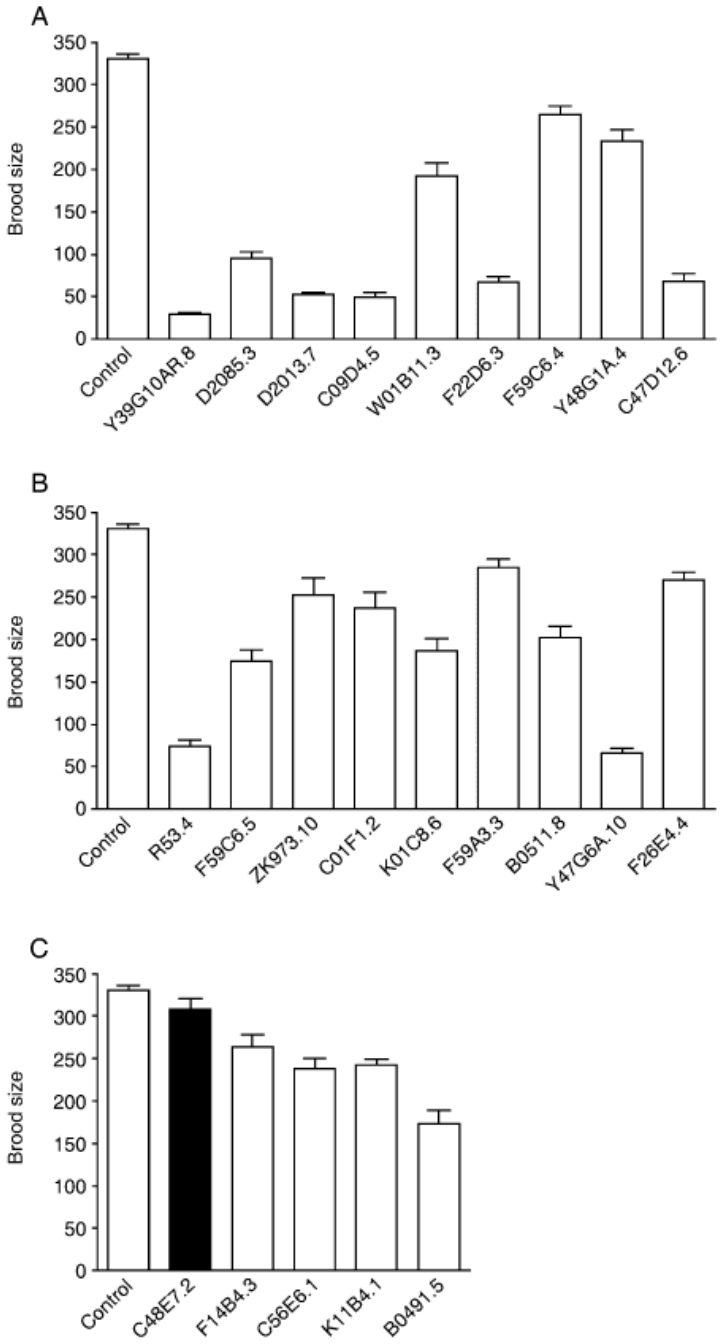

Fig. 5.

RNAi inactivation of most of the identified genes reduces fecundity. Brood sizes of animals treated with RNAi against genes involved in mRNA translation (A); mitochondrial functions (B); and transcription, signaling and unknown functions (C). RNAi inactivation of C48E7.2 does not affect brood size significantly.

Table 1

Lifespan extension in different genetic backgrounds

Gene	Brief description	Predicted functional group	N2 extension†	*daf-2* extension†	*daf-16; daf-2* extension†	*eat-2* extension†
Y39G10AR.8	eIF-2γ	Translation initiation	24%**	41%**	9%**	32%**
D2085.3	eIF-2Bε	Translation initiation	18%**	45%**	12%**	23%**
D2013.7	eIF-3f	Translation initiation	28%**	31%**	13%**	41%**
C09D4.5	rpl-19	Ribosome subunit	11%**	50%**	17%**	24%**
W01B11.3	Ribosome biogenesis protein Nop58p/Nop5p	Ribosome biogenesis	15%**	51%**	12%**	35%**
F59C6.4	3′–5′ exoribonuclease subunit involved in rRNA processing	Ribosome biogenesis	12%**	34%**	14%**	28%**
Y48G1A.4	Nucleolar protein involved in 40S ribosome biogenesis	Ribosome biogenesis	21%**	39%**	10%*	19%**
C47D12.6	Threonyl-tRNA synthetase	tRNA synthesis	12%**	19%*	8%*	11%*
F22D6.3	Asparaginyl-tRNA synthetase	tRNA synthesis	11%**	30%**	9%**	28%**
C48E7.2	RNA polymerase III subunit	Transcription	26%**	29%**	14%**	32%**
F14B4.3	RNA polymerase I subunit	Transcription	15%**	31%**	9%*	27%**
R53.4	Mitochondrial F1F0-ATP synthase subunit f	ATP synthesis	20%**	57%**	9%*	43%**
F59C6.5	NADH:ubiquinone oxidoreductase subunit NDUFB10/PDSW	ATP synthesis	16%**	49%**	10%*	23%**
ZK973.10	NADH:ubiquinone oxidoreductase subunit NDUFS4	ATP synthesis	17%**	31%**	11%**	30%**
C01F1.2	Cytochrome C oxidase assembly protein	ATP synthesis	23%**	24%**	15%**	17%**
K01C8.6	Mitochondrial rpl-10	Mitochondrial translation	17%**	38%**	13%**	26%**
F59A3.3	Mitochondrial rpl-24	Mitochondrial translation	14%**	28%**	12%**	28%**
B0511.8	Mitochondrial rps-30	Mitochondrial translation	21%**	44%**	17%**	25%**
Y47G6A.10	AAA+-type ATPase	Mitochondrial translation	24%**	43%**	13%**	25%**
F26E4.4	Cell death regulator Aven	Mitochondrial	17%**	27%**	9%**	20%**
C56E6.1	ABC superfamily transporter	Signaling	16%**	38%**	14%**	27%**
K11B4.1	Uncharacterized conserved protein	Unkown	16%**	29%**	11%**	28%**
B0491.5	Uncharacterized protein	Unkown	16%**	43%**	9%**	35%**

RNAi inactivation of the identified genes during adulthood cause lifespan extension in the wild-type N2, *daf-2(e1370)*, *daf-16(mu86)*; *daf-2(e1370)* and *eat-2(ad465)* animals. All lifespan experiments were performed at 20 °C. Lifespan of animals treated with different RNAi were compared with those treated with the control RNAi (empty vector) using the log-rank method.

†Mean lifespan extension compared to animals treated with the control RNAi.

$^{**}P < 0.001$;

$^{*}P < 0.01$.

P values were calculated using the log-rank method (Graphic Prism 4).

Table 2

Inactivation many of the 23 genes by RNAi during adulthood result in increased stress resistance and decreased fecundity

Gene	Brief description	Stress resistance		Reduced fecundity‡
		Heat*	Juglone†	
Y39G10AR.8	eIF-2γ	Yes	Yes	Yes
D2085.3	eIF-2Bε	No	No	Yes
D2013.7	eIF-3f	Yes	Yes	Yes
C09D4.5	rpl-19	Yes	Yes	Yes
W01B11.3	Ribosome biogenesis protein Nop58p/Nop5p	Yes	No	Yes
F59C6.4	3′–5′ exoribonuclease subunit involved in rRNA processing	Yes	No	Yes
Y48G1A.4	Nucleolar protein involved in 40S ribosome biogenesis	Yes	Yes	Yes
C47D12.6	Threonyl-tRNA synthetase	Yes	No	Yes
F22D6.3	Asparaginyl-tRNA synthetase	Yes	No	Yes
C48E7.2	RNA polymerase III subunit	Yes	Yes	No
F14B4.3	RNA polymerase I subunit	No	No	Yes
R53.4	Mitochondrial F1F0-ATP synthase subunit f	Yes	Yes	Yes
F59C6.5	NADH:ubiquinone oxidoreductase subunit NDUFB10/PDSW	Yes	Yes	Yes
ZK973.10	NADH:ubiquinone oxidoreductase subunit NDUFS4	Yes	No	Yes
C01F1.2	Cytochrome C oxidase assembly protein	Yes	Yes	Yes
K01C8.6	Mitochondrial rpl-10	Yes	Yes	Yes
F59A3.3	Mitochondrial rpl-24	No	No	Yes
B0511.8	Mitochondrial rps-30	Yes	Yes	Yes
Y47G6A.10	AAA+-type ATPase	Yes	Yes	Yes
F26E4.4	Cell death regulator Aven	Yes	No	Yes
C56E6.1	ABC superfamily transporter	No	No	Yes
K11B4.1	Uncharacterized conserved protein	Yes	No	Yes
B0491.5	Uncharacterized protein	Yes	Yes	Yes

*Two-day-old adults treated with various RNAi were incubated at 35 °C and their survival was measured. Significant heat stress resistance ($P < 0.05$, log-rank test) is highlighted.

†Two-day-old adults treated with different RNAi were incubated on nematode growth medium (NGM) plates containing juglone (360 mM) and their survival were measured. Significant oxidative stress resistance ($P < 0.05$, log-rank test) was highlighted.

‡Brood sizes of animals treated with RNAi were measured at 20 °C. *P* values were calculated using the *t*-test. Significant brood size decreases ($P < 0.05$) were highlighted.