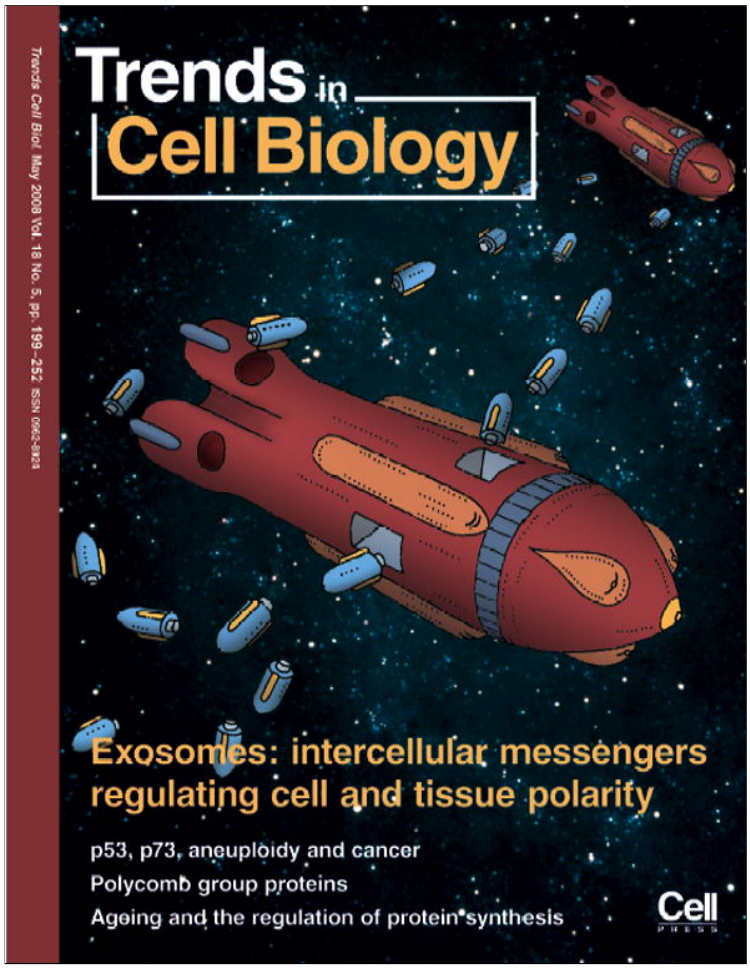

Review

Cell PRESS

Ageing and the regulation of protein synthesis: a balancing act?

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Ageing in diverse species ranging from yeast to humans is associated with extensive changes in both general and specific protein synthesis. Accumulating evidence now indicates that these alterations are not simply a corollary of the ageing process but, rather, they have a causative role in senescent decline. Indeed, interfering with mRNA translation significantly influences longevity. Interestingly, the mechanisms that control mRNA translation interface with intricate, conserved signalling pathways and specific conditions that regulate ageing, such as the insulin–insulin growth factor 1 signalling pathway and caloric restriction. This emerging relationship reveals that protein synthesis is a novel determinant of ageing in diverse organisms such as yeast, worms, flies and mice and can thus be considered as a universal component of the ageing process.

Introduction

Less than three decades of genetic and molecular scrutiny in simple model organisms such as *Caenorhabditis elegans*, *Drosophila melanogaster* and *Saccharomyces cerevisiae* have culminated in the delineation of several signalling pathways that influence ageing [1–4]. Understanding of these interlinked signal transduction cascades is becoming ever more detailed and comprehensive. However, insight into the cellular and biochemical processes upon which they impinge to modulate the rate of senescent decline and ageing has lagged considerably behind. Finding out which facets of cellular metabolism can be fine-tuned to ultimately promote longevity remains an important challenge of modern ageing research.

Early observations established that both protein synthesis and protein degradation decline during ageing. This effect could merely be a consequence of the general deterioration of the cellular functions that accompany ageing, or it could be a contributing factor in the process. Several recent studies have addressed this issue and have revealed a novel and intriguing link between protein synthesis and ageing [5–11]. Emerging findings suggest a causative relationship between the regulation of mRNA translation and ageing. Importantly, manipulations that lower the rate of protein synthesis also lower the rate of ageing, increasing the lifespan of different organisms. These observations suggest that protein synthesis constitutes a basic downstream cellular processes targeted by signalling pathways that modulate ageing. Here, I survey the link between the regulation of mRNA translation and senescent decline, highlighting recent discoveries that shed light on the basic molecular mechanisms underlying the effects of protein synthesis on ageing.

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Protein metabolism during ageing

Irreversible modifications on proteins accumulate during ageing. The most widely studied oxidative stress-induced modification is the formation of carbonyl derivatives. Carbonyl formation can occur through a variety of mechanisms, including direct oxidation of certain amino acid side chains, and oxidation-induced peptide cleavage. Several systems that generate oxygen free radicals catalyze the oxidative modification of proteins. Oxidation contributes to the pool of damaged proteins, which increases in size during ageing and in various pathological states [12]. In addition to generating oxidants, metabolism can produce other by-products, including glyoxal and methylglyoxal, both of which trigger the formation of advanced glycation end-products that also disrupt protein function and contribute to the ageing phenotype [13]. During physiological processes, proteins can also suffer additional modifications, such as racemization, isomerization and deamination. Such modifications can have mild to devastating effects in protein function. Ultimately, accumulation of modified proteins can contribute to senescent decline.

One of the main causes of age-related accumulation of damage is the limited capacity of the cellular maintenance, repair and turnover pathways. The rate at which a protein pool is refreshed is determined by turnover, the balance between protein synthesis and protein degradation, with protein synthesis providing fresh proteins, and protein degradation removing the existing and probably damaged protein molecules. Alterations in protein synthesis occur during embryonic development, cell growth, cell differentiation, and ageing. Both global and specific protein synthesis are markedly affected by ageing in organisms as distant as yeast and primates [14–19]. Signal transduction cascades, such as the insulin–insulin growth factor 1 (IGF-1) pathway, the kinase target of rapamycin (TOR) pathway, and the p38 mitogen-activated protein kinase (MAPK) pathway, play a key role in the global control of protein synthesis by targeting several components of the translation machinery (Box 1 and 2) [20]. For example, a variety of agents that promote cell growth and proliferation, including hormones, growth factors and nutrients, have stimulatory effects on protein synthesis [21] (see following sections below).

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Box 1. The process of mRNA translation in eukaryotes

Protein synthesis proceeds through three distinct phases, initiation, elongation and termination, which are tightly coordinated (Figure I). mRNA translation initiation involves several eukaryotic initiation factors (eIFs), which orchestrate the formation of the 43S pre-initiation complex (43S PIC) on the mRNA being translated. This complex incorporates the initiator methionyl-tRNA (Met-tRNA) bound on eIF2 and joins with the 60S ribosomal subunit at the ATG start codon to form the 80S initiation complex (80S IC), releasing the translation initiation factors. The 80S ribosome then commences elongation of the polypeptide chain. Two eukaryotic translation elongation factors (eEFs) participate in the process. eEF1 supplies the ribosome with the appropriate amino acid-loaded tRNAs, and eEF2 mediates translocation of the ribosome along the mRNA. eEF2 is regulated by the calcium–calmodulin-dependent eEF2 kinase (eEF2K). Specific aminoacyl-tRNA synthases load tRNAs with their cognate amino acids. Upon encountering a stop codon, mRNA translation is terminated. The eukaryotic release factor (eRF) mediates dissociation of the ribosome from the mRNA and the release of the two ribosomal subunits (40S and 60S). Control of global mRNA translation is mostly exerted at two crucial steps during initiation (shown in light blue rectangles). First, the activity of eIF2, which loads the 43S PIC with methionyl-tRNA, is regulated by phosphorylation of its α subunit. Phosphorylation interferes with the ability of the guanine nucleotide exchange factor (GEF) eIF2B to recycle GDP for GTP on eIF2. The activity of eIF2B itself is also regulated by phosphorylation. Second, the recruitment of the 43S PIC on the 7-monomethyl guanosine cap at the 5′ end of all nuclear mRNAs is regulated by the cap-binding protein eIF4E. The activity of eIF4E is modulated by direct phosphorylation and/or by association with the eiF4E-binding protein (4E-BP). 4E-BP sequesters eIF4E and limits its availability. Phosphorylation of 4E-BP releases eIF4E, which associates with the scaffold protein eIF4G, eIF4A, eIF4B and the poly-A binding protein (PABP) to promote PIC assembly and protein synthesis. The translation factors and ribosomal subunits that affect the ageing process when modified are shown in bold. Bar lines indicate negative (inhibitory) regulation events.

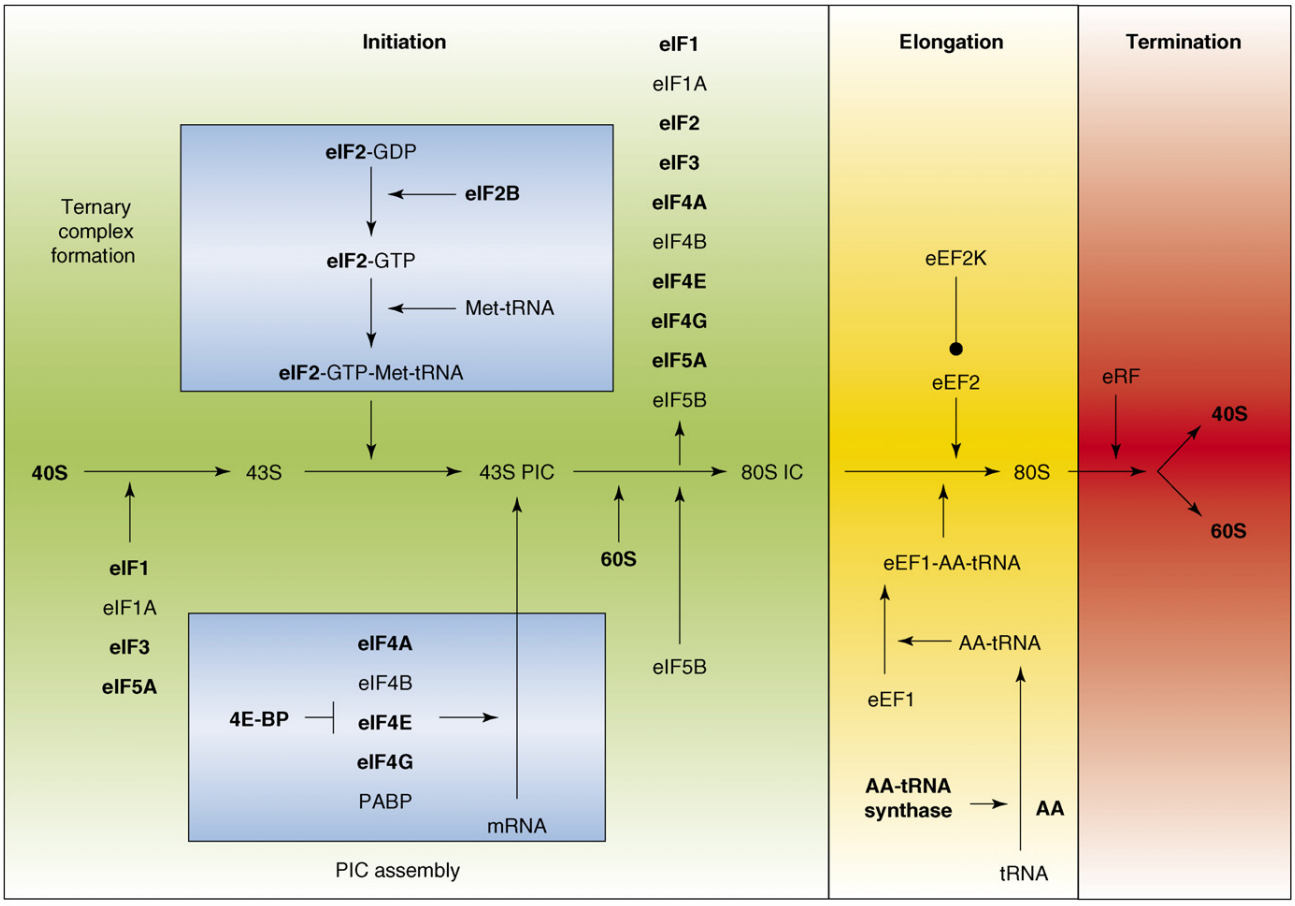

Figure I.

Although the fidelity of mRNA translation does not appear to deteriorate during ageing, numerous studies have established that general protein synthesis rates decline with age in a variety of organisms [14,17,22]. Mitochondrial protein synthesis activity also diminishes markedly during ageing [23]. Although the rate of bulk protein synthesis and the activity of key mRNA translation factors diminishes with age, it is not clear whether these alterations are a consequence of the ageing process – for example, as an adaptation to reduced mitochondrial function and energy production – or whether in fact they contribute to senescent decline. Furthermore, different tissues and cell types are characterized by dissimilar protein synthesis activity, and tissue-specific alterations have been reported [24].

General and specific degradation pathways, such as the proteasome system, autophagy and lysosomal degradation, remove damaged, non-functional proteins. Biochemical

Box 2. Ageing signalling pathways interfacing with the regulation of protein synthesis

Insulin–insulin growth factor 1 (INS–IGF-1) signalling through the insulin receptor (IRS) results in the activation of phosphatidylinositol 3 kinase (PI3K), which converts phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (1,4,5)-trisphosphate (PIP3) (Figure I). Phosphatase and tensin homologue (PTEN) antagonizes the PtdIns 3-kinase by degrading PIP3 to PIP2. PIP3, either directly or through the 3-phosphoinositide-dependent protein kinase (PDK), activates the serine–threonine protein kinase Akt (AKT8 virus proto-oncogene *akt*; also known as protein kinase B; Akt/PKB). Akt/PKB functions to activate the TOR kinase, either directly or through inhibition of the tuberous sclerosis complex 2 (TSC2) tumour suppressor, a negative regulator of the small GTPase dRheb (Ras homologue enriched in brain), which activates TOR. Upon activation, TOR phosphorylates the eukaryotic initiation factor 4E-binding protein (4E-BP), which inhibits protein synthesis by binding and blocking the eukaryotic translation initiation factor 4E (eIF4E), a key regulator of mRNA translation initiation. By contrast, phosphorylation of 4E-BP releases eIF4E and stimulates protein synthesis (Box 1). eIF4E and eukaryotic translation initiation factor 4G (eIF4G, a scaffold protein which recruits other essential mRNA translation initiation factors) are targets of the mitogen-activated protein kinase-interacting kinase (Mnk1). Mnk1 activity is under the control of p38 mitogen-activated protein kinase (MAPK) and the extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2; also called p44 and p42, respectively). ERK1 and ERK2 respond to INS–IGF-1 signalling and, in addition to Mnk1, also stimulate the S6 kinase (S6K), which phosphorylates the small ribosomal subunit S6. The α subunit of the eukaryotic translation initiation factor 2 (eIF2), which loads the 43S ribosomal pre-initiation complex with the initiator methionyl-tRNA, is phosphorylated by the general control nonderepressible 2 (GCN2) kinase and pancreatic endoplasmic reticulum eIF2α kinase (PERK), under conditions of low amino acid availability or endoplasmic reticulum stress, respectively. The amino acid transporter (AAT), which imports amino acids into the cytoplasm, also signals to the TOR kinase through the TSC1–TSC2 complex and Rheb. eIF2 recycling during successive rounds of mRNA translation initiation is mediated by the eukaryotic translation initiation factor 2B (eIF2B), a guanine nucleotide exchange factor. eIF2B activity is regulated by the serine–threonine protein kinase GSK3 (glycogen synthase kinase 3), which is under the control of the Akt/PKB kinase. Arrows indicate positive (stimulatory) regulation events. Bar lines indicate negative (inhibitory) regulation events. Protein synthesis regulators are shown in bold.

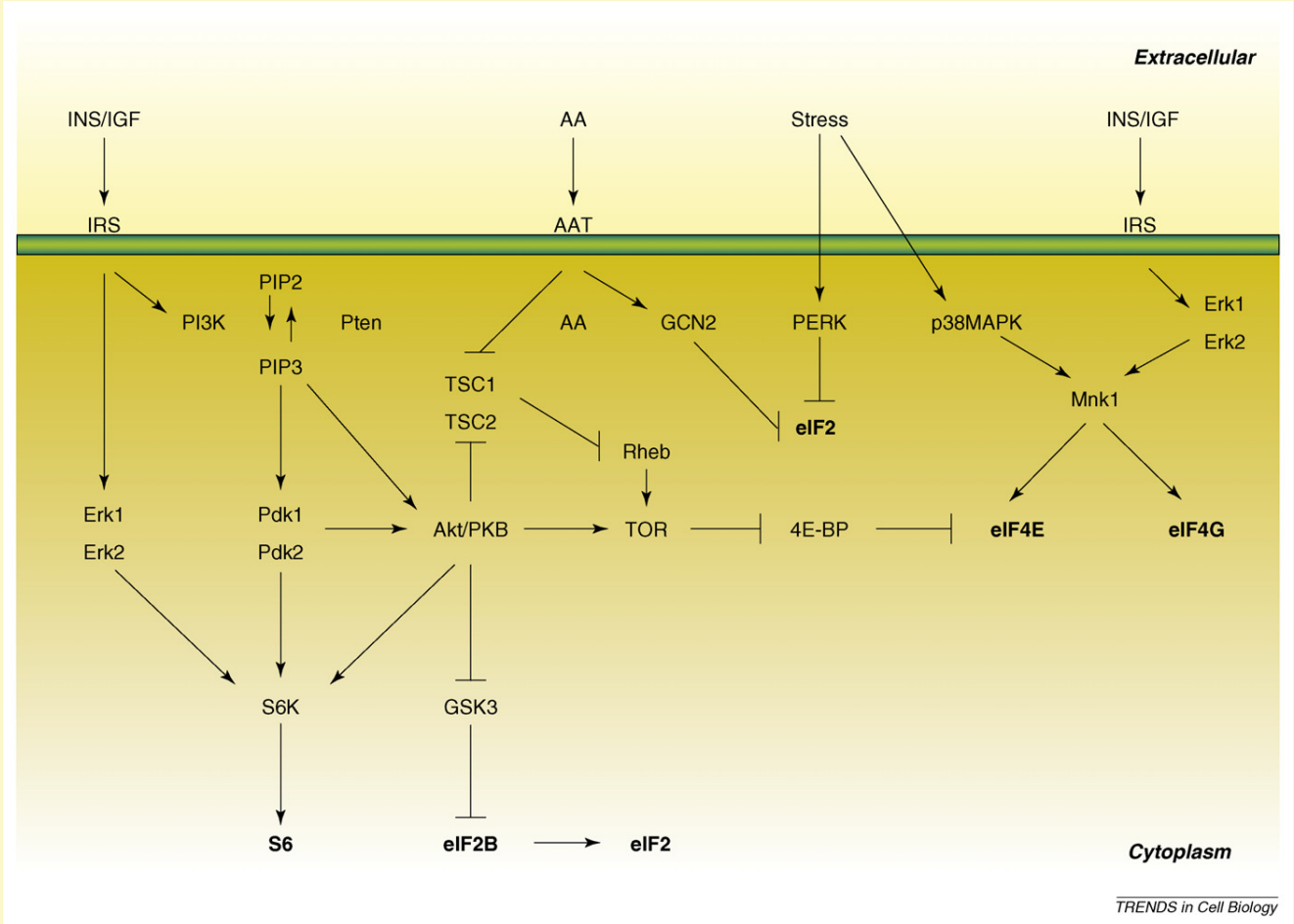

Figure I.

studies and microarray expression profiling correlate lowered protein degradation with ageing and senescent decline [18,25,26]. The activity of the cytosolic proteasomal system declines dramatically during postmitotic ageing and proliferative senescence in human fibroblasts, resulting in increased half-life of oxidized proteins [27]. Ageing also perturbs 26S proteasome assembly and function in *Drosophila* [28]. Interestingly, oxidized proteins and lipids (e.g. lipofuscin and ceroid), which directly inhibit the proteasome, accumulate during ageing and thereby further exacerbate the accumulation of damaged proteins [29]. Furthermore, the proteasomal E3 ligase complex is required for lifespan extension under reduced insulin signalling in *C. elegans* [30]. Thus, reduced proteasomal function emerges

as a common denominator of the ageing process in diverse organisms [31,32]. Autophagy has also been implicated in ageing. Genetic studies in *C. elegans* and *Drosophila* suggest that autophagy mediates, at least in part, the effects of insulin signalling and dietary restriction on longevity [33–38]. It should also be noted that, although a drop in bulk protein turnover emerges as a common theme coupled with the ageing process, not all proteins are uniformly affected, and individual protein levels do not always parallel the global trend.

Interventions that alter mRNA translation rates influence ageing

If the rate of protein synthesis is a determinant of ageing, then manipulation of mRNA translation should have an effect on longevity. However, because protein synthesis is essential for growth and development, it is not straightforward to dissect its specific role in ageing. Manipulation of general mRNA translation is likely to have pleiotropic effects, thus obscuring any explicit contribution to ageing. Nevertheless, several recent studies capitalize on the genetic malleability of model organisms such as yeast, *C. elegans* and *Drosophila* to investigate the link between protein synthesis and ageing. These studies demonstrate that, indeed, alteration of mRNA translation throughput has a significant impact on the ageing process. Targeting a battery of key protein synthesis regulators reduces protein synthesis and extends lifespan (Table 1 and references therein).

The process of protein synthesis involves three major, tightly regulated events: initiation of mRNA translation, elongation of the polypeptide chain, and termination of mRNA translation (Box 1). Initiation is the rate-limiting step in mRNA translation and is the most common target of mRNA translation control. Global control of protein synthesis is generally achieved by changes in the phosphorylation state of initiation factors or their regulators. The rate of protein synthesis is mainly determined by regulation of two discrete steps during mRNA translation initiation: recruitment of the 40S ribosomal subunit at the 5′ end of mRNA, and loading of the 40S ribosomal subunit with the initiator methionyl-tRNA. These events are coordinated by eukaryotic translation initiation factors (eIFs) eIF4E and eIF2–eIF2B, respectively [39]. Phosphorylation of the eIF2 α-subunit regulates dissociation of the eIF2B–eIF2 complex and subsequent eIF2 recycling. Similarly, phosphorylation of eIF4E binding proteins (4E-BPs) controls the availability of active eIF4E [40]. The 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. This is considered to be the rate-limiting step in translation initiation under

Table 1. Protein synthesis regulators implicated in ageing and their function

Regulator	Function	Refs
eIF1	Eukaryotic initiation factor 1, promotes 43S complex formation, crucial for stringent AUG selection	[10]
eIF2	Eukaryotic initiation factor 2, loads methionyl-tRNA onto the 43S pre-initiation complex	[5,8]
eIF2B	Eukaryotic initiation factor 2B, guanine nucleotide exchange factor, recycles eIF2	[8]
eIF3	Eukaryotic initiation factor 3, controls the assembly of 40S ribosomal subunit on mRNA	[8,10]
eIF4A	Eukaryotic initiation factor 4A, DEAD-box RNA helicase, part of eIF4F	[10]
eIF4E	Eukaryotic initiation factor 4E cap-binding, translation initiation, part of eIF4F	[7]
eIF4G	Eukaryotic initiation factor 4G, a scaffold protein that recruits other mRNA translation initiation factors onto mRNA, part of eIF4F	[5,6,10]
eIF5A	Eukaryotic initiation factor 5A, promotes the formation of the first peptide bond	[76,77]
4E-BP	Eukaryotic initiation factor 4E-binding protein, inhibitor of eIF4E	[78,79]
TOR	Target of rapamycin, serine–threonine protein kinase	[53,62,68,80]
S6K	40S ribosomal subunit S6 serine–threonine kinase (p70S6K), induces protein synthesis by phosphorylating S6	[5,6,68]
Mnk	Mitogen-activated protein kinase-interacting kinase	[81]
Thr-tRNA synthetase	Catalyzes the esterification of threonine to its cognate tRNAs	[8]
Asn-tRNA synthetase	Catalyzes the esterification of asparagine to its cognate tRNAs	[8]
Rps3	40S ribosomal subunit S3, ribosome biogenesis and function	[10]
Rps6	40S ribosomal subunit S6, ribosome biogenesis and function	[5,9]
Rps8	40S ribosomal subunit S8, ribosome biogenesis and function	[10]
Rps10	40S ribosomal subunit S10, ribosome biogenesis and function	[5]
Rps11	40S ribosomal subunit S11, ribosome biogenesis and function	[5,10]
Rps15	40S ribosomal subunit S15, ribosome biogenesis and function	[5]
Rps22	40S ribosomal subunit S22, ribosome biogenesis and function	[5]
Rps26	40S ribosomal subunit S26, ribosome biogenesis and function	[5]
Rps11	40S ribosomal-protein S11, ribosome biogenesis and function	[10]
Rpl4	60S ribosomal-protein L4, ribosome biogenesis and function	[5]
Rpl6	60S ribosomal-protein L6, ribosome biogenesis and function	[5]
Rpl9	60S ribosomal-protein L9, ribosome biogenesis and function	[5]
Rpl10	60S ribosomal-protein L10, ribosome biogenesis and function	[9]
Rpl19	60S ribosomal-protein L19, ribosome biogenesis and function	[5,8]
Rpl30	60S ribosomal-protein L30, ribosome biogenesis and function	[5]
Mitochondrial Rps30	Mitochondrial 28S ribosomal-protein S30, mitochondrial protein synthesis	[8]
Mitochondrial Rpl10	Mitochondrial 39S ribosomal-protein L10, mitochondrial protein synthesis	[8]
Mitochondrial Rpl24	Mitochondrial 39S ribosomal-protein L24, mitochondrial protein synthesis	[8]

most circumstances and is a primary target for translational control in many organisms [41]. Five eIF4E isoforms (IFE-1–IFE-5) are encoded in the *C. elegans* genome [42]. IFE-1, IFE-3 and IFE-5 are expressed in the germline, whereas IFE-2 and IFE-4 are expressed specifically in somatic cells. Loss of IFE-2 results in downregulation of protein synthesis in somatic cells and significant lifespan extension [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 lifespan. Depletion of other somatic or germline-expressed eIF4E isoforms does not cause similarly pronounced effects on nematode lifespan; however, depletion of IFE-1 during adulthood results in modest adult lifespan extension, suggesting that IFE-1 also modulates longevity [6].

Post-developmental 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 eIF2 beta subunit, by RNA interference (RNAi) during adulthood, leads to a ~30% increase in lifespan [5,6,8]. Similarly, using RNAi to reduce the levels of several ribosomal proteins or the ribosomal-protein S6 kinase (S6K) specifically during adulthood extends nematode lifespan. In all these cases, the rate of protein synthesis in RNAi-treated animals was reduced in comparison with that in wild type controls [5,6]. In addition, many genes encoding components of the eIF complex and components of the 40S and 60S subunits of the ribosome were recovered in an RNAi screen for essential genes that extend lifespan when inactivated post-developmentally [8,10].

What is the origin of lifespan extension by reduction of protein synthesis? IFE-2-depleted mutants are considerably more resistant than wild type animals to cellular oxidative stress induced by the herbicide paraquat (a methyl viologen) or the inhibitor of the respiratory chain, NaN_3. Furthermore, IFE-2 deficiency increases oxidative stress resistance and extends the lifespan of *mev-1* mutants, which experience chronic oxidative stress due to their lack of the cytochrome b large subunit in complex II of the mitochondrial electron transport chain. [7] Thus, depletion of a specific eIF4E isoform, IFE-2, expressed in somatic cells, increases oxidative stress resistance and extends lifespan. The fact that animals with compromised protein synthesis have increased resistance to several stressors further supports the link between translational regulation and general stress responses. Such animals are more resistant than wild type to various stresses, such as heat shock, oxidative stress, UV irradiation or starvation [5–7]. 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 [6]. Dauer larvae do not feed, and they have slowed metabolic rates and live longer than reproductive adults. Reduced protein synthesis might contribute to the longevity of these animals.

Hanging in the balance

If the turnover and replacement of damaged proteins is required to avoid senescent decline, how does lower protein synthesis augment stress resistance and extend lifespan? mRNA translation 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 [43]. For example, mRNA and ribosome biosynthesis, two processes controlled by insulin–IGF-1 and TOR signalling (Box 2), are highly energy-consuming [44]. Under favourable conditions, yeast cells synthesize ~2000 ribosomes per minute to maintain robust growth [45]. Therefore, reduction of protein synthesis rates under unfavourable stress-conditions would result in notable energy savings. Indeed, global translation is reduced in response to most, if not all, types of cellular stress [39]. This energy could then be diverted to cellular repair and maintenance processes, thus contributing to longevity.

However, a decline in turnover rates would delay the removal and replacement of damaged proteins, thus contributing to senescence. Indeed, protein synthesis is an essential process that has an impact on all cellular functions. Complete elimination of key mRNA translation factors cannot be tolerated. Genes encoding these proteins are essential for growth and development and are usually highly conserved in evolution. To investigate these questions without incurring the deleterious consequences of interfering with the expression of essential mRNA translation factors, researchers have used RNAi to target the corresponding genes after completion of development and during adulthood in *C. elegans* [8,10]. These experiments reveal that reduced expression of genes that are essential early in life actually enhances longevity when RNAi is initiated later into adulthood. Late-onset interference usually also results in increased stress resistance and decreased fecundity, indicating a possible trade-off between somatic maintenance and reproduction. Such a trade-off is postulated by the antagonistic pleiotropy theory of ageing. This theory proposes that pro-ageing alleles with adverse effects late in life – after the reproductive period – are, nevertheless, maintained in the population by natural selection owing to their beneficial functions early in life [46]. The totality of these observations suggests that perturbing protein synthesis is a double-edged sword, promoting either longevity or senescence, depending on whether or not a certain threshold is exceeded. A delicate balance exists between the maximal longevity benefit and the detrimental impairment of protein metabolism.

Signal transduction pathways that influence ageing associate with mRNA translation regulatory mechanisms

Several cellular signalling mechanisms converge to influence the rate of mRNA translation, in response to a variety of stimuli, by modulating the activity or the availability of important translational regulators (Box 2) [21,47]. The insulin–IGF-1 pathway modulates ageing in a variety of organisms [3]. For example, downregulation of insulin–IGF-1 signalling by mutations [e.g. in the genes encoding the insulin–IGF-1-like receptor DAF-2 (abnormal DAuer Formation 2) or the phosphatidylinositol-3 kinase (PI3K) AGE-1 (AGEing alteration 1)] extends lifespan in *C. elegans*. This extension depends on the activity of DAF-16, a

forkhead (FOXO) transcription factor [4]. DAF-16 controls a wide variety of downstream targets, thereby affecting stress resistance, fat accumulation, fertility and metabolism [48]. In *Drosophila*, mutations in both the insulin-like receptor (*InR*) and the insulin-receptor substrate (*chico*) prolong lifespan in homozygous female flies, with evidence that dFOXO is also required for longevity [49,50]. In the yeast *S. cerevisiae*, mutations in the protein kinase SCH9 (Suppressor of CDC25), which is a functional orthologue of S6K [51] and also shows sequence similarity to Akt (also known as protein kinase B [PKB]), increase lifespan and stress resistance [52]. SCH9, together with the TOR kinase (see below), mediates the effects of caloric restriction on ageing in yeast [53,54].

In mammals, separate receptors for insulin and IGF-1 mediate distinct signalling events in different tissues [55]. Insulin coordinates cellular metabolism, whereas IGF-1 regulates growth and differentiation. Mutations in either receptor gene or in upstream genes that regulate insulin or IGF-1 levels extend lifespan [56–58]. For example, the Ames and Snell dwarf mice, which have low levels of serum insulin, IGF-1 and growth hormone, live significantly longer than their normal siblings, as do dwarf mice mutant for the growth hormone receptor [56]. The underlying molecular mechanisms that mediate these effects on lifespan are not clear. However, the rate of protein synthesis is decreased significantly in long-lived Snell dwarf mice [59]. Downregulation of mRNA translation is the result of reduced insulin–IGF-1 signalling through Akt/PKB and p38 MAPK, which in turn control key translation regulators such as the mammalian target of rapamycin (mTOR) kinase, ribosomal S6K, the eIF4E kinase Mnk1 (mitogen-activated protein kinase-interacting kinase), and the translation initiation factors eIF4E and 4E-BP1 (Box 2) [59]. A similar mechanism mediating protein synthesis reduction appears to operate in long-lived Ames dwarf mice, in which PI3–Akt–mTOR signalling is attenuated [60]. Therefore, low protein synthesis is a common denominator of both Ames and Snell long-lived dwarf mice.

TOR, an evolutionarily conserved seronine–threonine kinase, has emerged as a central regulator of cell growth and proliferation, development, metabolism and ageing. Genetic and biochemical studies in yeast, *C. elegans*, *Drosophila* and mammalian cells have identified several upstream and downstream components of the TOR signalling pathway (Box 2) [44].

TOR activity is regulated by four major input signals: nutrient and energy availability, growth factors and stress. Low insulin–IGF-1 signalling, nutrient or energy deprivation and stress converge to downregulate the activity of TOR [61]. In *C. elegans*, TOR deficiency causes developmental arrest, but these arrested animals live longer than wild type arrested worms. Similarly, knockdown of TOR by RNAi in adult animals increases their lifespan compared with that of wild type [62]. Genetic data suggest that the TOR pathway can also mediate the life-extending effects of dietary restriction. [63,64]. Therefore, the TOR and insulin–IGF-1-like signalling pathways might integrate nutrient sensing and nutrient uptake to influence adult nematode lifespan.

How might TOR influence lifespan? TOR interfaces with several cellular processes such as DNA transcription, mRNA translation, protein turnover, autophagy, and actin cytoskeletal organization, among others [45]. However, one of the best-characterized functions of mTOR is its ability to regulate protein synthesis. Biochemical analysis has revealed an orchestrated sequence of events that promotes efficient protein synthesis under conditions that activate mTOR [65]. Two key downstream targets of mTOR are S6K and 4E-BP1 [20]. Deletion of either dTOR or dS6K in *Drosophila* results in small cell size and severely reduced body size, similar to the effects of loss-of-function mutations of positive regulators of the insulin–IGF-1 pathway [45].

S6K phosphorylates the 40S ribosomal protein S6 and selectively promotes the translation of 5′TOP mRNAs, a specific subset of mRNAs containing a terminal oligopyrimidine tract. Interestingly, these mRNAs often encode ribosomal components and translation elongation factors. 4E-BP acts as a translational repressor by competing with eIF4G for an overlapping binding site on eIF4E (Box 1). Phosphorylation of 4E-BP promotes its dissociation from eIF4E, enabling recruitment of eIF4G and eIF4A translational factors to the mRNA cap structure. mTOR also regulates other translation initiation factors, such as eIF4GI and eIF4B, in addition to elongation factors, such as eEF2 [44]. In addition to downregulation of translation, under nutrient deprivation or environmental stress such as heat shock, osmotic shock, or UV irradiation, reduced TOR activity induces autophagy, a lysosomal catabolic pathway for the degradation and turnover of proteins and organelles [66]. It is noteworthy that autophagy is elevated in long-lived *daf-2* nematode mutants; and *bec-1* (the worm homologue of mammalian beclin 1, a protein essential for autophagosome formation) is required for lifespan extension in these mutants [33]. Thus, the TOR and insulin–IGF-1-like signalling pathways might influence lifespan by relaying nutrient signals to modulate protein synthesis and turnover.

Conclusions

The finding that reduction of mRNA translation rates extends lifespan establishes a direct link between protein synthesis and ageing. The biological relevance of this relationship is underscored by the tight integration of the insulin–IGF-1 pathway and caloric restriction responses with mechanisms governing mRNA translation regulation [63,65,67–69]. Thus, the effects of insulin–IGF-1 signalling and caloric restriction on ageing could in part be mediated by appropriately modulating protein synthesis, among other processes, to promote longevity.

The distinct effects of protein synthesis modulation in somatic versus germline cells are intriguing. Ageing is a soma-specific phenomenon. Somata, which encapsulate immortal germlines, are disposable; by contrast, the germline is an immortal cell lineage [70–72]. Is protein synthesis a contributing factor towards this fundamental distinction? 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. Although in metabolically active

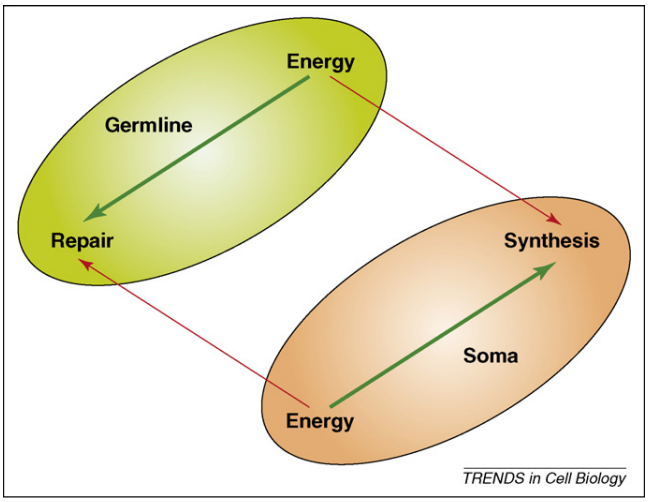

Figure 1. Different energy investment strategies between the soma and the germline, consistent with the 'disposable soma' theory of ageing. In somatic cells and tissues, more energy is relayed to biosynthetic activities (green arrow) and less is available for repair (red arrow), leading to progressive structural and functional deterioration, ageing and senescence. By contrast, in the germline, energy is mostly invested in maintenance and repair (green arrow), with less energy being channelled towards biosynthetic activities (red arrow); this scenario promotes germline immortality.

somatic tissues energy is mostly consumed in biosynthesis, in the germline, energy is invested in repair instead (Figure 1). In this context, lowering protein synthesis specifically in somatic tissues generates an energy surplus that can now be mobilized towards cellular repair and maintenance mechanisms, resulting in a net extension of lifespan.

In addition to moderating the large energy requirement of protein synthesis in the soma and facilitating cellular maintenance and repair, downregulation of mRNA translation, under appropriate conditions, can prevent the synthesis of unwanted proteins that could interfere with the cellular stress response. Remarkably, the stress-induced attenuation of global translation is often accompanied by a switch to the 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. Hormesis, a phenomenon in which mild stress stimulates maintenance and repair mechanisms [73], might in part depend on lowering mRNA translation to levels that increase energy availability but enable essential protein production. Hormesis is associated with reduced accumulation of damaged proteins, stimulation of proteasomal activity, and increased cellular resistance to toxic agents, and it often prolongs lifespan [74,75]. It remains to be seen whether moderating protein synthesis is an integral component of delayed ageing in hormesis.

The significance of the decrease in general protein synthesis that has been observed during ageing in many species is still an open issue [22]. What molecular mechanisms bring about the changes in protein synthesis during ageing? Would a sustained high rate of mRNA translation, specifically late in life, retard or accelerate senescent decline and ageing? Notwithstanding the overall reduction of protein synthesis, are any mRNAs differentially translated during ageing? A related question is whether translation of specific mRNAs is favoured under conditions that extend lifespan, such as caloric restriction. Addressing these questions should provide crucial insights into the biochemical basis of cellular ageing.

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