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# Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*

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## Summary

Protein synthesis is a regulated cellular process that links nutrients in the environment to organismal growth and development. Here we examine the role of genes that regulate mRNA translation in determining growth, reproduction, stress resistance and lifespan. Translational control of protein synthesis by regulators such as the cap-binding complex and S6 kinase play an important role during growth. We observe that inhibition of various genes in the translation initiation complex including *ifg-1*, the worm homologue of eIF4G, which is a scaffold protein in the cap-binding complex; and *rsks-1*, the worm homologue of S6 kinase, results in lifespan extension in *Caenorhabditis elegans*. Inhibition of *ifg-1* or *rsks-1* also slows development, reduces fecundity and increases resistance to starvation. A reduction in *ifg-1* expression in dauers was also observed, suggesting an inhibition of protein translation during the dauer state. Thus, mRNA translation exerts pleiotropic effects on growth, reproduction, stress resistance and lifespan in *C. elegans*.

### Keywords

*C. elegans*; eIF4G; lifespan; mRNA translation; protein synthesis; S6 kinase

## Introduction

The genetics of lifespan in model invertebrate organisms has yielded considerable information on the possible physiological determinants of aging rate. Many of the major genetic modulators of lifespan, including those in the insulin/IGF-1 and target of rapamycin (TOR) pathways, influence aging in organisms ranging from single-celled yeast, fruit flies, nematodes and, in the case of insulin/IGF-1 signalling, rodents (Kapahi & Zid, 2004; Kenyon, 2005). Protein synthesis is a key regulated cellular process that links nutrients to organismal growth, and is also modulated by the insulin and TOR pathways (Sonenberg *et al.*, 2000; Shamji *et al.*, 2003). The eukaryotic initiation factor 4F (eIF4F) initiation complex mediates mRNA translation (Sonenberg *et al.*, 2000; Harris & Lawrence, 2003; Shamji *et al.*, 2003). This is accomplished by regulating the association of the mRNA cap-binding protein eIF4E to the scaffold protein eIF4G, both components of the eIF4F complex (Supplementary Fig. S1). Eukaryotic initiation factor 4G helps assemble the eIF4F complex by bridging the poly(A)-binding proteins (PABPs) with eIF4E (Sonenberg *et al.*, 2000). This leads to the circularization of mRNAs, which has a synergistic effect on the rate of translation (Sonenberg *et al.*, 2000). Ribosomal S6 kinase (S6K) phosphorylates and thereby activates the ribosomal protein S6 on



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multiple serine residues, under the control of TOR, and is a key regulator of mRNA translation and growth (Thomas, 2002; Shamji *et al*., 2003; Ruvinsky & Meyuhas, 2006). S6K was also recently found to be associated with the eIF3 pre-initiation complex, which coordinates protein synthesis (Holz *et al*., 2005). In this study we examine the effect of modulating mRNA translation on various life history traits including lifespan, reproduction and stress resistance.

## Results

mRNA translation was inhibited using RNA interference (RNAi) of *ifg-1* (an eIF4G homologue in *Caenorhabditis elegans*) and the deletion mutant *rsks-1*(*ok1255*) (*C. elegans* ribosomal S6K, homologue of mammalian *p70S6K*). A 1.6-kb deletion in exon 4 of *rsks-1* was confirmed using nested polymerase chain reaction (PCR) (data not shown). The rate of protein synthesis was measured by incorporation of $^{35}$S-methionine from media. Radioactivity in worm protein extracts was measured as a function of exposure time to $^{35}$S-methionine-labelled OP50 *Escherichia coli* (Fig. 1a). The slope of this function indicates the rate of protein synthesis (see Supplementary Fig. S2 for feeding rates). These results demonstrate a significant decrease in the rate of incorporation of $^{35}$S-methionine upon inhibition of either *ifg-1* by RNAi or in the *rsks-1*(*ok1255*) mutant (Fig. 1a). Moreover, inhibition of *ifg-1* in the *rsks-1*(*ok1255*) mutant background resulted in an additive decrease in protein translation (Fig. 1a). Translation state was also analyzed by measuring polysomal distribution. Inhibition of *ifg-1* led to a reduction in total polysomal translation compared with controls (Fig. 1b). Mutation in *rsks-1* resulted in a smaller decrease in translation compared to that observed upon *ifg-1* inhibition. The most abundant polysome levels shifted from the sixth peak (A) in wild type (N2) to the fifth peak (B) in *rsks-1*(*ok1255*) animals (Fig. 1c), consistent with an overall reduction in translation. The data from both the methionine incorporation assay and the polysome profiles suggest a decrease in mRNA translation upon inhibition of *ifg-1* and *rsks-1* compared to controls.

Then we examined the effect on lifespan upon inhibition of genes that regulate protein translation. Inhibition of *ifg-1* during adulthood led to a 36% increase in mean lifespan (Fig. 2a). We also examined the effect on lifespan of inhibition of other genes that are part of the cap-binding complex. Inhibition of at least one eIF4E isoform and two of the three PABPs also has a significant effect on lifespan extension (Supplementary Fig. S3). Inhibition of *rsks-1* using RNAi during adulthood led to a 22% increase in mean lifespan (Fig. 2a). Mean lifespan was also extended in a deletion mutant of *rsks-1*(*ok1255*) compared to the parental control N2 strain (Fig. 2c). Together, these results support the view that inhibition of protein synthesis by capdependent translation and S6K extends lifespan in *C. elegans*.

Inhibition of *let-363* (worm homologue of TOR) has been shown to extend adult lifespan in *C. elegans* (Vellai *et al*., 2003). If effects of *let-363* on lifespan are mediated by its influence on translation rate, one might expect non-additive effects of *let-363* and *ifg-1* on lifespan. We therefore examined whether the lifespan extension caused by inhibition of *let-363* and *ifg-1* is additive. Inhibition of *let-363* increased lifespan in both the N2 background and when *ifg-1* was inhibited (Fig. 2b). These results could imply that lifespan extension by *let-363* and *ifg-1* involves different mechanisms. We also find that lifespan extension by inhibition of *ifg-1* and *rsks-1* is additive (Fig. 2c). In the *rsks-1*(*ok1255*) mutant background, inhibition of *ifg-1* led to a further extension of 46% in mean lifespan (Fig. 2c). However, inhibition of *let-363* by RNAi in *rsks-1* mutant background led to a slight shortening of lifespan (Fig. 2c). These results strongly imply that a common mechanism mediates lifespan extension due to inhibition of *let-363* and *rsks-1* but, surprisingly, suggests that this may not be the case for *let-363* and *ifg-1*, or *ifg-1* and *rsks-1*. Alternatively, additive effects could reflect a weak influence on a common mechanism in each case.

We tested the effects of inhibiting *ifg-1* or *rsks-1* on other lifespan extension pathways in worms. Down-regulation of *daf-2* (insulin/IGF-1-like receptor) causes lifespan extension that is dependent on *daf-16*, a forkhead transcription factor (Kenyon *et al*., 1993; Lin *et al*., 1997; Ogg *et al*., 1997). In the *daf-2*(*e1370*) mutant background, we observed that inhibition of either *ifg-1* or *rsks-1* further extended lifespan by 70% and 24%, respectively (Fig. 2d). To further test interaction with the insulin-like signaling (ILS) pathway, we examined lifespan extensions by *ifg-1*or *rsks-1* inhibition in *daf-16*(*mu86*) null mutant background. Inhibition of either *ifg-1* or *rsks-1* extended lifespan by 27% and 14%, respectively, in a *daf-16*(*mu86*) background (Fig. 2e). These findings show that knock-down of *ifg-1* or *rsks-1* does not increase lifespan by activating DAF-16, and also could imply that mutation of *daf-2* also extends lifespan via a different mechanism.

Then we tested if lifespan extension by *ifg-1* or *rsks-1* inhibition is additive with that resulting from mutation of the Clock gene *clk-1*. Inactivation of *clk-1*, a gene required for ubiquinone biosynthesis (Ewbank *et al*., 1997), has been shown to increase lifespan independently of the ILS pathway and displays the associated phenotypes of delayed development, slow pharyngeal pumping and defecation rates (Lakowski & Hekimi, 1996). Inhibition of either *ifg-1* or *rsks-1* in the *clk-1*(*e2519*) mutant background led to a further lifespan extension by 19% and 13%, respectively (Fig. 2f). This could imply that *clk-1*(*e2519*) extends lifespan via mechanisms distinct to *ifg-1* or *rsks-1*; however, it is worth noting that *clk* mutants show additive interactions with one another.

We also tested the role of *ifg-1* and *rsks-1* in lifespan extension by dietary restriction (DR) using the *eat-2*(*ad465*) mutant, which exhibits a mutationally induced form of DR (Lakowski & Hekimi, 1998). Inhibition of either *ifg-1* or *rsks-1* extended lifespan in the *eat-2* background by 54% and 11%, respectively (Fig. 2g). This could imply that effects of *ifg-1* and *rsks-1* in lifespan are distinct from those of DR. But again, it remains possible that additive effects involve a shared mechanism.

SIR2 is a conserved regulator of lifespan in yeast (Kaeberlein *et al*., 1999), worms (Tissenbaum & Guarente, 2001), and flies (Rogina & Helfand, 2004). In a *sir-2.1* mutant background, *ifg-1* inhibition extended lifespan by 93% and *rsks-1* inhibition by 33% (Fig. 2h). This clearly demonstrates that effects of *ifg-1* and *rsks-1* are not mediated by *sir-2.1*. Lifespan extension by inhibition of *ifg-1* and *rsks-1* is also independent of *bec-1* which has been shown to regulate autophagy (Melendez *et al*., 2003) (data not shown). This implies that effects on lifespan here are not dependent in a change in levels of autophagy.

Quantification of *ifg-1* mRNA levels at different developmental periods showed that it was significantly reduced in the dauer stage (Fig. 3a). These results suggest a down-regulation of cap-dependent translation due to inhibition of *ifg-1* during the dauer stage. We observe that the *rsks-1*(*ok1255*) mutant developed more slowly than the controls (Fig. 3b). Mutant animals are smaller at the same chronological age compared to controls (Fig. 3b) and show a delay in reaching adulthood (data not shown). Inhibition of *ifg-1* was reported earlier to lead to larval arrest in a manner similar to inhibition of *let-363* (Long *et al*., 2002). Inhibition of either *ifg-1* or *rsks-1* during adulthood also led to a decrease in fecundity (Fig. 3c,d). Taken together, these results show that *ifg-1* and *rsks-1* are required for normal growth and development during the early part of life in *C. elegans*.

It has been proposed that mechanisms that increase resistance to various environmental stressors also extend lifespan (Johnson *et al*., 1996; Martin *et al*., 1996). We examined the effect of inhibition of *ifg-1* and *rsks-1* on several stressors. Inhibition of *ifg-1* or *rsks-1* had a negligible effect on thermotolerance while inhibition of *daf-2* resulted in an increased thermotolerance (Fig. 4a) as previously reported (Lithgow *et al*., 1995). Similarly, no

significant increase was observed in resistance to the oxidant juglone, which leads to increased levels of superoxide *in vivo* (Fig. 4b). In fact, inhibition of *rsks-1* using RNAi (Fig. 4b) or the deletion mutant *rsks-1*(*ok1255*) caused increased sensitivity to juglone (data not shown). Increased resistance to UV stress was observed by inhibition of *ifg-1* but not *rsks-1* (Fig. 4c). In contrast to other stressors, we observed that inhibition of either *ifg-1* or *rsks-1* led to increased starvation resistance: a 56% and 28% increase, respectively, of median survival of starved animals compared to N2 (Fig. 4d). Selection for resistance to starvation leads to lifespan extension in *Drosophila*, implying a possible common mechanism for resistance to starvation and lifespan extension.

## Discussion

The nutrient-responsive TOR pathway is an evolutionarily conserved regulator of lifespan affecting yeast (Kaeberlein *et al*., 2005), worms (Vellai *et al*., 2003; Jia *et al*., 2004), and flies (Kapahi *et al*., 2004). Lifespan extension by inhibition of S6K was observed in *C. elegans* (Fig. 2a) and *Drosophila melanogaster* (Kapahi *et al*., 2004) suggesting that this enzyme also has a conserved role in determining lifespan. S6K1 null mice are smaller in size and show a reduction in ribosomal S6 phosphorylation (Pende *et al*., 2004). However, the mechanisms by which S6K regulates mRNA translation remain poorly understood (Pende *et al*., 2004). Mice lacking S6K1 are resistant to diet-induced obesity (Um *et al*., 2004). Moreover, both mice on high-fat diet and leptin-deficient mice show increased S6K activity, suggesting a role of S6K in fat metabolism.

To explore the role of *rsks-1* and *ifg-1* in known pathways affecting aging, we tested the effects of simultaneous knockdown of these and other genes. Such epistasis analysis is subject to certain limitations in terms of interpretation. While the absence of an additive effect between two treatments strongly implies that both act via a common mechanism, the occurrence of additive effects are consistent with both distinct and shared mechanisms (Gems *et al*., 2002). It is possible that the maximal lifespan extension possible due to perturbing a particular pathway may have not been achieved in a particular 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). We see additive effects of inhibition of *rsks-1* and *ifg-1* on longevity. Given than both regulate mRNA translation, this interaction may reflect additive effects on overall level of mRNA translation; alternatively, it could reflect distinct mechanisms. Similarly, our data also show that lifespan extension by inhibition of *ifg-1* or *rsks-1* is additive to the lifespan increases due to *daf-2, clk-1* and *eat-2*; taken alone, these observations are difficult to interpret in terms of single or multiple underlying mechanisms. By contrast, the lifespan extension observed in *daf-16* and *sir-2.1* background clearly demonstrates that the effects on lifespan of *ifg-1* and *rsks-1* are not mediated by these genes. Recently, inhibition of translation factors *ifg-1* and eIF2B were shown to extend lifespan in *C. elegans* (Henderson *et al*., 2006). The lifespan extension upon inhibition of *ifg-1* was dependent on *daf-16* when RNAi was applied during adulthood but not when applied throughout development. This contrasts with our results, where we see a significant increase in lifespan in a *daf-16* background resulting from inhibition of *ifg-1* during adulthood. Possibly, differences in RNAi constructs are responsible for this discrepancy.

It has been estimated that the fraction of genes devoted to translation may be as high as 35 to 45% (Sonenberg *et al*., 2000). We reason that inhibition of this costly process is likely to shift investment towards somatic maintenance and away from development, growth, and reproduction. The effects of inhibition of *ifg-1* and *rsks-1* on lifespan, growth, development and reproduction shown here are consistent with its role as an antagonistically pleiotropic mechanism that determines lifespan (Williams, 1957; Martin *et al*., 1996). Both S6K and eIF4G play a key role in growth in response to changes in nutrients in the environment in mammals

(Sonenberg *et al.*, 2000). Lifespan extension by DR has been proposed to be due to a shift in resources from growth and reproduction towards somatic maintenance allowing the animal to survive nutrient-poor conditions until they find conditions fit for reproduction. Hence, molecular mechanisms that lead to lifespan extension by DR may overlap with those that ensure increased survival during times of starvation. The data show that reduction in protein synthesis via inhibition of *ifg-1* or *rsks-1* leads to prolonged survival under starvation (Fig. 4d). Thus, reduction of protein synthesis may in part be responsible for the lifespan extension due to DR. The extension of lifespan that we observed in *eat-2* mutants upon RNAi of *ifg-1* or *rsks-1* is not inconsistent with this.

Our data suggest a possible link between translation regulation and the longevity of dauer larvae. The dauer stage involves a nonfeeding, nonreproducing alternative developmental state under which animals have prolonged survival. Possibly, *C. elegans* has evolved a method to inhibit cap-dependent translation in the dauer state by reducing the expression of *ifg-1*, since we observe lowered expression of this gene (Fig. 3a), in agreement with a previous genome wide expression study of dauers (Wang & Kim, 2003). As the scaffold protein eIF4G forms a bridge between PABPs and eIF4Es it would be an effective target for inhibition of cap-dependent translation. *Saccharomyces cerevisae* have also evolved an eIF4G-dependent mechanism to reduce protein synthesis under conditions of starvation. In this case, eIF4G degradation is observed when yeast cells undergo a diauxic shift (Berset *et al*., 1998). Overexpression of eIF4E has recently been shown to increase cellular senescence in mice as measured by β-galactosidase staining, implicating its potential involvement in mammalian aging (Ruggero *et al*., 2004). The regulation of cap-dependent translation may therefore be a conserved response to nutrient limitation in different species.

Longevity is a trait which shows a very high degree of variation between animal species. Could protein synthesis play a role in determining species-specific lifespan? If it did one would expect to see an inverse correlation across species between rate of protein synthesis and longevity. In fact, such an inverse correlation has been observed (Munro, 1969; Finch, 1990). Taken together with our results, this supports the notion that differences in rate of protein synthesis may contribute to evolved differences in lifespan between species.

## Experimental procedures

### Strains

*Caenorhabditis elegans* strains N2 and *rsks-1*(*ok1255*) were cultured at 20 °C on NGM plates seeded with OP50 bacteria as described in Riddle *et al*. (1997). *rsks-1*(*ok1255*) was obtained from the Gene Knockout Project at OMRF, which is part of the International *C. elegans* Gene Knockout Consortium. *rsks-1*(*ok1255*) was backcrossed into control N2 strain five times for all the experiments. Other strains used, *daf-2*(*e1370*), *daf-16*(*mu86*), *clk1*(*e2519*), and *eat-2* (*ad465*) were obtained from the Caenorhabditis Genetics Center.

### RNAi experiments

RNAi bacteria strains were cultured as previously described (Kamath *et al*., 2001b). 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was used for induction in all cases except Fig. 2b,c where 2 mM was used. Inhibition of *let-363* under 1 mM conditions did not extend lifespan (data not shown). Worms were staged at L4 and introduced to RNAi for 48 h using methods previously described (Kamath *et al*., 2001a). Clone identity of all RNAi bacteria was verified by sequencing. In addition, quantitative reverse transcriptase-PCR confirmed the inhibition of *ifg-1* and *rsks-1* (data not shown).

## Polysomal profiling

Two-day-old adult worms were used to generate polysomal profiles. Previously used method (Dinkova *et al*., 2005) was optimized to resolve the polysomes and ribosomal subunits. One hundred microliter of gently pelleted worms were homogenized on ice in 300 μL of solublization buffer [300 mM NaCl, 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 1 mM EGTA, 200 μg heparin per mL, 400 U RNAsin per mL, 1.0 mM phenylmethylsulfonyl fluoride, 0.2 mg cycloheximide per mL, 1% Triton X-100, 0.1% Sodium deoxycholate] by 60 strokes with a Teflon homogenizer. Seven hundred microliter additional solubilization buffer was added, vortexed briefly, and placed back on ice for 10 min before centrifuging the sample at 20 000 ***g*** for 15 min at 4 °C Approximately 0.9 mL of the supernatant was applied to the top of a 10-50% sucrose gradient in high salt resolving buffer [140 mM NaCl, 25 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$] and centrifuged in a Beckman SW41Ti rotor (Beckman Coulter, Fullerton, CA, USA) at 180,000 ***g*** for 90 min at 4 °C. Gradients were fractionated with continuous monitoring of absorbance at 260 nm.

## Lifespan analysis

Lifespan analysis was performed as previously described (Lithgow *et al*., 1995), except that 80 μL of 0.2 mM (+)-5-fluorodeoxyuridine (FUdR) was added onto plates daily during the reproductive phase to eliminate contamination from progeny. Worms were transferred onto fresh plates every 3 days. In all experiments, RNAi was introduced to 1-day-old adults except for the experiments in Fig. 2b, where RNAi was introduced to eggs. All trials were performed at 20 °C. Survival was scored by means of touch-provoked movement and pumping of the pharynx. All survival plots refer to lifespan beginning from adulthood including for *rsks-1* (*ok1255*) which develops slower than control. Animals that crawled off the plate, burrowed in the agar, or died from internally hatching progeny were censored.

## 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.

## $^{35}$S-methionine incorporation

OP50 was cultured in LB containing 100 μCi $mL^{-1}$ of $^{35}$S-methionine for 12 h and then spotted onto NGM plates, then spotted with approximately 200 worms in 0.2 mM FUdR and incubated at 20 °C for varying time points. We then measured radioactivity in protein extracts collected from these worms to determine translation rates. Worms were washed with S-basal and placed on normal OP50 for 30 min to purge undigested $^{35}$S-methionine-labelled OP50 out of their intestines. Worms were given another wash with S-basal and flash frozen in liquid nitrogen. Frozen samples were boiled in 1% SDS, centrifuged at 10,000 ***g***, and the supernatant was precipitated in 5% TCA on ice for 1 h. Protein was collected by centrifugation at 13 000 r.p.m. and washed with icecold ethanol. The ethanol was then allowed to dry completely and the protein pellet was resuspended in 1% SDS. Protein concentration was then assayed by bicinchoninic acid (BCA) protein assay (Pierce Chemical Co., Rockford, IL, USA) in 96-well plates. $^{35}$S activity was measured by liquid scintillation with a Beckman LS6500.

## Quantitative RT-PCR

Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) was used for total RNA preparations from *C. elegans*. Total RNA was treated with DNase I (New England Biolabs, Ipswich, MA, USA). The first strand cDNA was synthesized using the reverse transcription system (Promega, Madison, WI, USA). SYBR Green dye (Applied Biosystems, Foster City, CA, USA) was used for qRT-PCR. Reactions were run in triplicate on an ABI Prism 7000 real-time PCR machine

(Applied Biosystems). Relative-fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). The sequences of primers used were as follows: for *pmp-2* mRNA, forward, GAT TGT TTT ACC ACA AAC CAC; reverse, GTT CGA AAC GAT ATG ATC CAC; for *ifg-1* mRNA, forward, AGC AAA GAG ATC GTA TGC AC; reverse, ACA AAC TTC AAT AAC CGC AG.

### Nested PCR

The sequences of primers used were as follows: outer left GAG ATG CGG AAG CTA TGC TC, outer right GTT GAA TTC CTG CTC CTC CA, inner left ATT CAA CTG TGT GCC AGT GC, inner right TGG GGC TTC ACT ATT TGG TC. These primers were used to confirm the deletion in the *rsks-1* mutant.

### Fecundity analysis

Individual unmated hermaphrodites were transferred every 24 h and their daily progeny was scored.

### Pump rate analysis

Pump rates were measured as previously described (Avery & Horvitz, 1989).

### Body size measurement

N2 and *rsks-1*(*ok1255*) adults were allowed to lay eggs for 1 h on OP50. Eggs were then collected and grown at 20 °C. At 24 h intervals, twenty worms of each type were mounted onto slides in S-basal, heat killed at 65 °C for 2 min and then imaged on an Olympus 1 × 70 microscope. Worm lengths were measured from the center of the head to the tail, along the side of the worm, and converted into pixel numbers using Scion Image Beta 4.02 (Scion Corporation, Fredrick, MD, USA).

### Stress resistance assays

**Thermotolerance—**Thermotolerance assays were performed as previously described (Lithgow *et al*., 1995). Synchronous 5-day-old adults were shifted from 20 °C to 35 °C and survival scored by means of touchprovoked movement. Young adult worms were exposed to RNAi treatment for 48 h before the assay. Worms not responding to touch were scored as dead.

**Oxidative stress—**Juglone resistance was performed as described previously (de Castro *et al*., 2004). Ethanol dissolved juglone solution was added to liquid NGM to a final concentration of 240 $\mu_M$ and poured immediately onto plates to dry. One hundred microliter of OP50 was spotted on the plates and dried. Three hours after the plates were poured, 30 5-day-old adults were transferred to each plate after RNAi treatment during adulthood. Worms were incubated at 20 °C and scored hourly until death.

**UV resistance—**Worms synchronized at L4 stage were put on RNAi bacteria for 48 h. Then worms are transferred onto a NGM agar plate without any bacteria and exposed to 2000 J $m^{-2}$ UV using a UV Stratalinker® 1800 (Stratagene, La Jolla, CA, USA). Worms are then transferred back to plates containing their respective RNAi bacteria and scored for survival.

**Starvation resistance—**Synchronized L4 worms grown at 20 °C on OP50 were transferred onto large plates containing RNAi bacteria and 0.5 mL of 0.2 $m_M$ FUdR for 48 h. Then worms were washed from the plates and transferred to a 15-mL conical tube. Worms were washed five times in S-basal. Finally, worms were mixed in S-basal containing 0.2 $m_M$ FUdR and aliquots were placed into culture dishes. Worms were shaken continuously in this media. Scoring was done by placing 50 μL of the sample onto a NGM agar dish.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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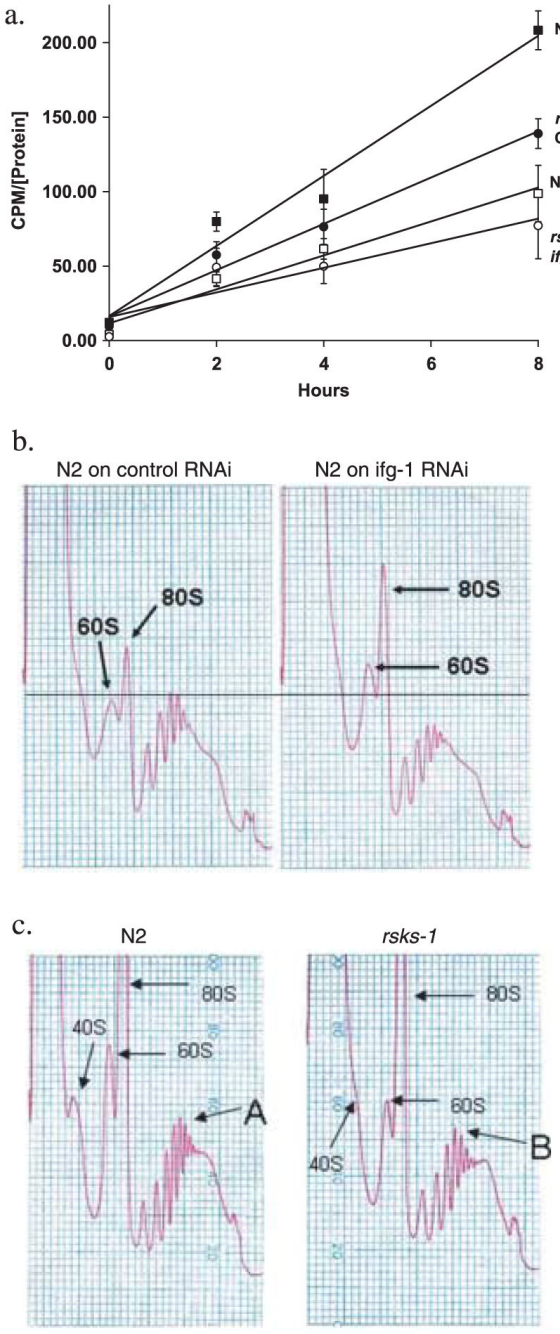


**Fig. 1.**

Inhibition of either *rsks-1* or *ifg-1* reduces the rate of protein translation. (a) Reduction in rates of protein synthesis measured by $^{35}$S-methionine incorporation upon inhibition of *ifg-1*and *rsks-1*. Age-matched adults were placed in $^{35}$S-methionine-labelled OP50 for 0, 2, 4, and 8 h. Radioactivity was measured in the TCA precipitated protein extract and standardized for protein concentration. Protein synthesis rate was evaluated by measuring the slope of the curves during incorporation. The following slopes were observed: N2 on L4440 (control RNAi), 23.5 (cpms (counts per minute) $\mu g^{-1}$) h; *rsks-1*(*ok1255*) on L4440 (control RNAi), 15.5 (cpms $\mu g^{-1}$) h; N2 on *ifg-1*(RNAi), 11.4 (cpms $\mu g^{-1}$) h; *rsks-1*(*ok1255*) on *ifg-1*(RNAi), 8.3 (cpms $\mu g^{-1}$) h. Each data point represents the mean of three biological replicates. Similar results were

obtained in two separate experiments. (b,c) Polysomal profiles were generated by measuring absorbance profiles at 260 nm of worm extracts separated on 10-50% sucrose gradients. (b) The N2 strain on control RNAi and *ifg-1*(RNAi) and from (c) N2 and *rsks-1*(*ok1255*). 40S, 60S, and 80S labels show the position of ribosomal subunits and whole ribosomes. There was an increase in the 60S and the 80S peaks upon inhibition of *ifg-1* accompanied by a decrease in the polysomal peaks suggesting a significant reduction in ribosomes that were involved in mRNA translation but an increase in free ribosomes (b). Maximum absorbance in N2 was detected in the sixth ribosome peak (A) vs. the fifth (B) in the *rsks-1*(*ok1255*) (c). Results are representative of three experiments performed.

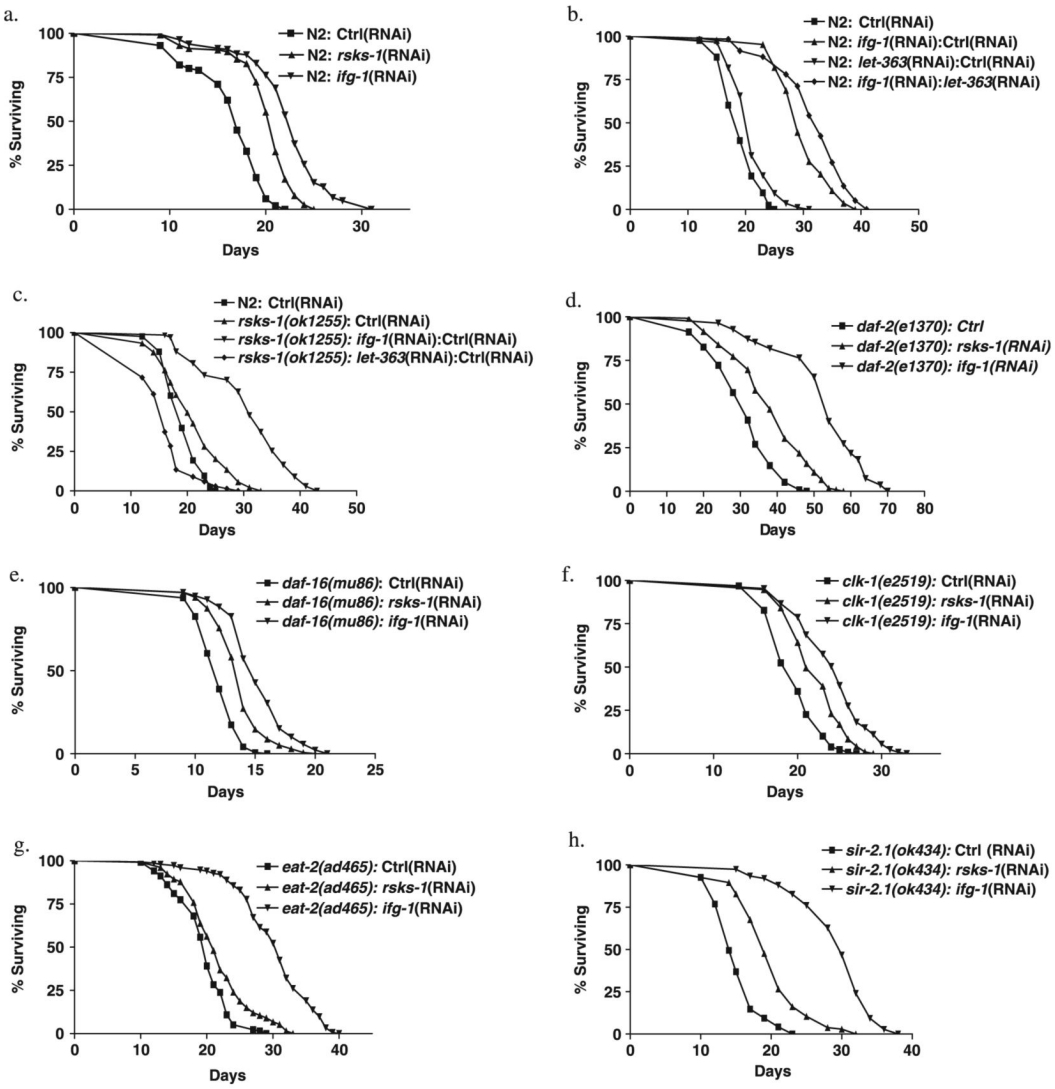


**Fig. 2.**

Lifespan extension by inhibiting *ifg-1* and *rsks-1* and their interaction with other longevity genes. (a) Mean lifespan was 16.4 days ($n$ = 100) for N2 on control RNAi; 20 days ($n$ = 116) for *rsks-1*(RNAi); and 22.3 days ($n$ = 164) for *ifg-1*(RNAi) ($P < 0.0001$). (b) Mean lifespan was 19.2 days ($n$ = 83) for N2 on control RNAi; 29.8 days ($n$ = 83) for N2 on 1: 1 *ifg-1*(RNAi) and control RNAi; 21.2 days ($n$ = 87) for 1: 1 *let-363*(RNAi) and control RNAi; and 32 days ($n$ = 59) for N2 on 1: 1 *ifg-1*(RNAi) and *let-363*(RNAi) ($P < 0.0001$). (c) Mean lifespan was 19.2 days ($n$ = 83) for N2 on control RNAi; 21 days ($n$ = 78) for *rsks-1*(*ok1255*) on control RNAi; 30.6 days ($n$ = 67) for *rsks-1*(*ok1255*) on 1: 1 *ifg-1*(RNAi) and control RNAi ($P <$ 0.0001); and 16.1 days ($n$ = 67) for 1: 1 *let-363* (RNAi) and control RNAi. (d) *daf-2*(*e1370*) on control RNAi, *rsks-1*(RNAi), and *ifg-1*(RNAi). Mean lifespan was 30.8 days ($n$ = 115) for *daf-2*(*e1370*) on control RNAi; 38.1 days ($n$ = 119) for *rsks-1*(RNAi); and 52.3 days ($n$ = 55) for *ifg-1*(RNAi) ($P < 0.0001$). (e) Mean lifespan was 12 days ($n$ = 144) for *daf-16*(*mu86*) on control RNAi; 13.7 days ($n$ = 136) for *rsks-1*(RNAi); and 15.2 days ($n$ = 138) for *ifg-1*(RNAi) ($P < 0.0001$). (f) Mean lifespan was 19.6 days ($n$ = 128) for *clk-1*(*e2519*) on control RNAi; 22.1 days ($n$ = 95) for *rsks-1*(RNAi); and 24.1 days ($n$ = 127) for *ifg-1*(RNAi) ($P < 0.0001$). (g) Mean lifespan was 19.5 days ($n$ = 138) for *eat-2*(*ad456*) on control RNAi; 21.7 days ($n$ =

133) for *rsks-1*(RNAi); and 30.1 days ($n = 153$) for *ifg-1*(RNAi) ($P < 0.0001$). (h) Mean lifespan was 15.2 days ($n = 95$) for *sir-2.1*(*ok424*) on control RNAi; 20.2 days ($n = 105$) for *rsks-1* (RNAi); and 29.3 days ($n = 75$) for *ifg-1*(RNAi) ($P < 0.0001$). Similar results were obtained in at least two separate experiments for each panel.

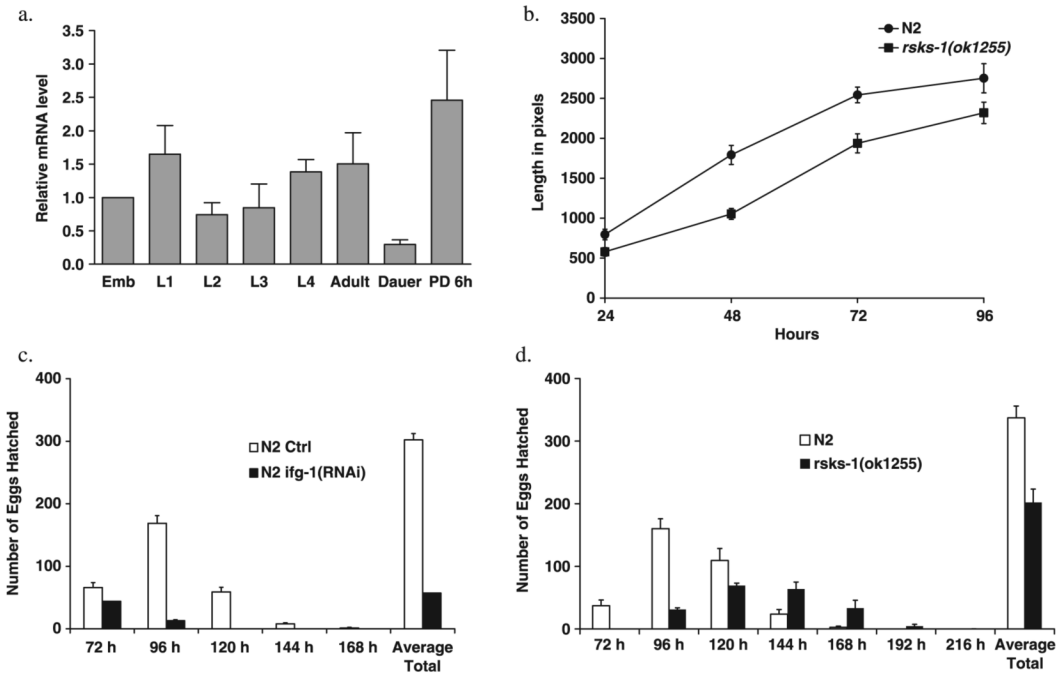


**Fig. 3.**

Pleiotropic effects on growth, development, reproduction and stress resistance upon inhibition of *ifg-1* and *rsks-1*. (a) *ifg-1* expression is reduced during dauer state. Quantification of relative levels of *ifg-1* mRNA in N2 animals at different developmental stages at 20 °C using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). *pmp-2* expression was used for internal reference. Mean and standard error of the relative *ifg-1/pmp-2* ratios based on three real-time RT-PCR trials are shown. Emb, embryos; PD, post dauer; 6 h, 6 h after dauer larvae were exposed to food. (b) *rsks-1* regulates rate of development in *C. elegans*. Average length of 20 worms was measured every 24 h till 96 h after egg lay in the N2 vs. *rsks-1*(*ok1255*) strain. Similar results were obtained in five trials. (c,d) Reduction of fecundity by inhibition of *ifg-1* and *rsks-1*. Progeny were counted every 24 h in (c) N2 on control RNAi and *ifg-1*(RNAi) (d) N2 and *rsks-1*(*ok1255*).

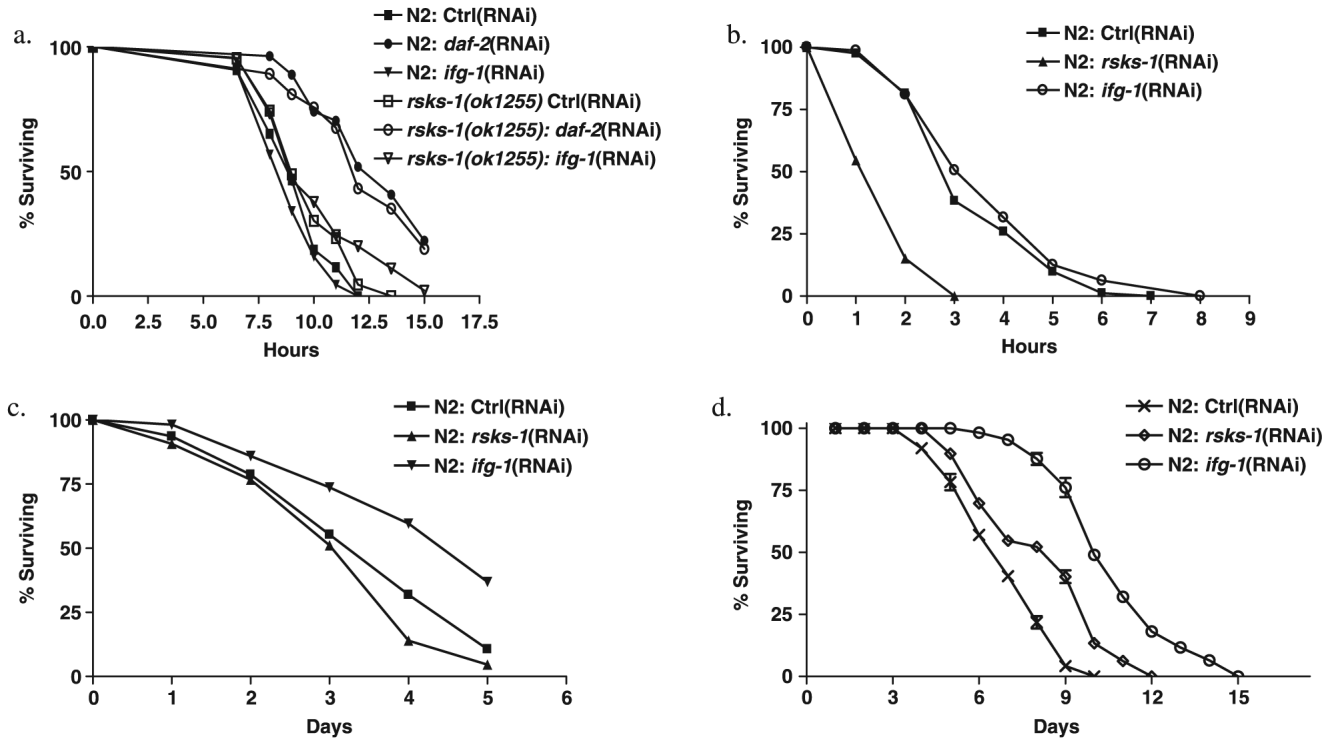


**Fig. 4.**

Effect of inhibition of *ifg-1* and *rsks-1* on resistance to various stresses. (a) One-day-old adult N2 and *rsks-1*(*ok1255*) worms were exposed to control RNAi, *ifg-1*(RNAi), and *daf-2*(RNAi) for 48 h in 20 °C before heat stressed at 35 °C. N2 on control RNAi ($n$ = 43) N2 *ifg-1*(RNAi) ($n$ = 44); N2 on *daf-2*(RNAi) ($n$ = 21) *rsks-1*(*ok1255*) on control RNAi ($n$ = 43), *rsks-1* (*ok1255*) *ifg-1*(RNAi) ($n$ = 44), *rsks-1*(*ok1255*) *daf-2*(RNAi) ($n$ = 30). Similar results were obtained in three experiments. (b) Adult worms were exposed to control RNAi, *rsks-1*(RNAi), and *ifg-1*(RNAi) for 48 h in 20 °C before being transferred to NGM agar plates containing 240 μM juglone. Mean survival was 3.5 h ($n$ = 81) for N2 on control RNAi; 1.7 h ($n$ = 66) for *rsks-1* (RNAi) ($P < 0.0001$); and 3.6 h ($n$ = 74) for *ifg-1*(RNAi). (c) Worms treated with RNAi bacteria for 48 from the L4 stage were exposed to 2000 J $m^{-2}$ of UV via a Stratalinker® 1800. Mean survival was 3.7 days ($n$ = 47) for N2 on control RNAi; 3.4 days ($n$ = 43) for *rsks-1*(RNAi); and 4.5 days ($n$ = 57) for *ifg-1*(RNAi) ($P < 0.0005$). (d) N2 and *rsks-1*(*ok1255*) were both exposed to control RNAi, *rsks-1*(RNAi), and *ifg-1*(RNAi) for 48 h before being starved in S-basal. Standard deviations were calculated from five biological replicates. Between 50 and 100 worms per replicate were scored each day. Median survival was 6.4 days for N2 on control RNAi; 8.2 days for *rsks-1*(RNAi); and 10 days for *ifg-1*(RNAi). Similar results were obtained in at least three experiments.