Protein Synthesis Is a Novel Determinant of Aging in *Caenorhabditis elegans*

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ABSTRACT: Protein synthesis is a tightly regulated cellular process that affects growth, reproduction, and survival in response to both intrinsic and extrinsic cues, such as nutrient availability and energy levels. A pronounced, age-related decline of the total protein synthesis rate has been observed in many organisms, including humans. The molecular mechanisms underlying this decline and their role in the aging process remain unclear. A series of recent studies in the nematode, *Caenorhabditis elegans*, have revealed a novel link between protein synthesis and aging. Remarkably, these research findings, in their totality, converge to indicate that reduction of mRNA translation prolongs life in worms. Signal transduction cascades implicated in aging, such as the insulin/insulin growth factor-1 pathway, interface with mechanisms regulating protein synthesis via a battery of key mRNA translation factors. Are the effects of these pathways on aging mediated, in part, by alterations in protein synthesis? This is an intriguing possibility in light of the latest discoveries. Whether attenuation of protein synthesis promotes longevity across different phyla is an additional important matter. Here, we survey work associating protein synthesis with aging and discuss the basis of life-span extension under conditions that attenuate protein synthesis.

KEYWORDS: *Caenorhabditis elegans*; eIF4E; insulin growth factor; life span; mRNA translation; oxidative stress; TOR kinase

INTRODUCTION

Research on simple model organisms has led to remarkable progress in understanding the molecular pathways that modulate aging and senescence. A multitude of single-gene mutations altering life span have been identified, providing evidence that aging can be modulated by evolutionary-conserved, regulatory pathways.[1] These pathways normally control growth, reproduction,

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stress response, and energy metabolism. Protein synthesis is critical for all biological processes and is influenced by aging. The activity of key mRNA translation factors declines with age in many organisms, resulting in reduction of protein synthesis rates.[2,3] However, the significance of this decline in senescence remains unclear. Is it a beneficial adaptation to reduced mitochondrial function and energy production, as a consequence of aging, or does it directly contribute to the aging process? Because protein synthesis is essential for growth and development, it is not straightforward to dissect its specific role in aging. Manipulation of general mRNA translation is likely to have pleiotropic effects, thus obscuring any explicit contribution to aging. Nevertheless, several recent studies capitalize on the genetic malleability of invertebrate models, such as *Caenorhabditis elegans* and *Drosophila*, to investigate the link between protein synthesis and aging. These studies provide an entry point into what might turn out to be an important aspect of the biology of aging.

REDUCTION OF PROTEIN SYNTHESIS EXTENDS LIFE SPAN IN *CAENORHABDITIS ELEGANS*

The eukaryotic mRNA translation initiation factor 4E (eIF4E) is a key regulator of protein synthesis that recognizes the 5′-end cap structures of most eukaryotic mRNAs and facilitates their recruitment to ribosomes.[4] This is considered to be the rate-limiting step in translation initiation under most circumstances and is a primary target for translational control in many organisms.[4] Five eIF4E isoforms (IFE-1 to IFE-5) are encoded in the *C. elegans* genome.[5] IFE-1, IFE-3, and IFE-5 are expressed in the germ line, whereas IFE-2 and IFE-4 are expressed specifically in somatic cells. IFE-3 and IFE-4 bind only 7-monomethyl guanosine caps whereas IFE-1, IFE-2, and IFE-5 can bind both 7-monomethyl guanosine and 2,2,7-trimethyl guanosine caps.[5] In *C. elegans*, the majority of mRNAs acquire a 2,2,7-trimethyl guanosine cap through the process of trans-splicing.[6]

Interestingly, depletion of IFE-2 results in significant (approximately 40%) life-span extension, whereas depletion of other isoforms does not alter nematode life span. Loss of IFE-2 results in downregulation of protein synthesis in somatic cells; *ife-2* deletion mutants show lower protein synthesis rates compared to wild type. Elimination of germline-specific eIF4E isoforms does not significantly alter animal life span.[7] Because IFE-2 is the most abundant eIF4E isoform in somatic *C. elegans* tissues, these findings suggest that reduction of protein synthesis specifically in the soma extends life span. This notion is further supported by experiments in *glp-4* mutant animals that lack a functional germ line at the non-permissive temperature.[8] These experiments show that the germ line is not required for life-span extension by IFE-2 deficiency.[7]

Postdevelopmental elimination of other translation initiation factors or their regulators has analogous effects on the longevity of the nematode. Reducing the

levels of the scaffold protein eIF4G or the eIF2β subunit, using RNAi during adulthood, leads to an approximately 30% increase in life span.[9,10] Similarly, reducing the levels of several ribosomal proteins or the ribosomal protein S6 kinase (S6K) during adulthood extends nematode life span. In all cases, the rate of protein synthesis in RNAi-treated worms are reduced compared to wild-type worms.[9,10] In addition, many genes encoding components of the translation initiation factor complex and components of the 40S subunit of the ribosome were recovered in an RNAi screen for essential genes that extend life span when inactivated postdevelopmentally.[11]

The insulin/insulin growth factor (IGF) 1 pathway is known to modulate aging in a variety of organisms, including *C. elegans*. Downregulation of the insulin/IGF-like signaling by mutations (e.g., in the insulin/IGF-like receptor DAF-2 or the phosphatidylinositol-3-OH kinase (PI3K) AGE-1) extends nematode life span. This extension is dependent on the activity of DAF-16, a forkhead transcription factor.[1] IFE-2 is not a target of the insulin pathway since depletion of DAF-16 or DAF-2 does not affect the expression of *ife-2*. Knockdown of IFE-2 further increases the life span of long-lived *daf-2* and *age-1* mutant animals in a *daf-16*-independent manner, suggesting that IFE-2 deficiency extends life span via a different mechanism.

In addition to the insulin/IGF pathway, an evolutionary-conserved determinant of aging is caloric/dietary restriction. In *C. elegans*, a dietary-restricted mutant is *eat-2*, which carries a loss-of-function mutation in the gene encoding a subunit of the nicotinic acetylcholine receptor.[12] The *eat-2* mutants have a pumping defect, eat less, and live longer than wild-type animals.[12] The life span of *eat-2* mutants is further increased by knocking down the expression of *ife-2*. Similarly, IFE-2 depletion further extends the life span of long-lived *clk-1* mutants lacking an enzyme necessary for the biosynthesis of ubiquinone, a redox factor of the mitochondrial electron transport chain. A ubiquinone intermediate accumulates in these mutants, which reduces extra-mitochondrial-produced, reactive oxygen species (ROS), extending life span.[13] The additive effects of IFE-2 deficiency in both dietary restricted animals and *clk-1* mutants indicate that protein synthesis reduction contributes in parallel to dietary restriction and mitochondrial function to extend life span.

eIF4E AND PROTEIN SYNTHESIS REGULATORY PATHWAYS

Low insulin/IGF-1 signaling, nutrient or energy deprivation, and stress converge to downregulate the activity of the protein kinase *target of rapamycin* (TOR).[14] Reduced TOR activity generally reduces protein synthesis and induces autophagy, a lysosomal catabolic pathway for turnover of proteins and organelles. TOR deficiency also increases life span but the mechanisms and downstream targets of TOR that affect aging are not well understood. In

C. elegans, TOR deficiency causes developmental arrest, but these arrested animals live longer than wild-type arrested worms.[15] Similarly, knockdown of TOR by RNAi in adult animals increases their life span compared to wild type.[15,16] Genetic data suggests that the TOR pathway interacts with the insulin/IGF-1-like pathway but can also mediate the life-extending effects of dietary restriction.[16,17] Animals deficient for both IFE-2 and TOR live longer than either single mutant.[7] This synergy between IFE-2 and TOR indicates distinct mechanisms of aging modulation by these two proteins.

In *Drosophila* and mammals, members of the eIF4E binding proteins (4E-BPs), a family of translational repressors, are well-known targets of TOR kinase and mediate the effects of TOR on eIF4E activity. However, there is no apparent structural 4E-BP homologue in the *C. elegans* genome. In addition to 4E-BP, TOR signaling regulates S6K and its targets, the ribosomal protein S6 and elongation factor 2. Knockdown of the *C. elegans* p70S6K homologue *rsks-1* and the closely related p90S6K homologue *rskn-1* does not abrogate life-span extension by IFE-2 depletion.[7] Thus, S6K is not required for longevity by reduced protein synthesis.

The activity of mammalian eIF4E is regulated by phosphorylation on Ser209 by the MAP kinase signal-integrating kinases (Mnk1/2), although the effects of eIF4E phosphorylation on mRNA translation are not clear.[18] In *Drosophila*, the Mnk homologue Lk6 regulates growth in response to nutrients via eIF4E.[19,20] Knockdown of the single nematode Mnk homologue, *mnk-1,* shortens the life span of both wild-type and *ife-2* mutant animals. Interestingly, the negative effect of MNK deficiency on longevity requires a functional germ line.[7] Hence, it is likely that Mnk-1 exerts its effect on life span through germline-specific eIF4E isoforms.

PROTEIN SYNTHESIS AND OXIDATIVE STRESS RESISTANCE

Why does reduction of protein synthesis extend life span? Given that protein synthesis is a highly energy-consuming process,[21] we hypothesize that reduction of this process leads to significant cellular energy savings, coupled with reduced production of toxic metabolic derivatives. In agreement with this hypothesis, *ife-2* mutants are considerably more resistant to cellular oxidative stress induced by the herbicide paraquat (methyl viologen) or the inhibitor of respiratory chain NaN_3. Furthermore, IFE-2 depletion increases oxidative stress resistance and extends the life span of *mev-1* mutants, which lack the cytochrome b large subunit (Cyt-1ceSDHC) in complex II of the mitochondrial electron transport chain.[7,22] Thus, depletion of a specific eIF4E isoform, IFE-2, expressed in somatic cells, increases oxidative stress resistance and extends life span.

The link between translational regulation and general stress responses is further supported by the increased resistance of animals with compromised

protein synthesis to several stressors. Such animals are more resistant to various stresses, such as heat shock, oxidative stress, UV irradiation, or starvation, compared to wild type.[7,9,10] Interestingly, eIF4G levels are reduced during the dauer stage, an alternative nematode larval form that is normally induced by stress conditions, such as crowding and food deprivation.[10] Dauer larvae do not feed, have slowed metabolic rates, and live longer than reproductive adults. Reduced protein synthesis may contribute to the longevity of these animals.

PERSPECTIVE

Studies in diverse organisms suggest that lowering the rate of translation under unfavorable or stressful conditions would result first, in notable cellular energy savings and second, in the generation of a lesser amount of toxic metabolic byproducts.[23] Both consequences of protein synthesis attenuation favor cellular maintenance and repair, thus contributing to cell survival under stress. This notion is consistent with the augmented oxidative stress resistance that accompanies reduction of protein synthesis.

Different tissues and cell types are characterized by dissimilar protein synthesis activity. Our findings indicate that reduction of protein synthesis, specifically in somatic cells, extends life span. What is the basis of this specificity? The "disposable soma" theory of aging[24] postulates that somatic maintenance and repair mechanisms play a central role in organismal aging. While these mechanisms cope with high metabolic activity in somatic cells, their capacity is not unlimited. Failure to keep up with accumulating damage and toxic metabolic waste contributes to senescent decline. We propose that reduction of protein synthesis, specifically in the soma, abrogates the deleterious consequences of metabolism by allowing for diversion of more cellular energy towards maintenance and repair mechanisms (FIG. 1). Thus, downregulation of protein synthesis may modulate aging by regulating somatic maintenance. In this context, the well-documented decline of protein synthesis with age may be a compensatory reflex in an attempt to conserve energy for somatic maintenance. The emerging link between protein synthesis and aging provides a framework for exploring these prospects.

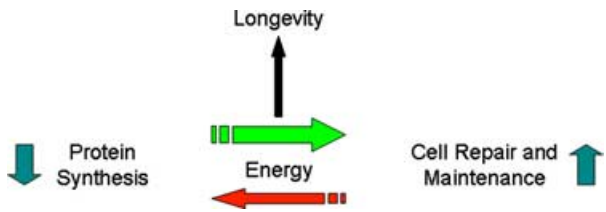

FIGURE 1. A working hypothesis linking protein synthesis and aging. A reduction in protein synthesis shifts the energy equilibrium toward cell repair and maintenance, promoting longevity.

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REFERENCES

1. KENYON, C. 2005. The plasticity of aging: insights from long-lived mutants. Cell **120:** 449–460.
2. MAKRIDES, S.C. 1983. Protein synthesis and degradation during aging and senescence. Biol. Rev. Camb. Philos. Soc. **58:** 343–422.
3. RATTAN, S.I. 1996. Synthesis, modifications, and turnover of proteins during aging. Exp. Gerontol. **31:** 33–47.
4. GINGRAS, A.C., B. RAUGHT & N. SONENBERG. 1999. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu. Rev. Biochem. **68:** 913–963.
5. KEIPER, B.D. *et al.* 2000. Functional characterization of five eIF4E isoforms in *Caenorhabditis elegans*. J. Biol. Chem. **275:** 10590–10596.
6. VAN DOREN, K. & D. HIRSH. 1990. mRNAs that mature through trans-splicing in *Caenorhabditis elegans* have a trimethylguanosine cap at their 5′ termini. Mol. Cell Biol. **10:** 1769–1772.
7. SYNTICHAKI, P., K. TROULINAKI & N. TAVERNARAKIS. 2007. eIF4E function in somatic cells modulates ageing in *Caenorhabditis elegans*. Nature **445:** 922–926.
8. BEANAN, M.J. & S. STROME. 1992. Characterization of a germ-line proliferation mutation in *C. elegans*. Development **116:** 755–766.
9. HANSEN, M. *et al.* 2007. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. Aging Cell **6:** 95–110.
10. PAN, K.Z. *et al.* 2007. Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. Aging Cell **6:** 111–119.
11. CURRAN, S.P. & G. RUVKUN. 2007. Lifespan regulation by evolutionarily conserved genes essential for viability. PLoS Genet. **3:** e56.
12. LAKOWSKI, B. & S. HEKIMI. 1998. The genetics of caloric restriction in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **95:** 13091–13096.
13. LAKOWSKI, B. & S. HEKIMI. 1996. Determination of life-span in *Caenorhabditis elegans* by four clock genes. Science **272:** 1010–1013.
14. WULLSCHLEGER, S., R. LOEWITH & M.N. HALL. 2006. TOR signaling in growth and metabolism. Cell **124:** 471–484.
15. VELLAI, T. *et al.* 2003. Genetics: influence of TOR kinase on lifespan in *C. elegans*. Nature **426:** 620.
16. JIA, K., D. CHEN & D.L. RIDDLE. 2004. The TOR pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. Development **131:** 3897–3906.
17. MEISSNER, B. *et al.* 2004. Deletion of the intestinal peptide transporter affects insulin and TOR signaling in *Caenorhabditis elegans*. J. Biol. Chem. **279:** 36739–36745.

18. WASKIEWICZ, A.J. *et al.* 1999. Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 *in vivo*. Mol. Cell Biol. **19:** 1871–1880.
19. ARQUIER, N. *et al.* 2005. *Drosophila* Lk6 kinase controls phosphorylation of eukaryotic translation initiation factor 4E and promotes normal growth and development. Curr. Biol. **15:** 19–23.
20. REILING, J.H. *et al.* 2005. Diet-dependent effects of the *Drosophila* Mnk1/Mnk2 homolog Lk6 on growth via eIF4E. Curr. Biol. **15:** 24–30.
21. PROUD, C.G. 2002. Regulation of mammalian translation factors by nutrients. Eur. J. Biochem. **269:** 5338–5349.
22. ISHII, N. *et al.* 1998. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature **394:** 694–697.
23. SYNTICHAKI, P. & N. TAVERNARAKIS. 2006. Signaling pathways regulating protein synthesis during ageing. Exp. Gerontol. **41:** 1020–1025.
24. KIRKWOOD, T.B. 1977. Evolution of ageing. Nature **270:** 301–304.