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LETTERS

eIF4E function in somatic cells modulates ageing in *Caenorhabditis elegans*

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Regulation of protein synthesis is critical for cell growth and maintenance. Ageing in many organisms, including humans, is accompanied by marked alterations in both general and specific protein synthesis[1]. Whether these alterations are simply a corollary of the ageing process or have a causative role in senescent decline remains unclear. An array of protein factors facilitates the tight control of messenger RNA translation initiation[2]. The eukaryotic initiation factor 4E (eIF4E), which binds the 7-monomethyl guanosine cap at the 5′ end of all nuclear mRNAs, is a principal regulator of protein synthesis[3]. Here we show that loss of a specific eIF4E isoform (IFE-2) that functions in somatic tissues[4] reduces global protein synthesis, protects from oxidative stress and extends lifespan in *Caenorhabditis elegans*. Lifespan extension is independent of the forkhead transcription factor DAF-16, which mediates the effects of the insulin-like signalling pathway on ageing. Furthermore, IFE-2 deficiency further extends the lifespan of long-lived *age* and *daf* nematode mutants. Similarly, lack of IFE-2 enhances the long-lived phenotype of *clk* and dietary-restricted *eat* mutant animals. Knockdown of target of rapamycin (TOR), a phosphatidylinositol kinase-related kinase that controls protein synthesis in response to nutrient cues, further increases the longevity of *ife-2* mutants. Thus, signalling via eIF4E in the soma is a newly discovered pathway influencing ageing in *C. elegans*.

The molecular mechanisms underlying age-associated changes in protein synthesis are largely unknown. In eukaryotes, the rate of cap-dependent protein synthesis is mainly determined by the translation initiation factor eIF4E[3]. We examined the role of eIF4E in *C. elegans* ageing. The *C. elegans* genome encodes five eIF4E isoforms (IFE-1 to IFE-5), which differ in cap-binding specificity and anatomical expression. Biochemical studies demonstrate that IFE-2 is the only eIF4E isoform in the soma that binds both 7-monomethyl guanosine and 2,2,7-trimethyl guanosine caps, present on nematode mRNAs. IFE-4 and the germline-specific IFE-3 only bind 7-monomethyl guanosine caps. IFE-1 and IFE-5, which are present in the germ line, also bind both 7-monomethyl guanosine and 2,2,7-trimethyl guanosine caps[4].

RNA interference (RNAi) knockdown of *ife-2* resulted in substantial extension of nematode lifespan (Fig. 1a; see also Supplementary Table 1). We observed similar lifespan extension in animals carrying an *ife-2* deletion (Fig. 1a). Depletion of other eIF4E isoforms had no effect on longevity (Fig. 1b; see also Supplementary Table 1). *ife-2* encodes the most abundantly expressed eIF4E isoform in *C. elegans* somatic tissues (Fig. 1c; see also Supplementary Fig. 1 and ref. 5). No *ife-2* expression is detected in the germ line, where *ife-1*, *ife-3* and *ife-5* are expressed[4,5]. *ife-4* encodes an additional eIF4E isoform expressed in the soma[4]. Consistent with RNAi experiments, we did not detect lifespan extension in animals carrying an *ife-4* deletion (Fig. 1b). We also measured the longevity of animals deficient for both soma-specific eIF4E isoforms. These nematodes showed lifespan equal to *ife-2(RNAi)* knockdown animals (Supplementary Table 1).

To establish further the somatic origin of longevity conferred by IFE-2 depletion, we used animals carrying a temperature-sensitive mutation in *glp-4* (*abnormal germline proliferation-4*), a zygotic gene required for normal post-embryonic proliferation of the germ line[6]. These mutants are germline-deficient at the restrictive temperature (25 °C). Similar to wild-type animals, knockdown of *ife-2* extends the lifespan of *glp-4* mutants (Fig. 1d; see also Supplementary Table 1). Therefore, lack of germ line does not suppress the effect of IFE-2 depletion on animal lifespan. We conclude that elimination of a specific eIF4E isoform, expressed in somatic cells, extends lifespan in *C. elegans*.

We considered whether IFE-2 depletion results in any other obvious defects that might account for the effects on animal lifespan. We examined both *ife-2* knockout mutants and wild-type animals subjected to *ife-2* RNAi for feeding behaviours, fertility, developmental abnormalities, movement defects and anatomical alterations. IFE-2-depleted animals grown at 20 °C were indistinguishable from wild-type animals for pharyngeal pumping of bacterial food into the intestine, defecation rhythms, fecundity, developmental timing, sinusoidal locomotion, body size and shape, and internal organ morphology (gonad, intestine, musculature and pharynx). IFE-2 deficiency induced significant embryonic lethality at 25 °C, whereas fecundity was relatively unaffected (Supplementary Table 2). We also tested the capacity of IFE-2-depleted animals to become dauer larvae, a stress-resistant, developmentally arrested larval form, induced by adverse environmental conditions. We did not observe any effects on dauer formation as a result of IFE-2 deficiency (Supplementary Table 2). In addition, we find that *ife-2* mutants are indistinguishable from wild-type animals for heat-shock resistance (Supplementary Fig. 2).

Ageing in *C. elegans* is mainly regulated by a conserved endocrine signalling pathway that involves the insulin/insulin-like growth factor (IGF) receptor DAF-2 (abnormal dauer formation-2) and the phosphatidylinositol-3-OH kinase (PI(3)K) AGE-1 (ageing alteration-1). Mutations that compromise the activity of DAF-2 or AGE-1 extend animal lifespan. Longevity conferred by *daf-2* and *age-1* mutations requires the forkhead box, sub-group O (FOXO) transcription factor DAF-16 (reviewed in ref. 7). We find that IFE-2 depletion further extends the lifespan of long-lived *daf-2* and *age-1* mutants (Fig. 2a; see also Supplementary Table 1). DAF-16 is not required for lifespan extension by IFE-2 deficiency (Fig. 2b; see also Supplementary Table 1). This observation indicates that IFE-2 functions downstream or independently of DAF-16 to control ageing. To distinguish between these two possibilities, we tested whether *ife-2* transcription is under the control of DAF-16. Expression of a full-length IFE-2::GFP fusion, driven by the *ife-2* promoter, is not affected by *daf-16* knockdown (Fig. 2c). Furthermore, depletion of DAF-2, which modulates DAF-16 activity via a protein phosphorylation cascade, does not affect *ife-2* expression (Supplementary Fig. 4). Therefore, *ife-2* is not a downstream target of DAF-16.

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Mutations in *clk-1* (*clock abnormality-1*), a gene required for the biosynthesis of ubiquinone (an essential component of the mitochondrial electron transport chain), also extend *C. elegans* lifespan[8]. Knockdown of *ife-2* further extends the lifespan of long-lived *clk-1* mutant animals (Fig. 2d; see also Supplementary Table 1). We observed a similar additive effect on lifespan in long-lived, dietary restricted *eat-2* (*eating, abnormal pharyngeal pumping-2*) mutants[9] (Fig. 2e; see also Supplementary Table 1). Taken together, our results suggest that regulation of eIF4E activity in somatic tissues constitutes a novel mechanism that influences longevity in *C. elegans*.

eIF4E activity is regulated by association with inhibitory eIF4E-binding proteins (4E-BPs) and by direct phosphorylation mediated by the mitogen-activated protein (MAP) kinase signal-integrating

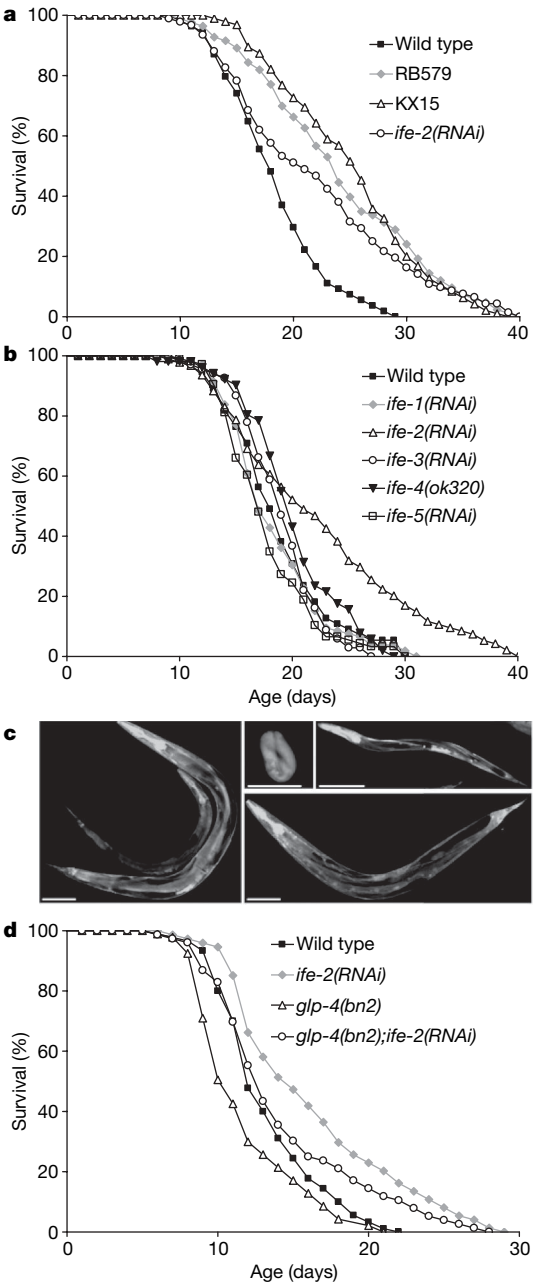

Figure 1 | eIF4E deficiency in somatic tissues extends *C. elegans* lifespan. The percentage of animals remaining alive is plotted against animal age. Lifespan values are given in Supplementary Table 1. **a**, Depletion of IFE-2 by gene deletion or RNAi extends animal lifespan. Strains RB579 and KX15 both harbour the *ife-2(ok306)* deletion allele. KX15 is an outcrossed version of RB579. Assays were carried out at 20 °C. **b**, Knockdown of the germline-specific *ife-1*, *ife-3* and *ife-5* genes does not alter animal lifespan. In addition, animals carrying the *ife-4(ok320)* deletion allele show normal lifespan. Assays were carried out at 20 °C. **c**, Images of animals expressing the $p_{ife\text{-}2}$IFE-2::GFP reporter fusion. *ife-2* is highly expressed in all somatic tissues of adult animals, including the pharynx, the intestine, body wall muscles, the hypodermis, neurons and the canal cell. Expression is high during all post-embryonic developmental stages, throughout adulthood, and is detectable in twofold stage embryos (details are shown in Supplementary Fig. 1; scale bars, 100 μm). **d**, Knockdown of *ife-2* extends the lifespan of *glp-4(bn2)* mutant animals lacking a germ line. Assays were carried out at 25 °C.

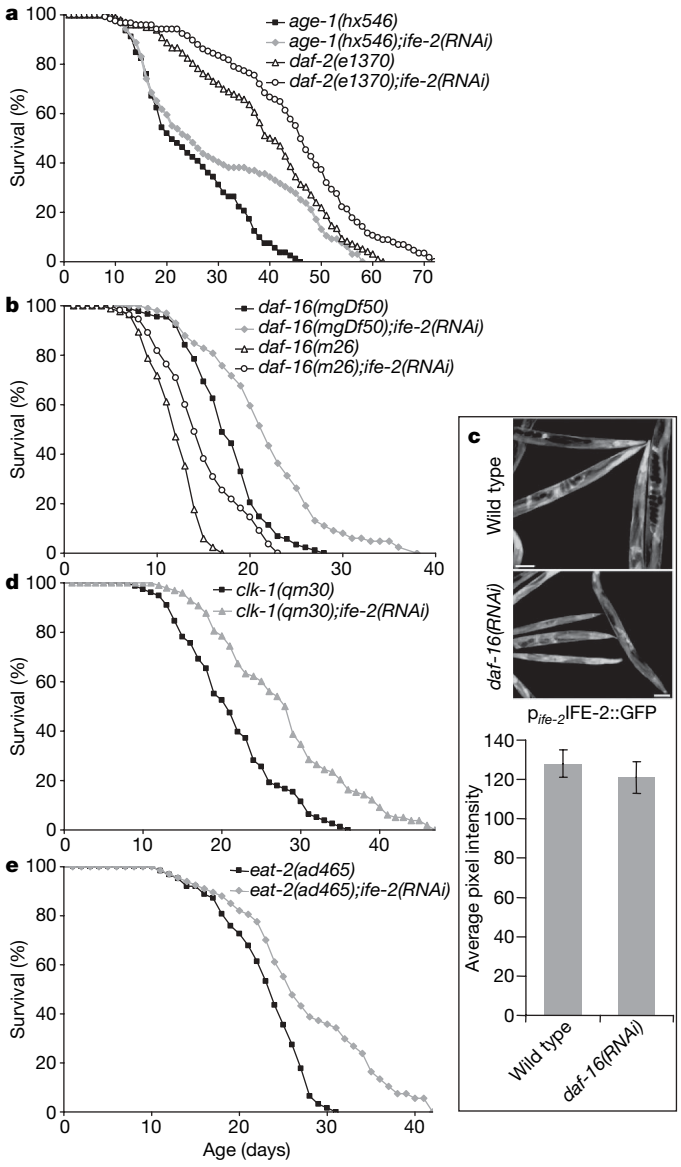

Figure 2 | IFE-2 and genes influencing ageing in *C. elegans*. Survival curves of mutant nematode populations subjected to *ife-2* RNAi are shown, with the percentage of nematodes remaining alive plotted against age. Lifespan values are given in Supplementary Table 1; assays were carried out at 20 °C. **a**, Knockdown of *ife*-2 further increases the lifespan of long-lived *age-1* and *daf-2* mutants. **b**, Lifespan extension by IFE-2 depletion is not suppressed by two *daf-16* mutant alleles. **c**, Expression of *ife-2* is independent of the DAF-16 transcription factor. Knockdown of *daf-16* does not affect expression of full-length IFE-2::GFP driven by the *ife-2* promoter ($p_{ife\text{-}2}$IFE-2::GFP). Representative images of nematodes bearing the $p_{ife\text{-}2}$IFE-2::GFP transgene subjected to *daf-16* RNAi compared to wild-type controls are shown. Quantification of animal fluorescence is also shown (five independent experiments, 50 animals total for each strain; error bars denote standard deviation; $P > 0.5$, unpaired *t*-test). **d**, **e**, Knockdown of *ife-2* further increases the lifespan of long-lived *clk-1* mutants (**d**) and long-lived *eat-2* mutant animals (**e**).

kinases (Mnk1/2)[10]. Phosphorylation of 4E-BP by the nutrient-sensing kinase TOR reduces its affinity for eIF4E and is thought to promote mRNA translation (reviewed in ref. 2). Notably, TOR deficiency, which dampens the rate of translation in yeast and mammals, extends *C. elegans* lifespan[11]. This observation is consistent with our findings that eIF4E downregulation confers increased longevity. Are the effects of TOR depletion on lifespan mediated by concomitant downregulation of eIF4E via 4E-BP? This scenario predicts that elimination of 4E-BP would cancel the effect that TOR deficiency has on ageing. However, no structural 4E-BP homologue is apparent in the *C. elegans* genome (P.S. and N.T., unpublished observations). Thus, we investigated the potential link between TOR signalling and eIF4E by assaying the lifespan of animals deficient for both TOR and IFE-2. Knockdown of *ife-2* further extends the lifespan of long-lived TOR-deficient mutants (Fig. 3a; see also Supplementary Table 1). Similarly, knockdown of TOR in animals carrying an *ife-2* deletion results in additional lifespan extension (Fig. 3b; see also Supplementary Table 1). The synergy between TOR and IFE-2 indicates that their effects on ageing are mediated by distinct mechanisms.

In addition to phosphorylating 4E-BP, TOR regulates S6K, a kinase that in turn phosphorylates the S6 ribosomal protein[12]. We asked whether S6K mediates the effects of TOR on ageing. Knockdown of the *C. elegans* p70S6K homologue *rsks-1* and the closely related p90S6K homologue *rskn-1* has no effect on animal lifespan (Fig. 3c; see also Supplementary Table 1). Furthermore, depletion of *rsks-1* or *rskn-1* does not suppress the lifespan extension brought about by IFE-2 deficiency (Fig. 3d; see also Supplementary Table 1). These observations suggest that the effects of IFE-2 on lifespan do not require S6K.

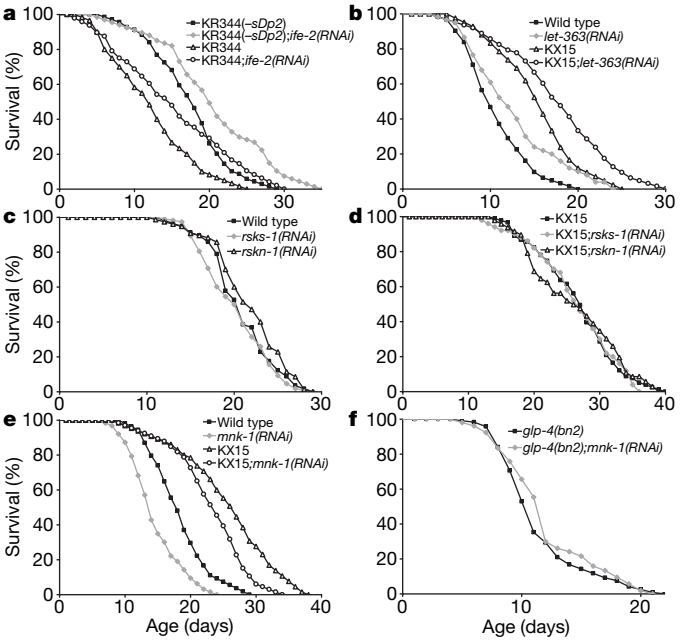

Figure 3 | Effects of regulatory kinase and IFE-2 removal on ageing. The percentage of animals remaining alive is plotted against animal age. Lifespan values are given in Supplementary Table 1. **a**, **b**, Knockdown of *ife-2* extends the lifespan of TOR-kinase-depleted mutants. **a**, Strain KR344 carries a loss-of-function allele of the *let-363* gene, which encodes the *C. elegans* TOR kinase, together with the chromosomal duplication *sDp2* that balances the *let-363* mutation. Loss of the *sDp2* genetic balancer (−*sDp2*) results in arrest at the L3 stage of development. Arrested *let-363* mutant animals are long lived[11]. Isogenic animals that have not lost the duplication (KR344) are included as a control (see also Methods and Supplementary Table 1; assays were carried out at 20 °C). **b**, RNAi knockdown of *let-363* was carried out in young adult animals exiting the L4 stage. Assays were carried out at 25 °C. **c**, **d**, S6K kinase knockdown does not affect wild-type animal lifespan and does not suppress *ife-2* mutant longevity. Assays were carried out at 20 °C. **e**, MNK-1 deficiency shortens the lifespan of wild-type and *ife-2* mutant animals. Assays were carried out at 20 °C. **f**, *mnk-1* knockdown does not alter ageing of *glp-4* germline-deficient animals. Assays were carried out at 25 °C.

In mammals, the Mnk1/2 kinases modulate the activity of eIF4E by phosphorylation at Ser 209 (ref. 10). In *Drosophila*, the Mnk homologue Lk6 regulates growth in response to nutrients via eIF4E[13,14]. We examined whether Mnk activity influences lifespan through eIF4E in *C. elegans*. RNAi knockdown of the nematode Mnk homologue *mnk-1* (*MAP kinase integrating kinase-1*) shortened both wild-type and *ife-2* mutant lifespan (Fig. 3e; see also Supplementary Table 1). We did not detect any concomitant developmental, behavioural or anatomical defects in *mnk-1* knockdown animals. Of note, no effect on lifespan is observed as a result of *mnk-1* knockdown in *glp-4* mutant animals lacking a germ line (Fig. 3f; Supplementary Table 1), indicating that MNK-1 function in the germ line is required to maintain normal lifespan.

Recent studies implicate eIF4E regulators such as 4E-BP, TOR and Mnk in the control of development, growth and ageing[15]. These regulators are coupled to the insulin/IGF signalling pathway. For example, transcription of 4E-BP is under the control of FOXO in *Drosophila*, whereas in mammals, the 4E-BP PHAS-I (phosphorylated heat- and acid-stable protein I) is regulated by insulin signalling (reviewed in ref. 2). In addition, genetic studies in *C. elegans* suggest that TOR may function downstream or independently of DAF-16 to mediate the effects of DAF-2 signalling on ageing[11]. However, the role of eIF4E in ageing remained unknown. We have shown that eIF4E deficiency in the soma, but not in the germ line, extends *C. elegans* lifespan. Our genetic analysis suggests that eIF4E could influence ageing independently of known mechanisms that involve insulin/IGF signalling, dietary restriction and respiratory chain components. Whether these mechanisms may alter protein synthesis by targeting other mRNA translation factors remains to be elucidated.

What is the mechanism underlying lifespan extension resulting from depletion of a specific eIF4E isoform? IFE-2 is the most abundant eIF4E isoform in all *C. elegans* somatic tissues and the only one in the soma that binds both 7-monomethyl guanosine and 2,2,7-trimethyl guanosine mRNA caps (Fig. 1c; see also Supplementary Fig. 1 and refs 4 and 5). Thus, we proposed that IFE-2 deficiency impairs mRNA translation initiation, resulting in protein synthesis reduction. To test this hypothesis, we developed a novel method to monitor and compare protein synthesis rates in *C. elegans*, based on fluorescence recovery after photo-bleaching (FRAP; see Supplementary Methods). We find that protein synthesis rates are lower in animals lacking IFE-2 (Fig. 4a). We used the protein synthesis inhibitor cycloheximide to confirm that the observed effects are due to alterations in protein synthesis (Fig. 4a).

Global mRNA translation is reduced in response to most, if not all, types of cellular stress. This results in notable conservation of cellular energy, given that the process of translation consumes up to an estimated 50% of the total energy, depending on the organism[16]. Similarly, reduction of protein synthesis by IFE-2 depletion may prolong lifespan by lowering energy demands and the associated generation of toxic metabolic by-products such as reactive oxygen species, which contribute significantly to the ageing process[17,18]. In addition, the concomitant increase in energy availability may allow diversion of critical resources to cellular maintenance and repair processes, thus promoting organism longevity. To test this hypothesis, we challenged *ife-2(ok306)* mutants with paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride) and sodium azide (NaN_3). The herbicide paraquat is a generator of superoxide anions[19]. NaN_3 is a potent and specific inhibitor of cytochrome *c* oxidase, which is part of the mitochondrial electron transport chain complex IV[20]. Paraquat treatment and inhibition of cytochrome *c* oxidase induce oxidative stress[21,22]. We find that long-lived *ife-2(ok306)* mutant animals are considerably more resistant to both paraquat and NaN_3 compared with wild-type animals (Fig. 4b). We also examined the effects of IFE-2 deficiency on the survival of *mev-1(kn1)* (*abnormal methyl viologen sensitivity-1*) mutants, which lack succinate dehydrogenase

cytochrome *b*, a component of complex II of the mitochondrial electron transport chain[23]. These animals are short lived and hypersensitive to oxidative stress induced by paraquat. Knockdown of *ife-2* renders *mev-1(kn1)* mutants markedly resistant to paraquat and also extends their lifespan (Fig. 4b, c). Hence, repression of protein synthesis in the soma renders animals capable of withstanding acute and chronic oxidative stress.

We next asked whether diminished IFE-2 activity in specific somatic tissues is sufficient to extend animal lifespan. We find that knockdown of *ife-2* by RNAi in all somatic cells except neurons is required to extend animal lifespan (Supplementary Fig. 3; RNAi is ineffective in the nervous system; see ref. 24). Thus, reduction of protein synthesis in neurons probably has negligible consequences on the lifespan of whole organisms.

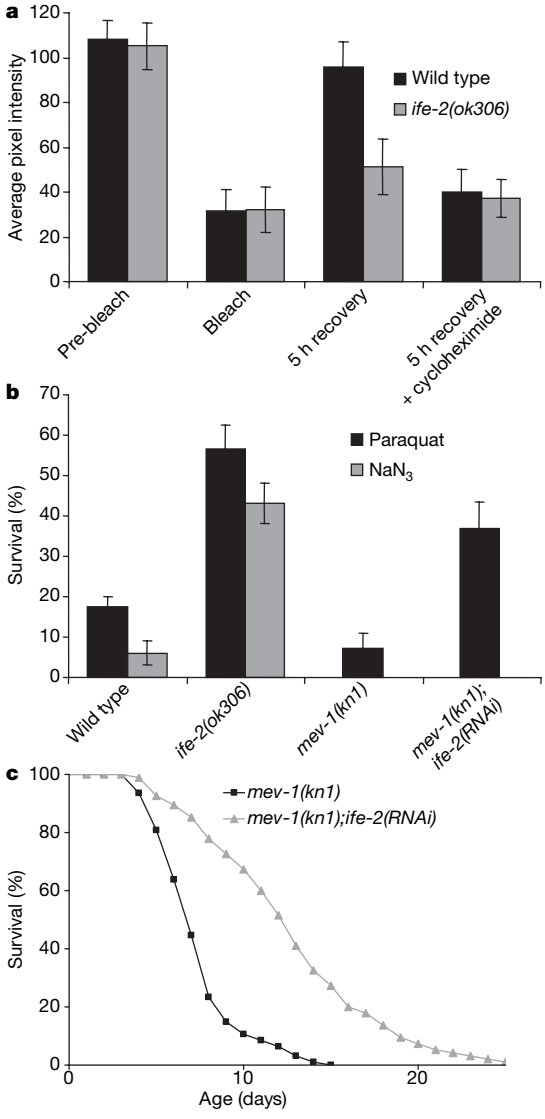

Figure 4 | eIF4E deficiency in the soma reduces protein synthesis and increases oxidative stress resistance in *C. elegans*. **a**, Quantification of fluorescence recovery after photo-bleaching in wild-type and IFE-2-deficient animals expressing GFP driven by the *ife-2* promoter ($p_{ife\text{-}2}$GFP; three independent experiments, 30 animals total for each strain; error bars denote standard deviation; $P < 0.005$, unpaired *t*-test; assays were carried out at 25 °C). **b**, Survival of synchronized animal populations under oxidative stress induced by treatment with paraquat and sodium azide (six independent experiments, 240 animals total for each strain; error bars denote standard deviation; $P < 0.005$, unpaired *t*-test). **c**, Knockdown of *ife-2* extends the lifespan of short-lived *mev-1* mutants. Assays were carried out at 20 °C.

Why is eIF4E activity in somatic tissues important for normal ageing? Studies in diverse species associate ageing with decreased protein synthesis[25,26]. This link suggests that a sustained high rate of mRNA translation may retard senescent decline and ageing[27]. Our work reveals a new link between protein synthesis and ageing. We demonstrate that depletion of eIF4E—a principal mRNA translation regulator—specifically in somatic cells increases oxidative stress resistance and extends lifespan. Our findings corroborate studies in mammals showing that overexpression of eIF4E promotes cellular senescence[28]. Therefore, downregulation of mRNA translation in the soma, under appropriate conditions, may facilitate cellular maintenance and repair by moderating the large energy requirement of protein synthesis. Whereas the germ line is an immortal cell lineage[29], somata, which encapsulate immortal germ lines, are mortal and frail. The 'disposable soma' theory of ageing provides an evolutionary framework for this fundamental distinction[30]. Failure to divert energy towards repairing stochastic damage that accumulates in the soma during life leads to inexorable decline of somatic functions and senescence. Our findings suggest that eIF4E is part of a novel mechanism, independent of insulin/IGF signalling, that modulates ageing of the soma by integrating environmental, reproductive and other cues to regulate protein synthesis and somatic maintenance.

Note added in proof: While this paper was under review, related work was published online[31,32].

METHODS

See Supplementary Information for detailed Methods.

Lifespan assays. Synchronous populations of nematodes were established by allowing 20 adult hermaphrodites to lay eggs for a limited time interval (4–5 h) on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50. For RNAi lifespan experiments, nematodes were placed on NGM plates containing 0.5–1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) and seeded with HT115(DE3) bacteria transformed with either the pL4440 vector or the test RNAi construct. Progeny were grown on HT115-seeded plates at 20 °C unless otherwise noted, through the L4 larval stage, and then transferred to fresh HT115-seeded plates at groups of 10–20 nematodes per plate for a total of 100–150 individuals per experiment. For strains bearing the *glp-4(bn2)* mutation, lifespan assays were initiated with freshly laid eggs transferred to 25 °C, to guard against germline proliferation. The first day of adulthood was used as $t = 0$. Animals were transferred to fresh plates every 2–4 days thereafter and were examined every day for touch-provoked movement and pharyngeal pumping, until death. Each survival assay was repeated at least three times and figures represent typical experiments.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions P.S., K.T and N.T. performed experiments; N.T designed experiments, analysed data and wrote the manuscript. All authors discussed the results and commented on the manuscript.

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