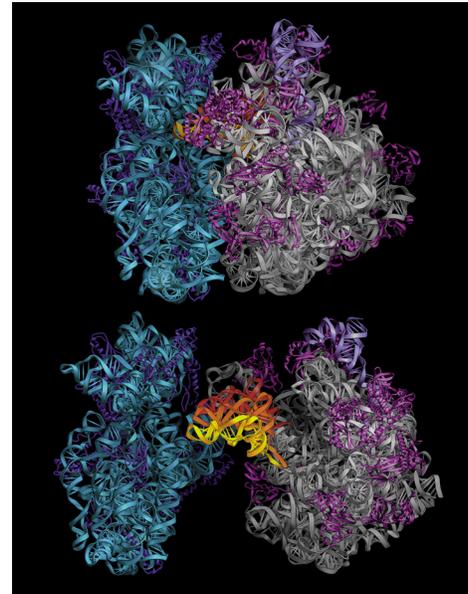


Protein synthesis

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Caption

Chapter Outline

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Summary

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20.1 Introduction

Key Concepts

- Protein synthesis is a fundamental cellular process that generates all proteins in a cell through translation of mRNAs.
- General protein synthesis declines during aging.
- Manipulations of protein synthesis can alter organismal lifespan.
- Signaling pathways that influence lifespan interface with protein synthesis.

The process of protein synthesis provides cells with building blocks and regulatory molecules essential for cellular function and survival. Protein synthesis impinges on all aspects of cellular life. Translating the genetic information encoded in mRNA molecules into polypeptide chains is a complex, multistep procedure involving numerous regulatory factors, auxiliary components, and specialized nanomachines (ribosomes). Therefore, not surprisingly, protein synthesis is highly sensitive to the physiological state of the cell and environmental conditions. Early studies in many organisms established that general protein synthesis declines during aging. This effect was initially considered a mere consequence of the general deterioration of cellular functions that accompanies aging. However, emerging findings suggest a causative relationship between the regulation of mRNA translation and aging. Indeed, manipulations that lower the rate of protein synthesis also lower the rate of aging, increasing the lifespan of different organisms. These observations suggest that protein synthesis is an important determinant of the aging process. In addition, several signaling pathways that modulate aging, such as the insulin/insulin-like growth factor 1 (IGF-1) pathway, the target of rapamycin (TOR) kinase pathway, and caloric restriction, directly interface with and regulate protein synthesis. Thus, protein synthesis constitutes a basic downstream cellular processes targeted by these signaling pathways, which exert their influence on lifespan, in part, by modulating mRNA translation. This chapter discusses our current understanding of the reciprocal relationship between aging and the molecular mechanisms that control protein synthesis.

20.2 The process of mRNA translation in eukaryotes

Key Concepts

- Protein synthesis proceeds through three phases: initiation of mRNA translation, elongation of the polypeptide chain, and termination of mRNA translation.
- Initiation of mRNA translation in eukaryotes is a tightly regulated process, involving numerous initiation factors and protein-ribonucleic complexes.
- Peptide synthesis in eukaryotes starts with the assembly of the 80S ribosome at the AUG start codon.

Translation of an mRNA molecule into a protein product is a tightly coordinated and conserved process that involves three distinct phases: mRNA translation initiation, polypeptide chain elongation, and mRNA translation termination ([Figure 20.1](#)). Numerous protein factors participate in each mRNA translation step. During the initiation phase, the initiation complex that scans for and recognizes the initiator codon is formed, by the association of the mRNA template with the small ribosomal subunit, numerous initiation factors, and the methionine-charged methionyl-tRNA. During elongation, the actual synthesis of the peptide chain takes place on a fully assembled ribosome that reads through mRNA and uses amino acid-charged tRNAs to catalyze polypeptide extension. mRNA translation termination concludes the polypeptide synthesis when a stop codon is encountered.

mRNA translation initiation involves several eukaryotic initiation factors (eIFs), which orchestrate the

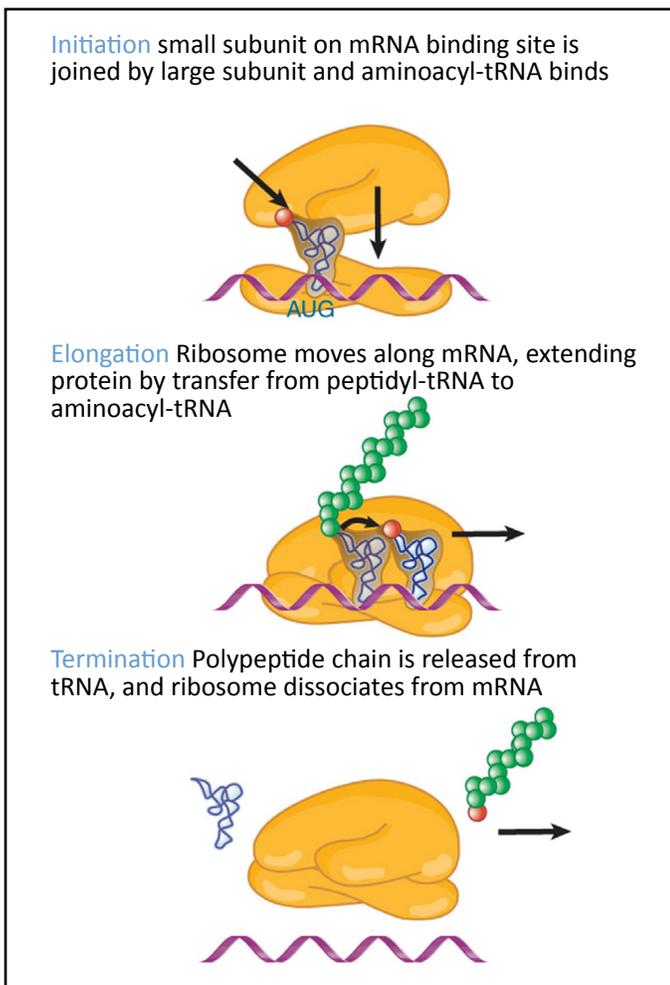


Figure 20.1 The three steps of mRNA translation in eukaryotes.

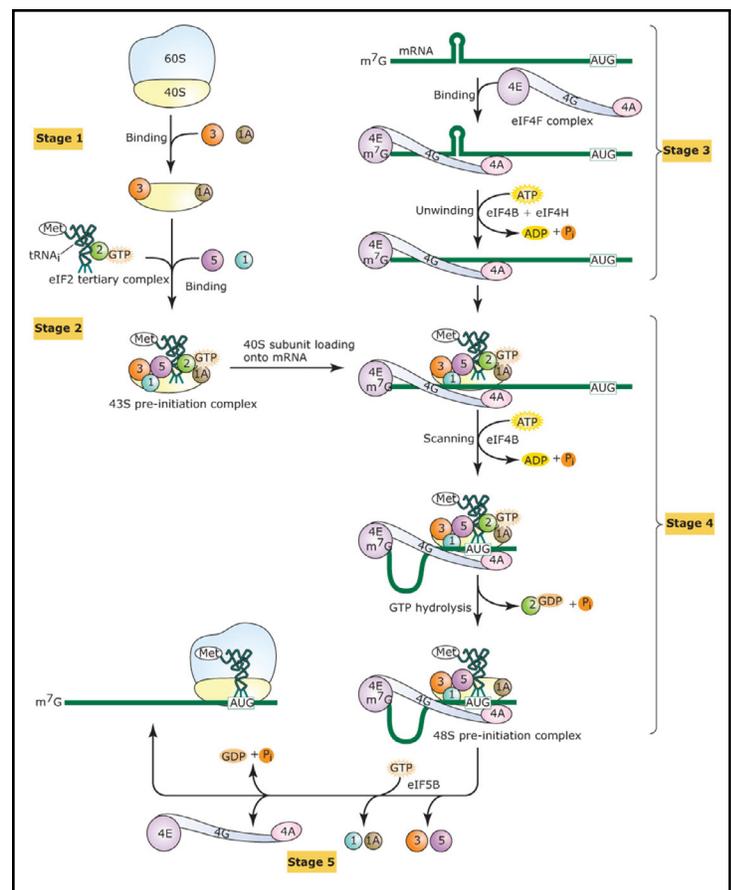


Figure 20.2 The eukaryotic translation initiation can be subdivided into five stages.

formation of the 43S preinitiation complex (43S PIC) on the mRNA being translated (Figure 20.2). This complex incorporates the initiator methionyl-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. Elongation of the polypeptide chain then commences by the 80S ribosome. 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 (Figure 20.3). eEF2 is regulated by the calcium/calmodulin-dependent eukaryotic elongation factor 2 kinase (eEF2K). Specific aminoacyl-tRNA synthetases load tRNAs with their cognate amino acids (AA). 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).

Core regulators of protein synthesis

mRNA translation initiation is the rate-limiting step in mRNA translation and is the most common target of mRNA translation control. Control of global mRNA translation is mostly exerted by changes in the phosphorylation state of initiation factors or their regulators involved in two critical steps during initiation; the recruitment of the 40S ribosomal subunit at the 5' end of mRNA and the loading of the 40S ribosomal subunit with the initiator methionyl-tRNA (Figure 20.4). These events are coordinated by initiation factors eIF4E and eIF2/eIF2B respectively.

First, the activity of eIF2, which loads the 43S PIC with methionyl-tRNA is regulated by phosphorylation of its alpha subunit. Phosphorylation interferes with recycling of GDP for GTP on eIF2 by the guanine nucleotide-exchange factor (GEF) eIF2B. The activity of eIF2B itself is also regulated by phosphorylation. Second, the

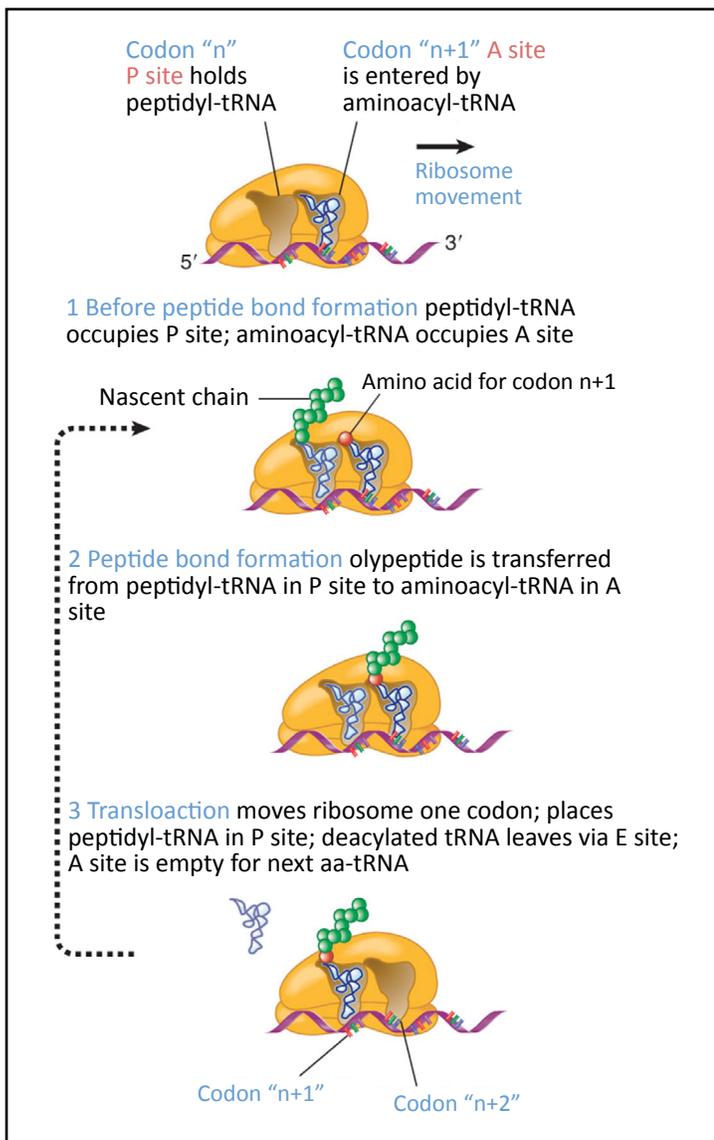


Figure 20.3 Polypeptide chain elongation.

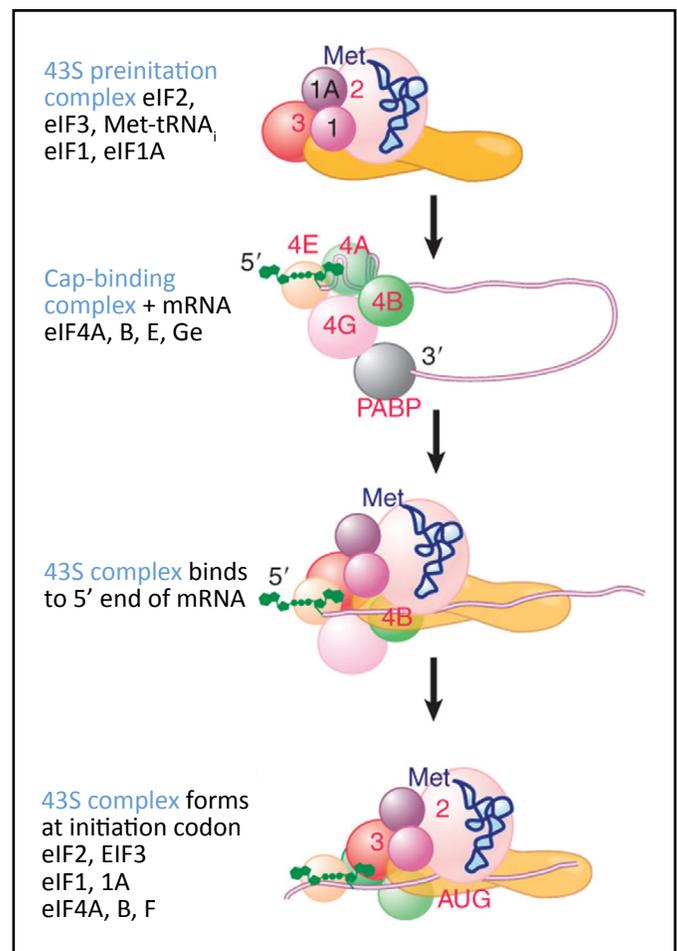


Figure 20.4 Nucleoprotein complexes formed during mRNA translation initiation.

and facilitates their recruitment to ribosomes. 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. The activity of eIF4E is modulated by direct phosphorylation and/or by association with the eukaryotic initiation factor 4E-binding proteins (4E-BPs). 4E-BP sequesters eIF4E and limits its availability. The availability of active eIF4E is controlled by phosphorylation of 4E-BP, which releases eIF4E. In turn, eIF4E associates with the scaffold protein eIF4G, eIF4A, eIF4B and the poly-A binding protein (PABP) to promote PIC assembly and protein synthesis.

mRNA processing

mRNA translation is tightly linked to the process of mRNA decay. Upon exiting translation, mRNAs enter a translationally repressed state via a transition into cytoplasmic structures, known as processing bodies, or P-bodies. P-bodies are sites of mRNA decapping and degradation, containing decapping enzymes and other proteins, such as key components of the RNA interference (RNAi) machinery (the RISC complex). P-bodies can also temporarily sequester mRNAs (naked or repressed by miRNAs) away from the translation machinery, probably for storage. Therefore, P-bodies appear to play a direct role in the regulation of protein synthesis, by

balancing two key events: active mRNA translation onto polysomes, and active repression into P-bodies. Due to limiting activity of translation initiation factors, most mRNAs are distributed between an actively translated and a non-translated pool in the cytoplasm of cells, and changes in the activity of these limiting translation factors elicit changes in global protein synthesis. Yeast cells lacking critical proteins for decapping/repressing translation of mRNAs, which also facilitate formation of P-bodies, are not capable of turning off protein synthesis under conditions where it would normally be repressed by the TOR pathway (for example, under glucose deprivation or amino acid starvation). In mammalian cells, repression of translation and targeting of mRNAs to P-bodies occurs through the interaction of eIF4E with the 4E-transporter (4E-T), via a conserved eIF4E-recognition motif, also found in eIF4G and 4E-BP. Colocalization of 4E-T with eIF4E and decapping factors appear to control mRNA stability and the transition from translation to decay. In addition to their important role in the global control of protein synthesis, P-bodies also facilitate mRNA-specific control by storing miRNA-repressed mRNAs.

20.3 Effects of aging on protein synthesis

Key Concepts

- The fidelity of mRNA translation is not significantly affected by aging.
- The rate of general protein synthesis declines during aging.
- mRNA translation in mitochondria becomes attenuated in old individuals.

All aspects of protein synthesis are tightly regulated and are executed with exquisite accuracy, which ensures the highest possible fidelity for the produced protein products. Nevertheless, aging impacts protein synthesis significantly. Early concepts, such as the error-catastrophe hypothesis and the somatic mutation theory, suggested that erroneous synthesis is responsible for the progressive accumulation of damaged macromolecules within cells. However, the fidelity of protein synthesis does not markedly decline with age. No age-related increase in amino acid misincorporation in proteins has been observed. For example, investigations of age-correlated errors in specific proteins such as hemoglobin also failed to support that aberrant proteins are synthesized in older cells.

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. Both biochemical data and microarray expression profiling correlate lowered protein synthesis rates with senescent decline. The levels of actively translating polysomes (large polysomes) are reduced with age in several organisms. When measuring the content and size distribution of membrane-bound and free polyribosomes in the mouse liver, old animals tend to show an increase in small polysomes and a decrease in large polysomes. This is consistent with a reduction in the rate of translation. Supporting studies in *Drosophila melanogaster* show that polyribosome levels exhibit a marked, age-related decrease. In the slime mold *Physarum polycephalum*, the efficiency of utilization of mRNA for translation decreases as the age of the mRNA population increases.

Mitochondrial protein synthesis activity also declines markedly with age. For example, the rate of mitochondrial protein synthesis in the rat heart is approximately 35% lower in 24-month-old rats compared to the 6-month-old rats. 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?

20.4 Molecular mediators of age-related effects on protein synthesis

Key Concepts

- Gradual reduction in the activity of key mRNA translation factors underlies attenuation of protein synthesis during aging.
- The mRNA translation initiation factor eIF2 and the elongation factor eEF-1 are particularly sensitive to age-related deterioration.

The activity of specific translation factors decreases with age, thus contributing to the decline of protein synthesis rates. 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 initiation factors eIF4E and eIF2/eIF2B respectively. Phosphorylation of the eIF2 α -subunit regulates dissociation of the eIF2B/eIF2 complex and eIF2 recycling. Similarly, the availability of active eIF4E is controlled by phosphorylation of eIF4E binding proteins. The activity of brain eIF-2, as well as that of other eukaryotic initiation factors that contribute to the binding of initiator aminoacyl-tRNA to ribosomes, decreases with age in the rat brain. This decline parallels the decrease in total protein synthesis.

Two specific eukaryotic elongation factors (eEF-1 and eEF-2) have also been implicated in the age-related decline in protein synthesis. In *Drosophila melanogaster*, peptide chain elongation rate decreases markedly with age. This decrease directly correlates with the age-related decline of overall protein synthesis. Of the three reactions (binding, translocation, and release) involved in peptide chain elongation, it is the binding of aminoacyl-tRNA to ribosomes that is most diminished with age, and the decrease parallels that of the decrease in peptide chain elongation and overall protein synthesis. Thus, decreased binding of aminoacyl-tRNA to ribosomes appears to be a major contributor to the age-related decreases in peptide chain elongation and overall protein synthesis. The decrease in the rate of protein synthesis in aging adult *Drosophila melanogaster* is mainly due to lowered activity of eEF-1. Interestingly, early reports indicated that transgenic flies overexpressing the eEF-1 alpha gene have an extended lifespan. However, subsequent studies failed to verify these results, thus uncoupling lifespan extension from eEF-1 overexpression in *Drosophila*. Nevertheless, *Podospira anserina* strains bearing high fidelity mutations in the eEF-1 alpha gene have a drastically increased longevity. eEF-1 activity has also been implicated in the age-related changes in protein synthesis in mammals. In liver and brain of 30-month-old rats eEF-1 activity is 30-40% lower than in three-month-old animals. The activity of brain eEF-1 decreases exponentially with age and declines in parallel to the age-dependent decrease in total protein synthesis in both mice and rats.

The activity of eukaryotic elongation factor 2 (eEF-2), which promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome, also undergoes age-related changes in mouse and rat liver. In conclusion, the decrease in protein synthesis that is generally observed in old animals is mainly due to age-related alterations of regulators and components of the mRNA translation machinery.

20.5 Perturbation of mRNA translation influences aging

Key Concepts

- Reduction of protein synthesis has pleiotropic effects on organismal physiology, development, and lifespan.
- Downregulation of key protein synthesis factors reduces protein synthesis and extends lifespan in several model organisms.
- Reduction of protein synthesis beyond a certain threshold is detrimental for development and longevity.
- In the nematode *C. elegans*, targeting a specific eIF4E isoform expressed in somatic tissues and in the germline, promotes longevity and increases resistance to oxidative stress.
- Lifespan extension and stress resistance by the attenuation of mRNA translation in *C. elegans* are, in part, mediated by SKN-1, the nematode homologue of mammalian Nrf1/2/3.

While the effects of aging on protein synthesis have been thoroughly characterized in many organisms, the reverse relationship—that of the effects of protein synthesis on aging—has only recently become the subject of experimental scrutiny. If the rate of protein synthesis is a determinant of aging, 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 aging. Indeed, complete elimination of key mRNA translation factors cannot be tolerated by most cells. Genes encoding these proteins are essential for growth and development and are usually highly conserved in evolution. Moreover, manipulation of general mRNA translation often results in pleiotropic effects, thus obscuring any explicit contribution to aging. Nevertheless, several recent studies capitalize on the genetic malleability of model organisms such as yeast, *C. elegans*, and *Drosophila* to probe the link between protein synthesis and aging. To avoid incurring the deleterious consequences of interfering with the expression of essential mRNA translation factors, their corresponding genes have been targeted by RNA interference (RNAi) after completion of development and during adulthood in *C. elegans*. 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 aging: pro-aging alleles with adverse effects late in life, after the reproductive period, are maintained in the population by natural selection, due to their beneficial functions early in life (see Chapter 2). Thus, similarly to a double-edged sword, perturbing protein synthesis can promote either longevity or senescence, depending on whether or not a certain threshold is exceeded. These studies have demonstrated that indeed, alteration of mRNA translation throughput has a significant impact on the aging process (Figure 20.5). A delicate balance exists between a maximal longevity benefit and a detrimental impairment of protein metabolism.

Targeting a battery of key protein synthesis regulators reduces protein synthesis and extends lifespan (Table 20.1). Five eIF4E isoforms (IFE-1 to IFE-5) are encoded in the *C. elegans* genome. IFE-1, IFE-3, and IFE-5 are expressed in germ cells, whereas IFE-2 and IFE-4 are expressed specifically in somatic cells. 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 cells, also bind both 7-monomethyl-guanosine and 2,2,7-trimethyl-guanosine caps. Loss of IFE-2 results in downregulation of protein synthesis in somatic cells and significant lifespan extension. 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, although depletion of IFE-1 during adulthood results in modest adult lifespan extension, suggesting that IFE-1 may also modulate longevity.

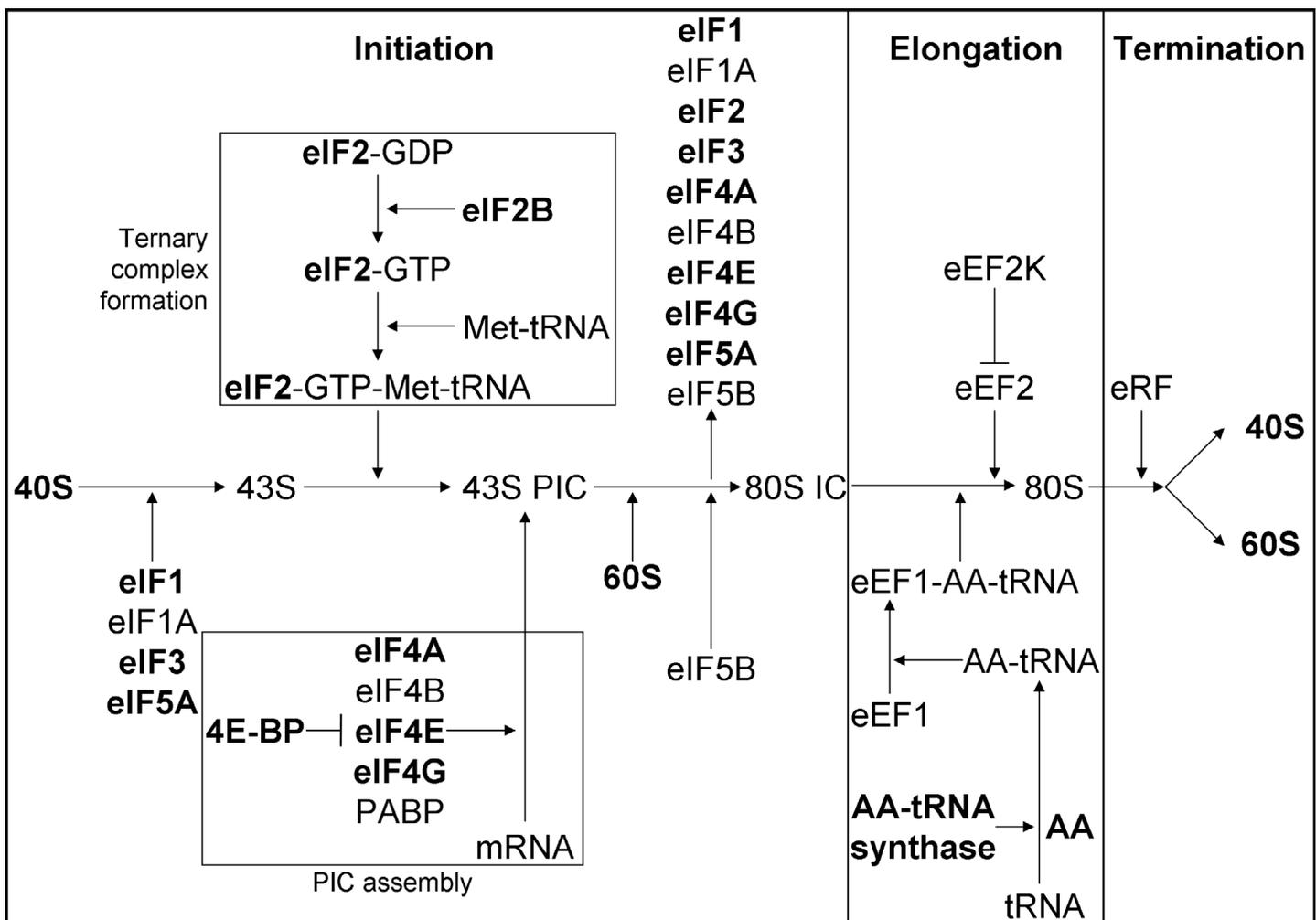


Figure 20.5 Key mRNA translation factors involved in each step of protein synthesis are indicated. The translation factors and ribosomal subunits that affect the aging process when modified are shown in bold. Bar lines indicate negative (inhibitory) regulation events.

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, using RNAi during adulthood, leads to a ~30% increase in lifespan. Similarly, reducing the levels of several ribosomal proteins or the ribosomal-protein S6 kinase (S6K) during adulthood by RNAi extends nematode lifespan. In all cases, the rate of protein synthesis in RNAi-treated animals was reduced compared to wild-type controls. In addition, many genes encoding components of the translation initiation factor (eIF) complex and components of the 40S and 60S subunit of the ribosome were recovered in an RNAi screen for essential genes that extend lifespan when inactivated post-developmentally.

What is the mechanism of lifespan extension by reduction of protein synthesis? IFE-2-depleted *C. elegans* 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 deficiency increases oxidative stress resistance and extends the lifespan of *mev-1* nematode mutants, experiencing chronic oxidative stress due to their lack of the cytochrome b large subunit in complex II of the mitochondrial electron transport chain. Thus, depletion of a specific eIF4E isoform, IFE-2, expressed in *C. elegans* somatic cells, increases oxidative stress resistance and extends lifespan. 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. These effects are, in part, mediated by SKN-1, the nematode ortholog of mammalian

NRF1/2/3. Perturbation of mRNA translation initiation induces SKN-1 expression, which mediates resistance to oxidative stress. 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. 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.

Table 20.1 Protein synthesis regulators implicated in aging and their function.

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

20.6 Aging signaling pathways interface with protein synthesis

Key Concepts

- Key signaling pathways that have been implicated in regulating lifespan interface with, and impinge upon, protein synthesis.
- Reduced insulin/IGF-1 signaling downregulates protein synthesis via the AKT/PKB and p38 MAPK kinases that, in turn, control the activity of the TOR, S6 and MNK1 kinases.
- The TOR kinase regulates mRNA translation by primarily targeting 4E-BP to influence the availability of eIF4E.

Several signaling pathways influence aging. While the understanding of these interlinked signal transduction cascades is becoming ever more detailed and comprehensive, the cellular and biochemical processes they impinge upon to modulate the rate of senescent decline and aging are still not well understood. Which facets of cellular metabolism can be fine-tuned to ultimately promote longevity remain an important challenge of modern aging research. Key cellular signaling pathways implicated in aging, such as the insulin/insulin growth factor 1 (IGF-1), the kinase target of rapamycin (TOR), and p38 mitogen-activated protein kinase (MAPK) pathways, 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. A variety of agents that promote cell growth and proliferation, including hormones, growth factors, and nutrients, have stimulatory effects on protein synthesis.

The insulin/insulin growth factor 1 (IGF-1) pathway modulates aging in a variety of organisms. For example, downregulation of insulin/IGF-1 signaling by mutations in the genes encoding the insulin/IGF-1-like receptor DAF-2 or the phosphatidylinositol-3 kinase (PI3K) AGE-1 extends lifespan in *C. elegans*. This extension depends on the activity of DAF-16, a forkhead (FOXO) transcription factor. DAF-16 controls a wide-variety of downstream targets, affecting stress resistance, fat accumulation, fertility, and metabolism. In *Drosophila*, mutations in both the insulin-like receptor (*InR*) and the insulin-receptor substrate (*chico*) prolong lifespan in homozygous female flies, with dFOXO also required for longevity. In the yeast *Saccharomyces cerevisiae* mutations in the protein kinase Sch9, which is a functional ortholog of the S6 kinase and shows sequence similarity to the AKT/protein kinase B (PKB), increase lifespan and stress resistance. Sch9, together with the TOR kinase, mediates the effects of calorie restriction on aging, in yeast. In mammals, separate receptors for insulin and IGF-1 mediate distinct signaling events in different tissues. Insulin coordinates cellular metabolism, whereas IGF-1 regulates growth and differentiation. Mutations in either receptor gene or in upstream genes that regulate insulin and IGF-1 levels extend lifespan. 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. 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. Downregulation of mRNA translation is the result of reduced insulin/IGF-1 signaling via the AKT/PKB and p38 MAPK kinases, which in turn control key translation regulators such as the mammalian TOR kinase, the ribosomal S6 kinase (S6K), the eIF4E kinase MNK1 (mitogen-activated protein kinase-interacting kinase), and the translation initiation factors eIF4E and 4E-BP (Figure 20.6).

The activity of eIF4E is under the control of many signaling cascades that mediate diverse cellular responses. These pathways converge mainly to modulate the association of eIF4E with inhibitory 4E-BPs and/or its direct phosphorylation by the MAP kinase signal-integrating kinases (MNK1/2). 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. Key eIF4E regulators such as 4E-BP, TOR, and Mnk, which have been implicated in the control of development, growth and aging, 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. The nutrient-sensing TOR kinase phosphorylates 4E-BP and enhances mRNA

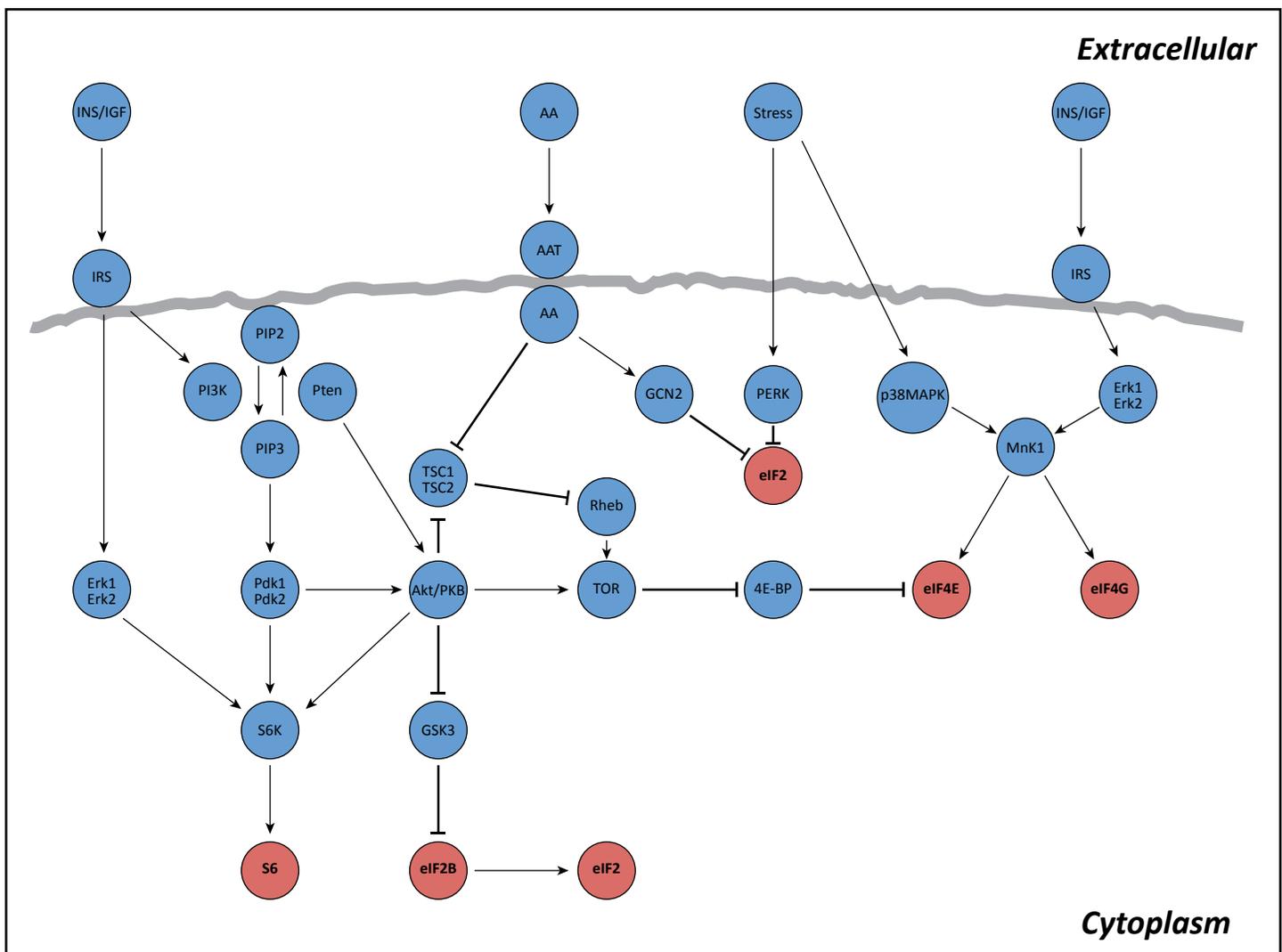


Figure 20.6 Aging signaling pathways interfacing with the regulation of protein synthesis. Arrows indicate positive (stimulatory) regulation events. Bar lines indicate negative (inhibitory) regulation events. Protein synthesis regulators are shown in red.

translation. 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. Furthermore, in *Drosophila*, the Mnk homolog Lk6 regulates growth in response to nutrients via eIF4E. Downregulation of eIF4E activity by these modulators extends lifespan. A similar mechanism mediating protein synthesis reduction appears to operate in long-lived Ames dwarf mice, where PI3/AKT/mTOR signaling is attenuated. Therefore, eIF4E interfaces with mechanisms influencing aging such as the insulin/IGF signaling and the TOR pathway to mediate at least part of their effects on lifespan.

TOR is an evolutionary conserved Ser/Thr kinase, which has emerged as a central regulator of cell physiology. TOR activity is regulated by four major input signals: nutrient and energy availability, growth factors, and stress (Figure 20.7). In turn, the TOR pathway modulates several cellular processes such as DNA transcription, mRNA translation, protein turnover, autophagy, and actin cytoskeleton organization, among others. Low insulin/IGF-1 signaling, nutrient or energy deprivation, and stress converge to downregulate the activity of TOR. Genetic screens in *Saccharomyces cerevisiae* have implicated several genes encoding components of the TOR signaling pathway in the regulation of both replicative and chronological aging (Chapters 3 and 4). Replicative (or mitotic) lifespan is defined by the number of daughter cells produced by an individual mother cell before senescence, whereas chronological (or postmitotic) lifespan represents the time a non-dividing cell population remains viable in liquid media. Pharmacological inhibition of the TOR pathway or removal of amino acids from the culture

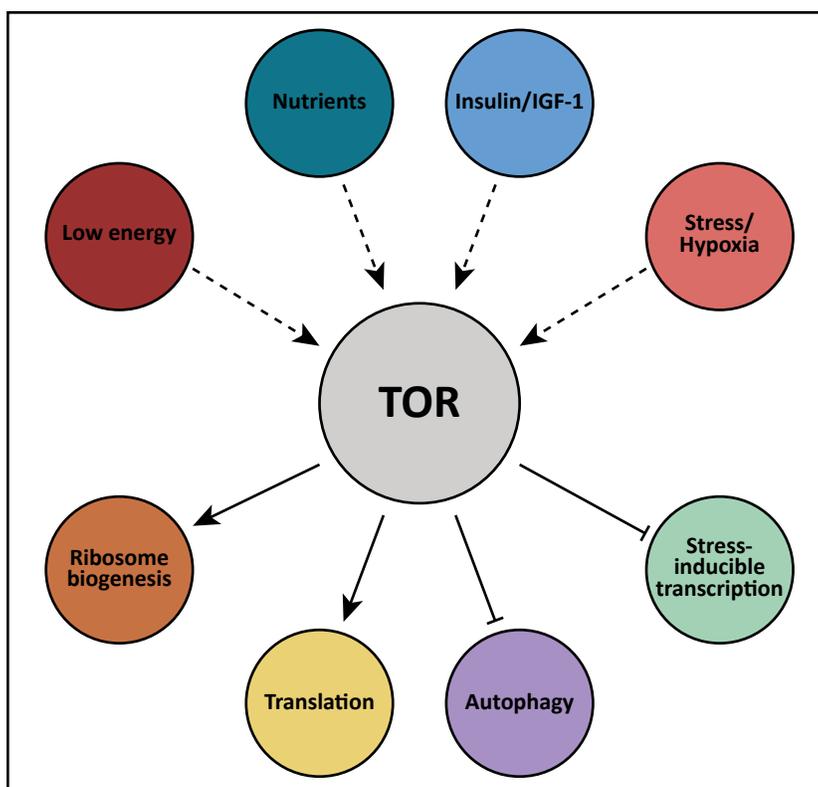


Figure 20.7 The TOR kinase integrates diverse inputs to modulate gene expression and protein turnover, under conditions of stress and during aging.

media significantly increases stationary phase survival. Importantly, rapamycin, an inhibitor of TOR, has been shown to also extend lifespan in several model organisms (Chapter 34). Given that TOR responds to nutrient availability, it has been proposed that reduced TOR activity extends yeast lifespan by simulating dietary restriction (Chapter 32). Indeed, superimposition of CR fails to further increase the replicative lifespan of cells lacking TOR.

In *C. elegans*, deficiency of TOR and the associated regulatory protein Raptor, encoded by the *let-363* and *daf-15* genes respectively, causes developmental arrest but these arrested animals live longer than wild-type arrested worms. Although lifespan extension is independent of DAF-16, the downstream effector of the insulin/IGF-1-like signaling pathway in worms, mutations in *let-363* and *daf-2* synergize to increase animal longevity. Thus, crosstalk between the TOR and DAF-2/insulin signaling pathways appears to regulate development, metabolism, and lifespan in worms. The TOR pathway is also involved in mediating the life-extending effect of dietary restriction in *C. elegans*. What are

the targets of TOR signaling that mediate aging effects in *C. elegans*? There is no apparent 4E-BP ortholog in the nematode genome. However, the *let-363* and the *raptor* mutant phenotypes are phenocopied by general mRNA translational initiation factor deficiency. For example, elimination of the *C. elegans* eIF4G homolog or subunits of eIF2 yields phenotypes that resemble CeTOR deficiency, indicating that TOR functions as a regulator of protein synthesis in worms. One additional effector of TOR signaling related to protein turnover that influences aging in *C. elegans* is autophagy, a lysosomal catabolic pathway for the degradation and turnover of proteins and organelles (Chapter 18). It is noteworthy that autophagy is increased in long-lived *daf-2* mutants, and *bec-1* (the worm homolog of mammalian beclin 1, a protein essential for autophagosome formation) is required for lifespan extension in these mutants. Therefore, the TOR and insulin/IGF-1-like signaling pathways may integrate nutrient sensing and nutrient uptake with protein synthesis and turnover to influence nematode lifespan.

Genetic studies in both *Drosophila* and mammals have established a functional link between TOR and the insulin/IGF-1 signaling pathway in controlling cell growth and overall organ size. Deletion of the single dTOR or dS6K in *Drosophila* results in small cell size and severely reduced body size, similarly to loss-of-function mutations in positive regulators of the insulin/IGF-1 pathway. Both pathways interact to modulate adult lifespan by regulating reproductive and metabolic genes. This is likely accomplished via systemic, humoral mechanisms, since downregulation of the dTOR pathway or activation of dFOXO specifically in the fat body of *Drosophila* extends lifespan of the fly. Interestingly, dTOR deficient flies display phenotypes characteristic of amino acid-deprived animals, suggesting that effects of dietary restriction on lifespan results are mediated by TOR signaling. Survival during starvation is also promoted by induction of autophagy in the *Drosophila* larval fat body, a process normally inhibited by TOR signaling.

20.7 mRNA translation regulators implicated in aging and their function

Key Concepts

- Phosphorylation of the 40S ribosomal protein S6 by the S6 kinase, a target of the TOR kinase, promotes the translation of 5' TOP mRNAs, a specific subset of mRNAs often encoding ribosomal components and translation elongation factors.
- Amino acid deprivation is sensed by the GCN2 and PERK kinases that reduce general protein synthesis by targeting eIF2, the mRNA translation factor that loads the 43S ribosomal preinitiation complex with the initiator methionyl-tRNA.

In addition to a multitude of downstream effectors, aging signaling pathways also impinge on key regulators of protein synthesis to exert their effects on lifespan. Insulin/IGF-1 signaling via the insulin receptor (IRS) results in the activation of a phosphatidylinositol 3 kinase (PI3K), which converts phosphatidylinositol (4,5)-bisphosphate (PIP₂) to phosphatidylinositol (1,4,5)-trisphosphate (PIP₃). The phosphatase and tensin homologue (PTEN) antagonizes the PI 3-kinase by degrading PIP₃ to PIP₂. PIP₃, either directly or via 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 via inhibition of the tuberous sclerosis complex 2 (TSC2) tumour suppressor, a negative regulator of the small GTPase *rheb* (RAS homolog enriched in brain), which activates TOR (Figure 20.6).

As noted earlier, the TOR kinase downregulates translation under nutrient deprivation or environmental stress such as heat shock, osmotic shock, or UV irradiation. Two key downstream targets of the TOR pathway are the ribosomal S6 kinase (S6K) and 4E-BP (Figure 20.8). 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. TOR also regulates other translation initiation factors, such as eIF4GI and eIF4B, as well as elongation factors, such as eEF2. eIF4E, together with eIF4G, a scaffold protein that recruits other essential mRNA translation initiation factors, are targets of Mnk1. Mnk1 activity is under the control of the mitogen activated protein kinases (MAPK) p38 and the extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2). ERK1 and ERK2 respond to insulin/IGF-1 signaling and, in addition to Mnk1, stimulate the S6 kinase (Figure 20.6).

The alpha subunit of the eukaryotic translation initiation factor 2 (eIF2), which loads the 43S ribosomal preinitiation complex with the initiator methionyl-tRNA, is phosphorylated by the GCN2 (general control nonderepressible 2) kinase and PERK (pancreatic endoplasmic reticulum eIF2alpha kinase; Table 20.2), GCN2 and PERK become activated under conditions of low amino acid (AA) availability or endoplasmic reticulum stress, respectively. The amino acid transporter (AAT), which imports amino acids into the cytoplasm, also signals to the TOR kinase via the TSC1/2 complex and Rheb. The activity of eIF2B, which recycles eIF2 during

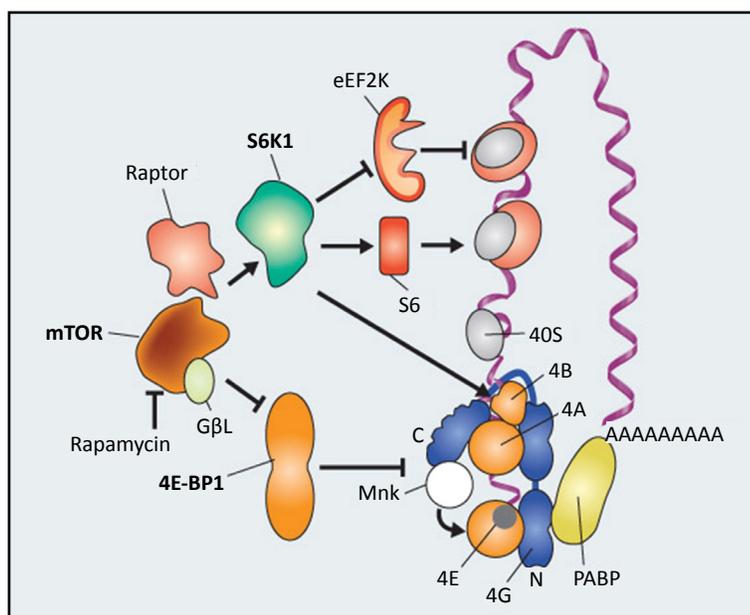


Figure 20.8 The mammalian TOR kinase (mTOR) regulates mRNA translation at multiple steps.

successive rounds of mRNA translation initiation, is regulated by the serine/threonine protein kinase GSK3 (glycogen synthase kinase 3), which is under the control of the Akt/PKB kinase (Figure 20.6).

Table 20.2 Regulatory modifications on key translation factors, executed by effector kinases.

Translation factor	Function	Phosphorylated by
eIF2 α	Loading the initiator methionyl-tRNA	GCN2, PERK
eIF2B	eIF2-GTP exchange/Translation initiation	GSK3
eIF4B	mRNA translation initiation	S6K
eIF4E	Cap-binding/Translation initiation	MNK1
4E-BP	Inhibitor of eIF4E/Translation initiation	TOR
eIF4G1	eIF4F assembly/Translation initiation	TOR
eEF2K	Polypeptide chain elongation	S6K
S6K	Ribosome biosynthesis	TOR
S6	Ribosome biosynthesis	S6K

20.8 Protein synthesis and protein turnover

Key Concepts

- Proteins accumulate damage during their functional life.
- Cells possess elaborate protein homeostasis mechanisms, involving protein repair, protein degradation, and protein synthesis.
- Cellular protein homeostasis capacity declines with aging.
- Fortification of protein homeostasis systems promotes longevity.

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. During their functional life, proteins are subject to numerous irreversible modifications (see also Chapter 22). Addition of carbonyl groups is a common type of oxidative stress-induced protein modification. Carbonyl derivative formation can occur through a variety of mechanisms including direct oxidation of certain amino acid side chains and oxidation-induced peptide cleavage. Such modifications normally mark proteins for degradation by cytosolic neutral alkaline proteases or the proteasome. Oxidation contributes to the pool of damaged proteins, which increases in size during aging and in various pathological states. In addition to oxidizing free radicals, metabolism generates other protein-modifying byproducts including glyoxal and methylglyoxal, both of which can contribute to the formation and accumulation of advanced glycation end

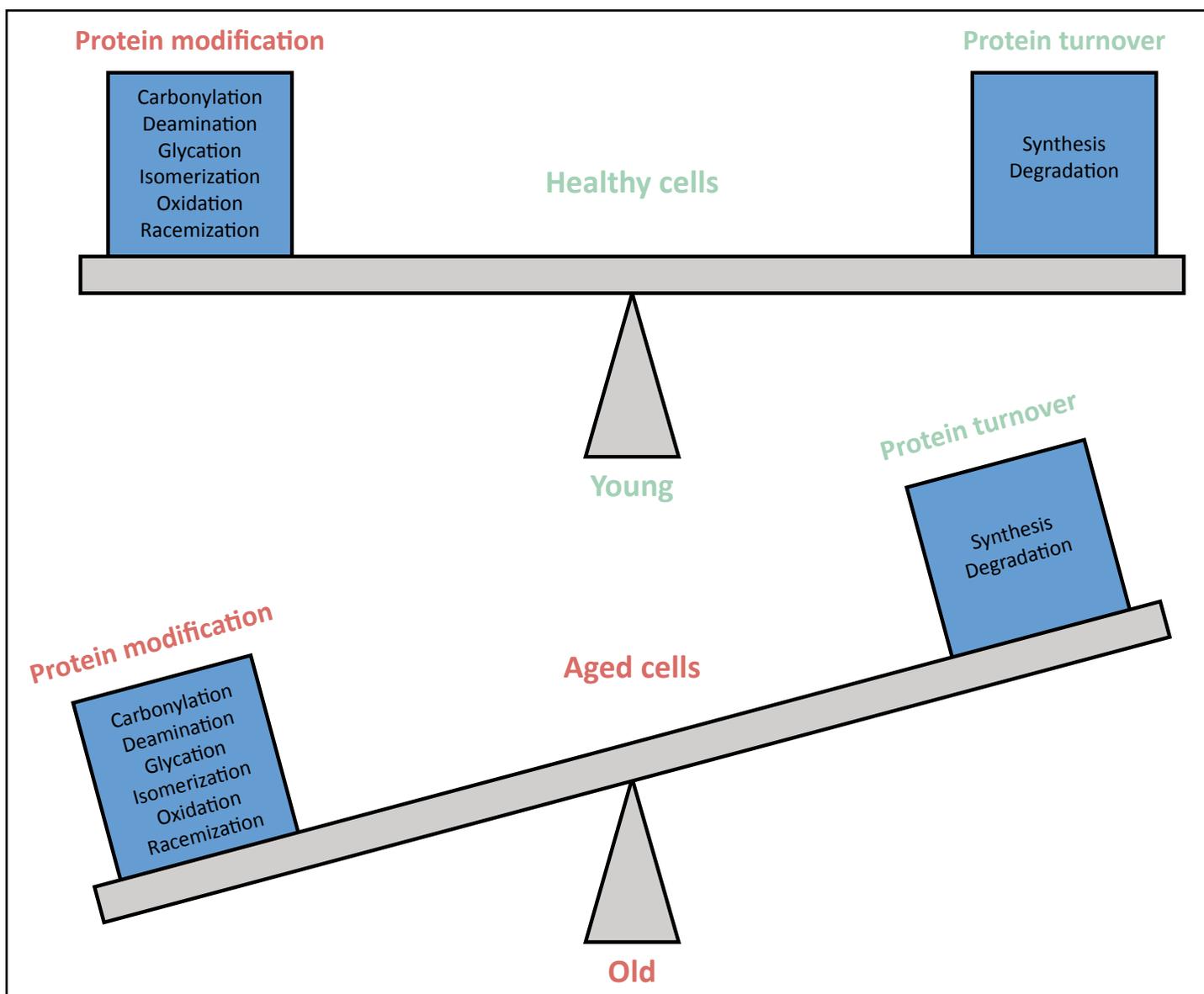


Figure 20.9 Aging disturbs the balance between protein modification and turnover.

products during aging. Proteins can also suffer additional modifications during their lifespan processes such as racemization, isomerization, and deamination. Such modifications can have mild to devastating effects on protein function. Accumulation of these modified forms of proteins both accompanies and contributes to senescent decline.

A main cause of age-related accumulation of damage is the limited capacity of cellular maintenance, repair, and turnover pathways. Damaged, non-functional proteins are removed by general and specific degradation pathways such as the proteasome system, autophagy, and lysosomal degradation. The overall efficiency of protein degradation declines with aging. Indeed, the activity of the cytosolic proteasomal system diminishes during postmitotic aging and proliferative senescence, resulting in increased half-life of oxidized proteins. In addition, oxidized proteins and lipids (such as lipofuscin/ceroid), which accumulate during aging, directly inhibit the proteasome, further exacerbating accumulation of damaged proteins. Reduced proteasomal function emerges as a common denominator of the aging process in diverse organisms. Autophagic degradation of proteins has also been implicated in aging. Genetic studies in *C. elegans* and *Drosophila* suggest that autophagy mediates, at least in part, the effects of insulin signaling and dietary restriction on longevity (Chapter 18).

The rate at which a protein pool is refreshed is determined by protein turnover, the balance between protein

synthesis and protein degradation, with protein synthesis providing fresh proteins and protein degradation removing the existing and likely damaged protein molecules. While aging is associated with a general decline in mRNA translation in a wide variety of organisms and tissues, the levels of most proteins remains relatively constant with increasing age. The decline in protein synthesis is accompanied by age-related decline in protein degradation. The combined reduction of turnover rates delays the removal and replacement of damaged proteins. Thus, the delicate balance between detrimental protein modification and protein turnover that exists early in life is tipped in favor of deleterious protein modification during late stages of life (Figure 20.9). Protein turnover cannot keep up with ever-increasing accumulation of damaged proteins. Decline in protein turnover is a major contributor to the accumulation of modified proteins that accompanies aging. It should be noted that while a drop in bulk protein turnover emerges as a common theme coupled with the aging process, not all proteins are uniformly affected, and individual protein levels do not always parallel the global trend.

Conditions that preserve the balance by augmenting protein synthesis and degradation have been associated with longevity. For example, caloric restriction, a significant reduction in calorie intake without essential nutrient deprivation, which extends lifespan in organisms ranging from yeast to primates, is associated with elevated protein turnover rates. However, the simplistic assumption that maintenance of high protein turnover rates generally has a beneficial effect on lifespan 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. For example, mRNA and ribosome biosynthesis, two processes controlled by insulin/IGF-1 and TOR signaling, are highly energy-consuming. Under favorable conditions, yeast cells synthesize ~2000 ribosomes per minute, in order to maintain robust growth. 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. The concomitant increase in energy availability may allow diversion of critical resources to cellular maintenance and repair processes, thus promoting organism longevity. In addition, reduction of protein synthesis may prolong lifespan by lowering the generation of toxic metabolism by-products such as reactive oxygen species associated with high energy demands.

What's next?

The finding that reduction of mRNA translation rates extends lifespan establishes a direct causative link between protein synthesis and aging. The biological relevance of this relationship is underscored by the tight integration between key signaling pathways influencing aging and mechanisms governing mRNA translation. Is the reduction of protein synthesis an integral component of a broader response to stress? Hormesis, a phenomenon where mild stress stimulates maintenance and repair mechanisms, may in part depend on lowering mRNA translation to levels that increase energy availability but allow essential protein production. Hormesis is associated with reduced accumulation of damaged proteins, stimulation of proteasomal activity, increased cellular resistance to toxic agents, and often prolonged lifespan. Whether moderating protein synthesis is an integral component of hormetic effects on aging remains to be investigated.

Aging is a soma-specific phenomenon, while the germline is an immortal cell lineage. Somata that encapsulate immortal germ cells are disposable. What molecular mechanisms underlie this fundamental distinction? Within the evolutionary framework of the disposable soma theory of aging (Chapter 2), 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 (Figure 20.10). 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, may prevent the synthesis of unwanted proteins that could interfere with the cellular stress response. Both consequences of protein synthesis attenuation favor cellular maintenance and repair, thus contributing to cell survival under stress. This notion is consistent with the

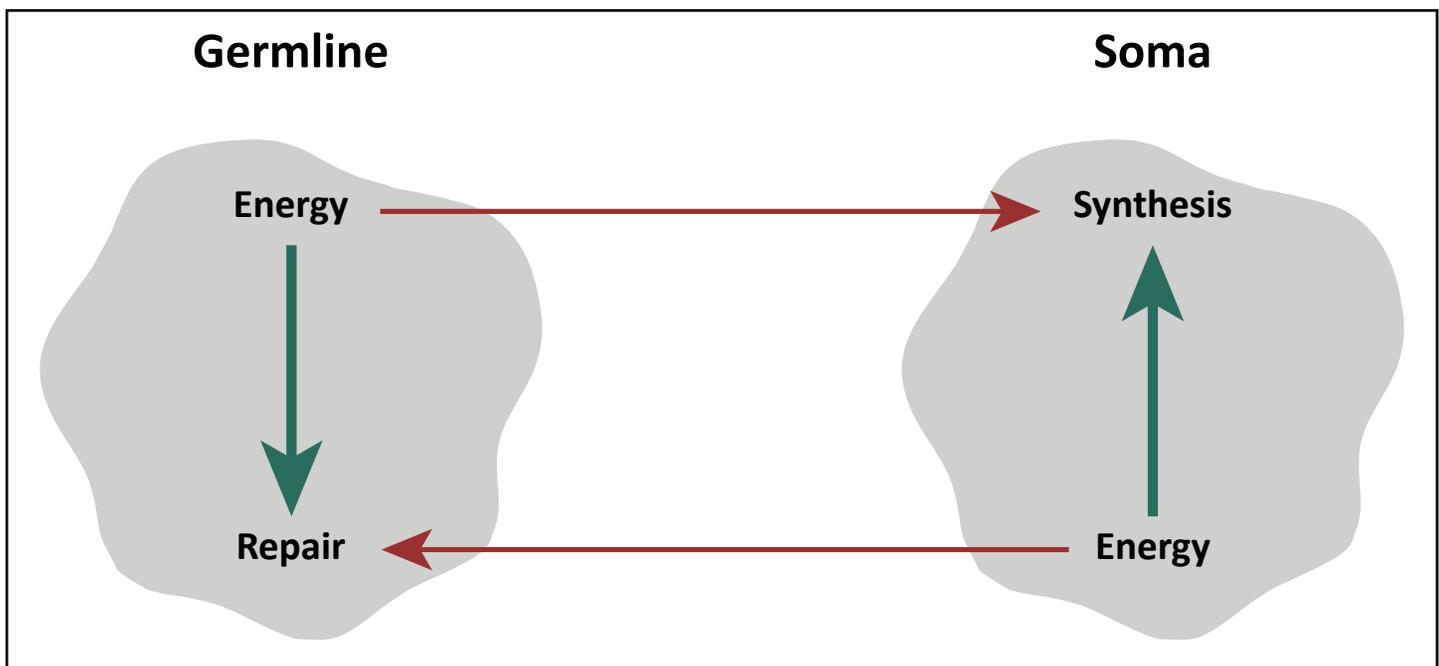


Figure 20.10 Protein synthesis and different energy investment strategies between the soma and the germline. In somatic cells and tissues, more energy is relayed to biosynthetic activities (green arrow) and less is available for repair (red arrow) leading to progressive structural and functional deterioration, aging and senescence. By contrast, in the germline, energy is mostly invested in maintenance and repair (green arrow), with less channeled towards biosynthetic activities (red arrow).

augmented oxidative stress resistance that accompanies the reduction of protein synthesis. 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. Moreover, different tissues and cell types are characterized by dissimilar protein synthesis activity.

The significance of the attenuation of protein synthesis during aging is also still an open issue. How general is it? Are there specific mRNAs that are differentially translated during aging or under conditions that extend lifespan? Would a sustained high rate of mRNA translation late in life retard senescent decline and aging? These questions can readily be addressed 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 humans.

Summary

Protein synthesis is a fundamental cellular process, responsible for generating both structural components for the cell and key regulators of cellular function. Together with protein degradation, protein synthesis also contributes to the turnover of aged and damaged proteins throughout the lifespan of a cell. Translating the information stored in an mRNA molecule into a polypeptide chain that will become a mature protein requires a large and elaborate set of regulatory factors, as well as specialized nanomachines, the ribosomes. Somatic cells invest a large part of their energy synthesizing proteins. Depending on cell type and growth conditions, more than half of the total energy output of a cell may be used for protein synthesis. Not surprisingly, protein synthesis is highly sensitive to the process of aging. Extensive changes in both general and specific protein synthesis accompany aging in diverse species, ranging from yeast to humans. These alterations are not simply a corollary of the aging 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 signaling pathways and specific conditions that regulate aging, such as the insulin/IGF-1

signaling pathway, the TOR kinase pathway, and caloric restriction. Protein synthesis has emerged as a novel determinant of aging in different organisms such as yeast, worms, flies, and mice and can thus be considered a universal component of the aging process.

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