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Perspective

Protein Synthesis and Aging

eIF4E and the Soma vs. Germline Distinction

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KEY WORDS

Caenorhabditis elegans, caloric restriction, eIF4E, germline, insulin, longevity, mRNA translation, ribosome, senescence, TOR

ABBREVIATIONS

eEF	eukaryotic elongation factor
eIF	eukaryotic initiation factor
4E-BP	eIF4E binding protein
FOXO	forkhead box sub-group O
IGF	insulin-like growth factor
MNK	MAP kinase signal-integrating kinase
PHAS-I	phosphorylated heat- and acid stable protein regulated by insulin 1
PI3K	phosphatidyl inositol-3 kinase
RNAi	RNA interference
S6K	S6 ribosomal protein kinase
TOR	target of rapamycin

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ABSTRACT

Classic studies in diverse organisms, including humans, have demonstrated that aging is accompanied by marked alterations in both general and specific protein synthesis. These early observations established a link between the aging process and the regulation of protein synthesis. However, two important questions remained. First, what are the molecular mechanisms underlying the changes in protein synthesis during aging? Second, are these changes simply a consequence of aging or do they actually have a causative role in senescent decline? We have recently shown that elimination of a specific isoform of the eukaryotic mRNA translation initiation factor 4E (eIF4E) that functions in somatic cells, reduces protein synthesis and extends lifespan in the nematode *Caenorhabditis elegans*. Depletion of eIF4E in the soma extends lifespan via a mechanism independent of the insulin/IGF pathway that modulates aging in diverse species. Our findings suggest that regulation of protein synthesis is an important determinant of longevity and provide a framework for elucidating the mechanisms by which the rate of protein synthesis influences the process of aging.

CHANGES IN PROTEIN SYNTHESIS DURING AGING

Protein synthesis is a tightly regulated process involving multiple steps, which are executed with extreme accuracy.[1] While the fidelity of mRNA translation does not appear to deteriorate during aging, numerous studies have established that general protein synthesis rates decline with age in a variety of organisms.[2-7] Both, biochemical data[7] and microarray expression profiling,[8] correlate lowered protein synthesis rates with senescent decline. Mitochondrial protein synthesis activity also diminishes markedly during aging.[9] To assess the significance of these alterations in the aging process, we need to consider two questions. First, what molecular mechanisms bring about these changes during aging? Second, is the decrease in protein synthesis a mere consequence of aging or does it play a causative role in age-related decline?

The rate of mRNA translation is mainly determined by a battery of translation initiation factors.[10] The activity of specific initiation factors such as the eukaryotic initiation factor 2 (eIF-2) has been found to decline with age.[11,12] Similarly, translation elongation factors have been implicated in the reduction of protein synthesis during aging. The activity of eukaryotic elongation factor 1 (eEF-1) in the liver and brain of rats declines with age.[13,14] Lower eEF-1 activity also correlates with old age in *Drosophila*.[15] In addition, the activity of another elongation factor, eEF-2, was found to undergo age-related changes in mouse and rat livers.[16] In their totality, these studies suggest that down regulation of specific translation factors mediates, at least in part, the effects of aging on protein synthesis across species. The ribosome and its components have also been implicated in aging-associated changes in protein synthesis,[17-19] although these studies do not converge to a common effector.

It is likely that additional, yet to be discovered, mechanisms mediate the reduction of protein synthesis during aging. The important questions, however, that still remain are first, how the process of aging modulates the activity of various mRNA translation factors to elicit a drop in protein synthesis and second, whether this drop contributes to senescent decline or is merely a corollary of the relentless deterioration of cellular function during aging.

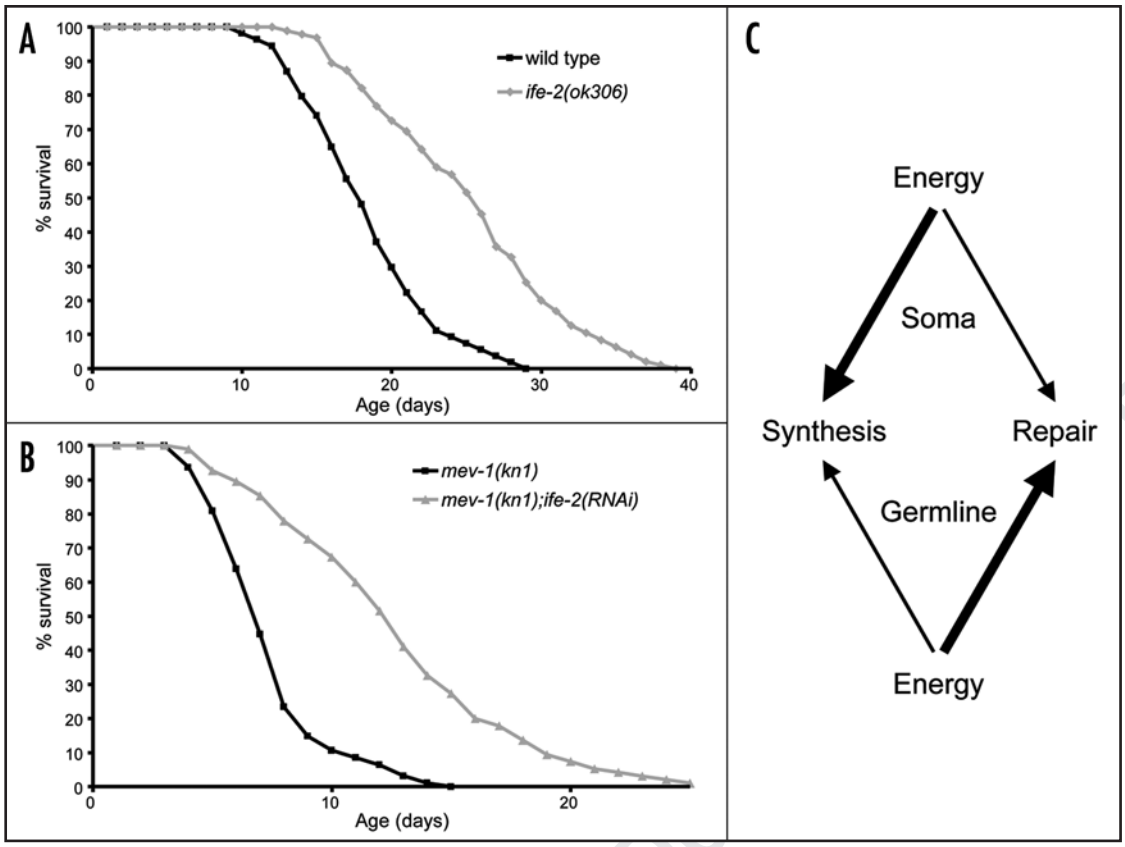

Figure 1. Reduction of protein synthesis specifically in somatic tissues of *C. elegans* extends lifespan and increases resistance to oxidative stress. (A) *ife-2(ok306)* mutants, deficient for IFE-2, the most abundant eIF4E isoform in the soma, outlive wild type animals The percentage of animals remaining alive is plotted against animal age. (B) Knockdown of *ife-2* by RNAi extends the lifespan of short lived *mev-1(kn1)* mutants, experiencing oxidative stress due to the of lack succinate dehydrogenase cytochrome b, a component of complex II of the mitochondrial electron transport chain. The percentage of animals remaining alive is plotted against animal age. (C) A working hypothesis inspired by the "disposable soma" theory of aging. In somatic tissues, more energy is relayed to biosynthetic activities and less is available for repair. In the germline, energy is mostly invested in maintenance and repair.

OBSTRUCTING mRNA TRANSLATION IN THE SOMA EXTENDS LIFESPAN

If the rate of protein synthesis is a determinant of aging then manipulation of mRNA translation should have an effect on longevity. Indeed, three recent studies show that reduction of protein synthesis extends lifespan in the nematode *Caenorhabditis elegans*.[20-22] We focused on eIF4E, a central regulator of mRNA translation, integrating several, diverse signaling inputs to control protein synthesis.[23,24] eIF4E binds the 7-methyl guanosine cap at the 5' end of nuclear mRNAs to mediate the formation of the mRNA translation initiation complex.[25] Five genes (*ife-1* to *5*) encode eIF4E isoforms in *C. elegans*.[26] These isoforms differ in cap-binding specificity and anatomical expression. *ife-1*, *3* and *5* are expressed in the germline, while *ife-2* and *4* are specific for somatic tissues. 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 germline also bind both 7-monomethyl guanosine and 2,2,7-trimethyl guanosine caps.[26,27] The multiplicity of eIF4E isoforms with different cap-binding and anatomical expression specificity in *C. elegans* provides the unique opportunity to selectively manipulate mRNA translation by interfering with the function of specific isoforms. By systematically knocking down each of the five *ife* genes in worms we were surprised to find that elimination of a specific eIF4E isoform expressed only in somatic tissues (IFE-2), extends lifespan in *C. elegans* (Fig. 1A).[22] Interfering with the three eIF4E isoforms expressed in the germline or IFE-4, an additional soma-specific isoform, which regulates translation of a small set of mRNAs,[28] did not alter animal lifespan. The observed lifespan extension does not require a functional germline since lack of germline does not suppress the effect of IFE-2 depletion. This finding, coupled with the specific expression of IFE-2 in somatic tissues, corroborates the somatic origin of longevity conferred by IFE-2 depletion and underscores the importance of somatic protein synthesis in aging.

Is there a link between protein synthesis and other mechanisms or manipulations known to influence longevity? In *C. elegans* aging is mainly regulated by a conserved endocrine signaling pathway that involves the insulin/insulin-like growth factor (IGF) receptor DAF-2 and the phosphatidyl inositol-3 kinase (PI3K) AGE-1.[29] Mutations that reduce the activity of DAF-2 or AGE-1 extend animal lifespan.[30-32] These effects on aging require the DAF-16/FOXO transcription factor.[33,34] By contrast, we find that DAF-16 is not required for lifespan extension by IFE-2 deficiency; knock down of *ife-2* extends the lifespan of *daf-16* loss-of-function mutants. This indicates that IFE-2 functions downstream or independently of DAF-16 to control aging. Consistent with this observation,

IFE-2 depletion further extends the lifespan of long-lived *daf-2* and *age-1* mutants. Furthermore, similar additive affects are observed in long-lived *clk-1* mutant animals[35] and dietary restricted *eat-1* and *eat-2* mutants. *clk-1*, is a gene required for the biosynthesis of ubiquinone, an essential component of the mitochondrial electron transport chain.[35] *eat-1* and *eat-2* mutants are dietary-restricted because pharyngeal pumping of bacterial food into the intestine is compromised in these animals.[36] Thus, eIF4E deficiency in the soma extends lifespan via a mechanism, at least in part, independent of insulin/IGF signaling, respiratory chain function and dietary restriction.

Why does IFE-2 deficiency extend lifespan? IFE-2 deficiency impairs mRNA translation initiation in the soma.[22] The rate of protein synthesis is decreased in long-lived *ife-2(ok306)* mutants, compared to wild type animals. This is not surprising since 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.[26,37] Therefore, protein synthesis reduction results in longevity.

The activity of eIF4E is under the control of many signaling cascades that mediate diverse cellular responses.[24,25] These pathways converge to mainly modulate the association of eIF4E with inhibitory eIF-4E-binding proteins (4E-BPs) and/or its direct phosphorylation by the MAP kinase signal-integrating kinases (Mnk1/2).[38] 4E-BPs act as translational repressors by competing with eIF4G for an overlapping binding site on eIF4E. Phosphorylation of 4E-BP promotes its dissociation from eIF4E, allowing for recruitment of eIF4G and eIF4A translational factors to the mRNA cap structure (Gingras et al.). For example, the nutrient-sensing kinase TOR (target of rapamycin) phosphorylates 4E-BP and enhances mRNA translation.[39]

It is intriguing that key eIF4E regulators such as 4E-BP, TOR, and Mnk, which have been implicated in the control of development, growth and aging,[40,41] are coupled to the insulin/IGF signaling pathway. 4E-BP transcription is under FOXO control in *Drosophila*, while in mammals, 4E-BP/PHAS-I (Phosphorylated heat- and acid-stable protein I) is regulated by insulin signaling.[42] In addition, genetic studies in *C. elegans* suggest that TOR may function downstream or independently of DAF-16/FOXO to mediate the effects of DAF-2/IGF signaling on aging. TOR deficiency, which dampens the rate of translation, extends *C. elegans* lifespan.[43] Furthermore, in *Drosophila*, the Mnk homolog Lk6 regulates growth in response to nutrients via eIF4E.[44-46] The totality of these findings is consistent with the notion that down regulation of eIF4E activity by these modulators extends lifespan. Thus, eIF4E interfaces with other mechanisms influencing aging such as the insulin/IGF signaling pathway to mediate at least part of their effects on longevity.[47] Indeed, knockdown of TOR in animals carrying an *ife-2* deletion results in additional lifespan extension.[22] Such synergy indicates that TOR effects on aging are mediated in part by an IFE-2-independent mechanism.

PROTEIN TURNOVER: REPAIR VS. RENEWAL

A major hallmark of aging is the progressive accumulation of molecular damage in nucleic acids, proteins, lipids and other macromolecules. This, in turn, leads to inexorable deterioration of essential cellular functions and consequently, to senescence. A main cause of age-related accumulation of damage is the limited capacity of cellular maintenance, repair and turnover pathways. Protein turnover determines the rate at which protein pools are getting refreshed, with protein synthesis providing fresh proteins and protein degradation removing the existing and likely damaged protein molecules. A decline in turnover rates would delay the removal and replacement of damaged proteins thus contributing to senescence. Why is then possible to extend lifespan by reducing protein synthesis?

The notion that high protein synthesis rates have a beneficial effect on longevity is a simplistic assumption that bears significant caveats. Protein synthesis is one of the most energy-consuming cellular processes, devouring an estimated 50% of the total cellular energy, depending on the organism and cell growth state.[48] For example, mRNA and ribosome biosynthesis, two processes controlled by insulin/IGF-1 and TOR signaling are highly energy-consuming.[49] Under favorable conditions, yeast cells synthesize ~2000 ribosomes per minute, in order to maintain robust growth.[50] Therefore, reduction of protein synthesis rates under unfavorable, stress conditions would result in notable energy savings. Indeed, global translation is reduced in response to most, if not all, types of cellular stress.[10] This energy could then be diverted to cellular repair and maintenance processes, thus contributing to longevity. Similarly, reduction of protein synthesis by IFE-2 depletion may prolong lifespan by lowering energy demands and associated generation of toxic metabolism by-products such as reactive oxygen species, which contribute significantly to the aging process.[51,52] In addition, the concomitant increase in energy availability may allow diversion of critical resources to cellular maintenance and repair processes, thus promoting organism longevity.

Resistance to oxidative stress is known to parallel the capacity for detoxification and repair.[53] The herbicide paraquat is a generator of superoxide anions[54] and induces acute oxidative stress in worms.[55] Interestingly, long-lived *ife-2* mutant animals are considerably more resistant to paraquat, compared to wild type.[22] IFE-2 deficiency also significantly improves the survival of *mev-1(kn1)* mutants (Fig. 1B),[22] which lack succinate dehydrogenase cytochrome b, a component of complex II of the mitochondrial electron transport chain.[56] 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. Therefore, repression of protein synthesis in the soma renders animals capable of withstanding both acute and chronic oxidative stress.

PERSPECTIVE

The unexpected finding that reduction of protein synthesis in the soma extends lifespan in worms provides new insights on the basic cellular processes that play a role in senescent decline. At the same time, several new questions now emerge. Why is eIF4E activity in somatic tissues, but not in the germline, important for normal aging? Aging is a soma-specific phenomenon; the germline is an immortal cell lineage.[57] Somata which encapsulate immortal germlines are disposable. What molecular mechanisms underlie this fundamental distinction? Within the evolutionary framework of the "disposable soma" theory of aging,[58,59] failure to divert energy towards repairing stochastic damage that accumulates in the soma during life is responsible for the inexorable decline of somatic functions and senescence. Thus, while in metabolically active somatic tissues energy is mostly consumed in biosynthesis, the germline invests in repair, instead (Fig. 1C). It remains to be determined whether regulation of protein synthesis is part of this scenario.

In addition to moderating the large energy requirement of protein synthesis in the soma and facilitating cellular maintenance and repair, down regulation of mRNA translation, under appropriate

conditions, may prevent the synthesis of unwanted proteins that could interfere with the cellular stress response. Remarkably, stress-induced attenuation of global translation is often accompanied by a switch to selective translation of proteins that are required for cell survival under stress. However, the mechanisms that govern preferential translation of specific mRNAs under stress are poorly understood.

The significance of the protein synthesis decrease during aging is also still an open issue.[60] How is it effected? How general is it? Are there mRNAs which are differentially translated during aging? Is the translation of specific mRNAs favored under conditions that extend lifespan? Would a sustained high rate of mRNA translation late in life retard senescent decline and aging? The list of questions can go on but the tools are available to address them in simple model organisms such as yeast, worms and flies. Given the remarkable conservation of the core protein synthesis machinery, its accessories and regulatory pathways, the outcome of this endeavor is likely to be relevant to aging in mammals.

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