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Keywords (separated by “ - ”)	mRNA translation - Polysome - Stress response - Cap-dependent - Ternary complex - Differential translation - Proteostasis	

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Chapter 13 Translational Control of Longevity

Jarod Rollins and Aric Rogers

Abstract There is accumulating evidence to suggest that that certain methods of limiting translation, while slowing growth and development, enhance somatic maintenance and lifespan. Much of this work has been based on studies in the model organism *C. elegans*. With its abundant genetic toolbox, rapid lifecycle, and transparency with diverse yet tractable tissues, many physiological responses to translation modulation were first characterized utilizing *C. elegans*. Translational regulation is complex and governed by hundreds of factors and noncoding RNAs, far too many to discuss in any one chapter. Instead, we explore the basic concepts of translational responsiveness to environment changes and inputs from signalling pathways associated with longevity regulation. We also discuss how studies aimed at diagnosing translation in *C. elegans* are enhancing efforts to understand mechanisms underlying the pro-longevity effects of attenuating translation.

Keywords mRNA translation • Polysome • Stress response • Cap-dependent • Ternary complex • Differential translation • Proteostasis

13.1 Introduction

The production of proteins via mRNA translation is critical for organismal growth and proper development. This process is energetically expensive and has been estimated to consume as much as 50 % of the available energy pool [1]. Due to its importance for survival and its metabolic cost, translation has evolved to be regulated by mechanisms that are highly conserved among eukaryotes, including well-studied invertebrate and mammalian models. Translation is regulated at three stages: initiation, elongation, and termination. Across different species, mRNA translation changes in response to developmental cues and environmental inputs. Periods of growth necessitate rapid translation, while various forms of stress are accompanied by an overall reduction in the synthesis of new proteins, as well as by differential

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© Springer International Publishing Switzerland 2016
A. Olsen, M.S. Gill (eds.), *Ageing: Lessons from C. elegans*, Healthy Ageing and Longevity 5, DOI 10.1007/978-3-319-44703-2_13

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translation (i.e., translation that changes differently for different genes) that leads to increased production of certain proteins important for withstanding stress and restoring homeostasis. The relative importance of quantitative and qualitative changes in translation has become a major focus of research into cellular adaptation to stress and, more recently, to physiological ageing. A growing body of evidence suggests that environmental and genetic interventions that result in negative translational regulation are associated with increased longevity. What remains unresolved is how attenuating translation mechanistically promotes longevity, especially in organisms with complex tissues of highly varied function, and whether these effects are dominated by global changes in protein synthesis or by relative changes in translation of specific mRNAs in specific tissues. In this chapter, we present examples of what model organisms have taught us about the biology underlying changes in ageing regulated at the level of translation. We will focus especially on the contributions made in work carried out in *C. elegans*, where the revolution in our understanding of the genetics of ageing began and where many of the physiological responses to translational modulation in a multicellular system were originally characterized.

13.2 Environmental Inputs and Signalling Pathways That Modulate Translation

13.2.1 Translation Changes in Response to Environmental Stress

In order to respond to sudden changes in environmental conditions, translation is rapidly altered in single cells and invertebrate models. A number of different conditions lead to altered translation, including thermal stress [2], hypoxic stress [3], oxidative stress [4], osmotic stress [5] and nutrient stress [6] (Fig. 13.1). Although the mechanisms linking longevity and translation are still not fully understood, protective effects of modulating translation during stress are frequently attributed to mitigating toxicity from unfolded proteins, redirecting energy expenditure, and differential translation that selectively enhance translation of genes important for recovering homeostatic balance [7]. The role of these phenomena in increased longevity associated with genetic attenuation of translation is discussed in Sect. 13.3.3.2. Interestingly, sub-lethal stress actually increases lifespan in *C. elegans* [8]. Thus, despite the temporary acute translation effects of many stressors, one or more of these phenomena may be responsible for long term effects that impact organismal health.

The paradigm that lowering translation in response to stress is important for mitigating damage is generally well supported by studies in which translation is attenuated genetically. For instance, down regulation of ribosome subunits, translation initiation factors, or pathways that positively regulate translation results in increased

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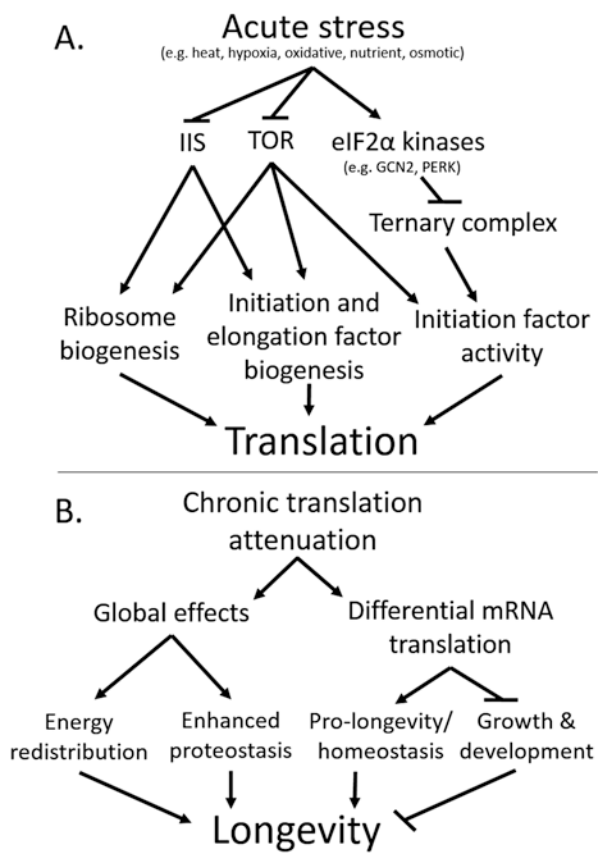

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Fig. 13.1 (**a**) Stress signalling impinges translation to promote longevity. A variety of abiotic stresses can decrease insulin-like signalling (IIS) or target of rapamycin (TOR) signalling, which leads to a decrease in ribosome subunit and initiation factor biogenesis. Reduction of TOR activity also leads to reduced initiation factor activity. Stress can activate the kinases GCN2 and PERK which act to inhibit translation through the ternary complex. These responses to stress lead to a decrease in protein translation. (**b**) Chronic translation attenuation promotes longevity through the global decrease in protein synthesis or through the preferential translation of specific transcripts. The global decrease in translation can increase longevity due to enhanced proteostasis or shifts in energy expenditure from growth to maintenance mechanisms. The preferential translation of pro-longevity genes can lead to their relative up-regulation during periods of stress

thermotolerance [6] and resistance to nutrient stress [9] in *C. elegans*. Similarly, oxidative stress resistance has also been shown to be increased in a *C. elegans* strain bearing a mutant allele of the regulatory translation initiation factor eIF4E/IFE-2 [10]. Results from these studies are consistent with the long-standing paradigm that reduced translation is not merely a side-effect of stress but a protective mechanism in stress acclimatization and recovery.

Although initiation is the rate-limiting step of translation, translational changes resulting from exposure to stress can involve factors controlling different stages of

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76 translation to help fine-tune protein synthesis [11]. Limiting translation rates at this
77 stage makes sense if organismal survival is enhanced by mitigating energy expendi-
78 ture associated with this costly process. However, changes can also be observed
79 through stoichiometric alterations in the translation machinery, itself, including
80 changes in the abundance and distribution of ribosomal subunits [12]. Expression at
81 this level is modulated, in part, downstream of pathways controlling longevity that
82 help orchestrate organismal response to environmental conditions, including the tar-
83 get of rapamycin (TOR) and insulin/IGF signalling (IIS) pathways. The following
84 section elucidates the role of certain stress-sensing kinases and signalling pathways
85 that influence translational responses.

86 *13.2.2 Translational Regulation via Stress-Sensing Kinases* 87 *That Target the Ternary Complex*

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88 An important cellular response to environmental challenges is regulated by the
89 translation ternary complex (Fig. 13.1a), which consists of the eukaryotic transla-
90 tion initiation factor 2 (eIF2), charged initiator $tRNA^{Met}$, and guanosine-5′-
91 triphosphate (GTP) [13]. The ternary complex delivers the charged tRNA to the 40S
92 ribosomal subunit [14] to initiate formation of an active 80S complex capable of
93 translation. Upon delivery of the tRNA and subsequent assembly of the 80S com-
94 plex, eIF2-bound GTP is hydrolyzed to GDP and both are released as an inactive
95 complex. eIF2 is a trimer comprised of three subunits, α, β, and γ. The β subunit is
96 necessary for reactivation of the inactive complex through the exchange of the GDP
97 for GTP. However, under various forms of stress, certain kinases targeting eIF2α are
98 activated, phosphorylating this subunit and preventing recharging by the exchange
99 catalyst eIF2B [15]. The result is potent attenuation of global translation initiation
100 [16] but also selective translational upregulation of factors important for responding
101 to the activating stressor [17, 18].

102 Two of the most highly conserved kinases that phosphorylate eIF2α are PERK
103 [PKR (RNA-dependent protein kinase)-like ER kinase] and GCN-2 (general control
104 nonderepressible 2) (Fig. 13.1a). PERK is activated by accumulation of unfolded
105 proteins in the ER, which is influenced by a number of factors, including, but not
106 limited to inhibition of glycosylation, calcium depletion, hypoxia, oxidative stress,
107 energy imbalance, and inflammation [19]. PERK is normally kept inactive by
108 dimerizing with the protein chaperone BiP, known in *C. elegans* as HSP-3 and HSP-
109 4. To prevent aggregation, BiP associates with unfolded proteins as they accumulate
110 in the ER, dissociating from PERK and activating this kinase resulting in phos-
111 phorylation of eIF2α and suppression of translation. One study in *C. elegans* found
112 that activation of this particular arm of the ER stress response pathway accounted
113 for 23 % of differential gene expression observed in the inducible response [20].

114 Osmotic and nutrient stress also lead to eIF2α phosphorylation in the ternary
115 complex by stimulating activity of GCN-2. When free amino acid levels are

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sufficient, GCN-2 assumes an auto-inhibitory conformation, but when amino acid levels become limiting, depleted tRNAs accumulate and bind to a histidyl-tRNA synthetase-like domain within GCN-2 [21]. Upon binding of depleted tRNAs, GCN-2 undergoes a conformational change resulting in the activation of its kinase activity [22]. GCN-2 then phosphorylates the α subunit of initiation factor eIF2 to inhibit translation in a manner similar to PERK. In *C. elegans*, hypertonic conditions have also been shown to activate GCN-2, and translation inhibition during this response includes signalling through other kinases to induce osmoprotective gene expression [5].

13.2.3 Growth and Developmental Pathways Regulating Longevity Help Orchestrate Translational Responses to Stress

The TOR pathway integrates a number of cellular cues to influence protein production and turnover (Fig. 13.1a). It is among the most well-documented longevity pathways and the interplay between TOR, translation, and longevity has been the subject of several exemplary reviews [23–25]. In brief, TOR is a nutrient responsive kinase associated with a number of other subunits to form a complex that integrates environmental cues with cellular responses [24]. TOR activity is downregulated under nutrient restriction and other stresses. Decreased kinase activity of TOR leads to reduced phosphorylation of two translation regulatory proteins, ribosomal subunit S6 kinase (S6K/RSKS-1) and eIF4E binding protein (4EBP).[1] Reduced phosphorylation of S6K/RSKS-1 leads to reduced biogenesis of ribosomal subunits [28]. In comparison, hypophosphorylation of 4EBP enhances its ability to bind to the translation initiation factor eIF4E. When eIF4E is bound by 4EBP, it is prevented from forming a complex with the methyl-guanosine cap at the beginning of the 5′ untranslated region (UTR). Loss of this complex abrogates cap-dependent translation, a key rate-limiting event in translation initiation [11].

The IIS pathway is a robust regulator of longevity [29] (see Chap. 4). Under conditions that are not ideal for development, IIS signalling is reduced, stress resistance is enhanced and longevity is extended [30] (Fig. 13.1a). The pro-longevity effects of IIS are largely dependent on nuclear translocation of the FOXO transcription factor DAF-16 in *C. elegans* [31], which increases transcription of several pro-longevity genes with functions involved in regulating stress responses, metabolism, lipid synthesis and peptide degradation [32]. Reduced IIS also results in reduced S6K/RSKS-1 activity and overlaps with TOR signalling in this regard, which may help explain an observed decrease in polysomes [2] (mRNA bound by two or more

[1] 4EBP as it pertains to translation inhibition has not been identified in *C. elegans* [10]. Another notable caveat with implications for translation is 5′ UTR trans-splicing [26], in which native 5′ UTRs are replaced with a spliced-leader sequence 22 nucleotides in length. An in depth appraisal of the merits and caveats of *C. elegans* use in translation research is found in Rhoads et al. [27].

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ribosomes, representing the translated pool of mRNA) and reduced synthesis of ribosomal subunits and translation initiation factors [33, 34]. Thus, the effects of IIS on gene expression are not limited to transcription alone.

13.2.4 The Influence of Noncoding RNA on Translation and Longevity

Just because a transcript is manufactured does not guarantee it will be used to synthesize the protein it encodes. A number of factors control the availability and propensity of a transcript for translation. These involve *cis*-regulation, which is determined by sequence-specific characteristics of the mRNA, as well as by *trans*-factors that help guide mRNA species to their fates (degradation, storage, or translation). MicroRNA (miRNA) and long noncoding RNA (lncRNA) are mechanisms of post-transcriptional regulation that illustrate what *C. elegans* has helped teach us about this ever-expanding mode of regulation and its influence on organismal ageing.

miRNAs are a species of small non-coding RNA that regulate and fine-tune expression of target genes post-transcriptionally [35]. Although evidence shows that part of the way miRNAs may influence expression is by diminishing transcript stability, they may also impair translation prior to degradation of the RNA message [36]. The existence of miRNAs was first discovered in *C. elegans* during characterization of the gene *lin-4* [37]. In this pivotal work, *lin-4* was determined to play a role in developmental timing by down-regulating *lin-14*. Based on the complementation of *lin-4* to sequences in the 3′UTR of *lin-14,* investigators suggested that *lin-4* regulated translation of *lin-14* through anti-sense RNA-RNA interactions. At the same time, another study confirmed post-transcriptional regulation via repeated sequences found in the *lin-14* 3′ UTR [38]. Another miRNA, *let-7* was later discovered to also regulate development in *C. elegans* [39], and was found to be highly conserved, including in humans [40]. These studies paved the way for understanding miRNA as a broadly applicable mechanism governing gene expression.

Since the discovery of their role in developmental timing, miRNAs have also been shown to mediate longevity in *C. elegans*. In the case of *lin-4*, loss-of-function reduces lifespan while overexpression extends lifespan in a DAF-16/IIS-dependent manner [41]. The expression of miRNAs changes with age [42], and some miRNAs, like *mir-71* and *mir-246* are predictors of lifespan in *C. elegans* [43]. miRNAs respond to environmental cues to regulate longevity, as has been seen with *mir-80* in response to dietary restriction [44]. Under periods of limited food, *mir-80* is downregulated in *C. elegans* and a deletion mutant of *mir-80* was shown to be long-lived. The longevity phenotype of *mir-80* mutants was dependent on the activity of the DAF-16 and the transcriptional co-factor CBP-1. These and other observations led the authors to formulate a model where *mir-80* represses *cbp-1* under well-fed conditions, which results in reduced transcriptional activity of DAF-16. However,

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under dietary restriction lower levels of *mir-80* allowed the translation of *cbp-1* mRNA, the product of which could then act as a co-factor to DAF-16 to promote transcription of pro-longevity genes. The presence of miRNAs related to *mir-80* have been observed in *D. melanogaster* and humans. Thus, research investigating the role of miRNAs on longevity in humans [45–47] was inspired and informed by research pioneered in *C. elegans*.

Another class of non-coding RNAs with emerging roles in translational regulation and longevity are lncRNAs, which are distinguished from other non-coding RNAs in that they are typically 200-bp or longer in length. There are several established ways that lncRNAs can modulate to affect gene expression [48]. For example, lncRNAs can promote stability and translation of target genes through extended base-pairing with them or can elicit reduced translation via partial base-paring. Additionally, lncRNA may play a role in alternative splicing by acting as ‘sponges’ for splicing factors [48]. A role for lncRNA in ageing was recently shown using high-throughput sequencing of transcripts associated with polysomes [49]. In *C. elegans*, long-lived *daf-2* IIS-deficient nematodes exhibit significantly reduced polysome activity compared to wild-type N2 worms [34, 49], which is indicative of overall reduced translation. The lncRNA *tts-1* (Transcribed Telomerase-like Sequence) was found to be specifically enriched in mono- and polysomal fractions of *daf-2* mutants as compared to wild-type or *daf-2;daf-16* double mutants [49]. When *tts-1* was knocked down via RNAi, polysome levels were returned to near wild-type levels. Additionally, upon reduction of *tts-1* the longevity of *daf-2* mutants was significantly reduced. Although the precise nature of the interaction between *tts-1* and ribosomes has yet to be fully elucidated, results suggest that *tts-1* negatively influences translation in a manner that contributes to enhanced longevity in this model.

13.3 Towards a Mechanistic Understanding of Translation’s Role in Lifespan Regulation

Before there were studies directly linking translation and longevity, there was evidence associating lifespan regulation with genetic and environmental conditions that influence translation. As early as 1976, dietary restriction associated with carbohydrate or nitrogen (protein) restriction, both of which increase lifespan [50], were observed to decrease protein synthesis in rat heart, lung, and liver tissue [51].[2] The TOR pathway, which was already known to modulate translation [23], was linked to longevity regulation in yeast [54, 55], Drosophila [56], and *C. elegans* [57] by the mid-2000s. The timing of these discoveries regarding the TOR pathway came on the heels of a major discovery in *C. elegans* related to genetic screening

[2] Results for acute or short-term dietary effects should not be confused with long-term studies showing that protein synthesis is better maintained with age under dietary restriction [52, 53].

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229 that would enable rapid establishment of a direct connection between translation
230 and longevity.

231 *13.3.1 RNAi in* C. elegans *Helped Establish the Connection*
232 *Between Translation and Longevity*

233 By the mid-2000s, an exceptional tool available in *C. elegans* allowed investigators
234 to easily implement genetic screens in this intact multicellular system. Unlike other
235 models, *C. elegans* can be fed bacteria containing double-stranded (ds) RNA to
236 rapidly knock-down particular genes [58], which subsequently led to the construc-
237 tion of large dsRNA libraries [59, 60]. These resources were used to test regulators
238 of longevity and helped identify translation attenuation as a positive regulator of
239 lifespan [6, 61, 62]. For example, two independent RNAi screens of genes known to
240 be required for development for their effects on lifespan were carried out by initiat-
241 ing dsRNA feeding *after* development was complete. In these studies, Chen et al.
242 [61] and Curran and Ruvkin [62] found several genes encoding factors involved in
243 ribosome biogenesis, tRNA synthesis, and translation initiation that result in lifes-
244 pan extension when suppressed via RNAi. Hansen et al. [6] also employed dsRNA
245 feeding in *C. elegans* to show that reduction of ribosomal subunits, translation ini-
246 tiation factors, and the ribosomal S6 Kinase *rsks-1* resulted in decreased levels of
247 newly synthesized proteins and lifespan extension. Concurrently, Pan et al. [9]
248 showed that targeting factors encoded by *rsks-1* and *ifg-1* (a.k.a. eIF4G, a cap-
249 binding complex scaffold important for mRNA-ribosome association) decreased
250 translation and increased longevity.

251 *13.3.2 Translation Diagnostics: Methods to Understand*
252 *the Connection Between Translation and Lifespan*

253 Over the last decade, the link between longevity and translation (as well as transla-
254 tion and age-related diseases) has grown substantially [63]. In order to understand
255 how downstream biological processes are affected to alter lifespan, efforts have
256 been applied towards determining associated changes in global and gene-specific
257 (i.e., differential) mRNA translation. One classic method of ascertaining global
258 translation rates is through pulsed metabolic labelling as performed in translation-
259 longevity regulation studies carried out by Hansen et al. [6] and Pan et al. [9]. Using
260 this method, the amount of protein synthesis is quantified by measuring the incor-
261 poration of radiolabeled methionine into de novo synthesized proteins [64]. This
262 labelling approach may be used in combination with 2D-gel electrophoresis to
263 quantify individual proteins [65]. More recently, stable isotope labelling by amino
264 acids in cell culture (SILAC) was developed using the incorporation of 'light' and

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'heavy' versions of amino acids into newly synthesized proteins [66]. When coupled with mass-spectrometry, SILAC allows the identification and quantification of newly translated proteins compared to those previously synthesized.

Although methods of proteomic analysis can be used to approximate translatomic changes, most studies do not distinguish whether changes in specific proteins arise from altered synthesis or from altered turnover. In addition, changes in protein synthesis with respect to a particular gene may arise from changes in translation efficiency of the mRNA and/or from transcript abundance. Distinguishing the effects of transcription and translation on protein synthesis can be realized by combining transcriptional analysis with polysome profiling [67] or ribosome profiling [68] technologies. When coupled with microarray analysis or mRNA sequencing, these profiling methods enable the global quantification of individual mRNAs that are actively being translated. By comparing the abundance of a transcript in the translated fraction against the abundance in the total RNA fraction, the propensity of that transcript to be translated can be estimated.

Polysome and ribosome profiling are similar in that they both use ultracentrifugation of lysed tissue over a sucrose density gradient to separate free mRNAs, mRNAs that are bound by single ribosomes (monosomes), and mRNAs actively translated by two or more ribosomes (polysomes) (Fig. 13.2a, b). It is at this point that the two profiling methods diverge; ribosome profiling introduces an RNAase step which degrades all mRNA not protected by the ribosome, leaving the "ribosome footprint" behind (Fig. 13.2c). In polysome profiling, translated mRNA is isolated intact and quantified via mRNA-seq. While both methods can be used to ascertain information about differential mRNA translation between sets of conditions, each has its advantages with respect to resolving specific characteristics of translational regulation.

Ribosome profiling ascertains the position of the ribosome within the mRNA as the nascent peptide is elongated. Thus, this technique excels at determining changes in elongation rate associated with codon usage [69]. Although translation elongation may not be a limiting factor in healthy organisms under optimal conditions, it can be slowed or paused in response to stress and depletion of charged tRNAs. For example, ribosome profiling was used to show that ribosomes accumulate near the open reading frame (ORF) in response to proteotoxic stress in mouse and human cells lines [70]. Similar results were obtained using heat shock as a stress [71]. In addition to providing information about pauses in elongation, ribosome profiling also facilitates identification of alternative upstream ORF usage [72]. For example, translation at repressive upstream ORFs can be distinguished from translation at productive ORFs by quantifying the ribosome footprints aligning to those sequences [69]. Additionally, since each short sequencing read from ribosome profiling represents the binding of a single ribosome, more exact measurements of translation (elongation) rates are achievable as compared to polysome profiling [69], which provides relative abundances. However, due to the short read length of ribosome footprints (28–32 bp), many reads are discarded due to ambiguous alignments leading to reduced coverage of the transcriptome [73].

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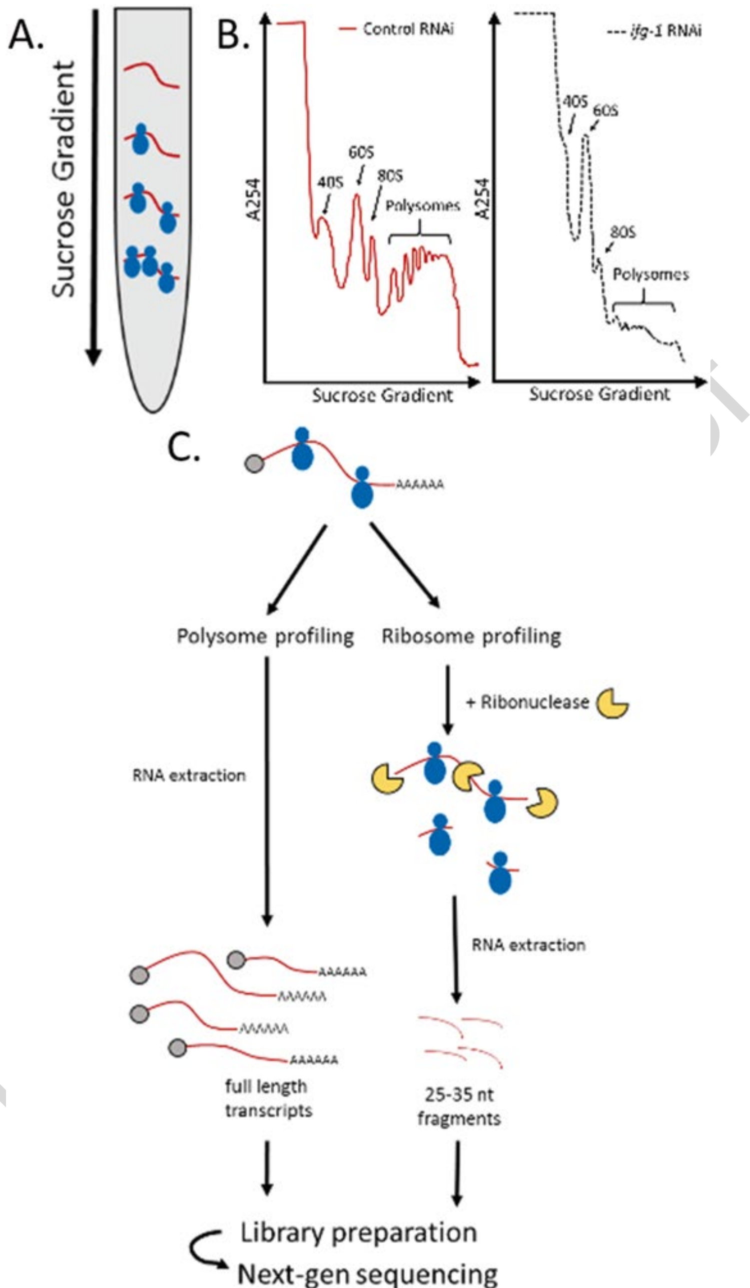

Fig. 13.2 Polysome and Ribosome profiling as diagnostics of translation. (**a**) Cell lysate is separated over a sucrose gradient to resolve free RNA, monosome bound RNA, and RNA bound to polysomes. (**b**) Sucrose gradients are fractionated based on the absorbance of RNA at 254 nm. A representative profile of *C. elegans* lysates treated with control RNAi is on the *left*. The peaks cor

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Polysome profiling leaves mRNA intact, which means that the length of transcript reads are only limited by the sequencing technology. While typical read lengths from high-throughput sequencers currently range from 50 to 1000 bps, the upper limit of this range has steadily increased. Longer reads lengths can be used to more reliably map and discover exon-exon junctions [74]. The ability to align reads across exon-exon junctions is important in distinguishing transcript isoforms that arise due to changes in alternative splicing. Therefore, polysome profiling is well suited for isoform-specific quantification of mRNA translational efficiency [75]. In addition, the sequencing of intact mRNA also preserves 5′ and 3′ UTR sequences, which contain *cis*-regulatory elements that help determine mRNA stability and translatability [76–78]. For example, binding of the *trans*-factor ELAVL1 to such elements within 3′ UTRs increases their stability [79]. Despite their differences and potential pitfalls, both ribosome profiling and polysome profiling provide a wealth of information about the status of the translatome and have allowed researchers to quantify global changes in translation as well as transcript specific changes.

13.3.3 Phenomena Associated with Attenuating Translation as Potential Mediators of Increased Longevity

Why do so many conditions that attenuate translation result in increased lifespan? Almost from the beginning, the link between the two phenomena led investigators to speculate on the causative factors behind this association. These early suppositions invoked the idea of enhanced proteostasis as a contributing factor [80, 81]. Since translation requires molecular chaperones and other proteostasis factors important for folding and turnover, lower flux through the translation apparatus may reduce the burden on protein fidelity assurance factors and, in so doing, result in enhanced proteostasis. Another idea is that, because translation is an energetically expensive process, lowering translation allows energy to be redirected to enhance somatic maintenance [82]. In addition, there is evidence of differential mRNA translation [coincident with reduced translation] geared towards maintaining the integrity of existing proteins and molecular complexes [82, 83]. None of these

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Fig. 13.2 (continued) responding to 40S and 60S subunits, monosomes (80S), and polysomes are labelled. A representative profile of *C. elegans* lysates treated with eIF4G/*ifg-1* RNAi is shown for comparison on the right. Knockdown of *ifg-1* results in a decrease in active polysomes and an increase in 40S and 60S subunits. (**c**) Samples corresponding to the translated (polysomal) fractions are processed by one of two methods. For polysome profiling, full length mRNA is extracted from polysomes and submitted for library preparation, which typically includes a fragmentation step, and next-generation (next-gen) sequencing. For ribosome profiling, fractions are treated using ribonucleases to digest mRNA not bound by ribosomes. The resulting RNA 'footprint' represents the position of the ribosome along the transcript. These fragments are then subjected to library preparation and sequencing

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338 theories are mutually exclusive and are included in the translation-based longevity
339 model in Fig. 13.1. Here, we talk about studies that helped form these theories,
340 along with new studies that are adding resolution to these paradigms.

341 #### 13.3.3.1 Proteostasis

342 Proper regulation of translation is essential during development and in response to
343 environmental inputs [11]. Too much synthesis or an inability to turn down produc-
344 tion during times of stress may cause proteotoxicity due to the inability of the cell
345 to properly fold, re-fold, and turn over what is manufactured [84]. Ageing leads to a
346 reduced ability to maintain or rebalance proteostasis after it is perturbed, resulting
347 in accumulation of protein aggregates [85] (see Chap. 12). Thus, it may be that
348 lowering translation helps offset losses in proteostatic capacity due to ageing
349 (Fig. 13.1b). In *C. elegans,* negative regulation of growth and development associ-
350 ated with reduced translation and increased lifespan has been shown to improve
351 resistance to forms of unfolded protein stress. For example, attenuating the IIS path-
352 way or invoking dietary restriction ameliorates aggregation-related toxicity [86,
353 87]. Hypoxic conditions attenuate translation, and stimulation of the hypoxic
354 response in *C. elegans* via knockdown of the ubiquitin ligase *vhl-1* increases lon-
355 gevity and enhances resistance to polyglutamine and β-amyloid toxicity [88].
356 However, each of these interventions is also associated with differential gene
357 expression. How do we separate these effects?
358 Cycloheximide is a chemical that binds to mRNA and blocks elongating ribo-
359 somes. Recent evidence in tissue culture suggests that cycloheximide is an equal
360 opportunity translation antagonist that does not result in significant differential
361 translation bias in ribosome profiling [89]. Thus, it is possible that effects of dif-
362 ferential expression may be less of a confounder in systems using this reagent. One
363 study in *C. elegans* showed that that hyperosmotic stress-induced polyglutamine
364 aggregation and toxicity were reduced in response to the translation inhibitor cyclo-
365 heximide [90]. Additionally, in yeast resistance to the ER-stress invoking compound
366 tunicamycin was increased upon cycloheximide treatment [91]. However, there is
367 little or no evidence that cycloheximide can increase lifespan. This may indicate
368 that, while blocking translation improves survival under acute stress, increased
369 lifespan requires accessing adaptive response pathways that may be invoked through
370 changes in translation factors, translation machinery, or translational substrates, and
371 not just translation attenuation, itself. Conversely, chemical factors like cyclohexi-
372 mide may have off-target or other effects that counteract longevity benefits.
373 One of the ways reducing translation may enhance proteostasis is by lowering
374 demand on protein turnover governed by the proteasome. Indirectly, this idea may
375 be tested by augmenting proteasomal function in the absence of altered translation.
376 In order to recycle normal and damaged proteins, the ubiquitin-proteasome system
377 has, as its downstream effector, the 20S proteasome. A recent study showed that
378 increasing 20S proteasomal activity was sufficient to increase lifespan in *C. elegans*

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[92], although it was noted that the effect was dependent on the genetic context and required known positive regulators of longevity. Although the precise role of altered proteasome efficiency in mediating translational effects on longevity requires further study, separating aspects of proteostatic mechanisms as done for this study will help interrogate the relative contributions of altered homeostasis associated with attenuating translation.

Yet another way proteostasis might be improved by attenuating translation in a manner that increases lifespan is by improving the fidelity of folding that occurs on a nascent peptide. For example, it was shown in bacteria that slowing translation elongation leads to enhanced proper folding of eukaryotic proteins [93]. A recent study to look for whether this phenomenon also exists in eukaryotic systems utilized mammalian tissue culture to show that slowing (but not stopping) translation dramatically improved the fidelity of protein folding [94]. Furthermore, slowing translation actually improved function of mutant proteins that normally display a high level of misfolding. Interestingly, the most dramatic improvements in protein folding were obtained by slowing translation elongation [94], suggesting that the mode of translational regulation is important for enhanced proteostasis contributing to longevity.

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13.3.3.2 Differential Translation as a Mechanism Governing Longevity Responses to Translation Attenuation

When translation is reduced as part of an adaptive mechanism to changing environmental conditions, the preference of particular transcripts for translation may change (Fig. 13.1b). Even if total transcript levels do not change, differences in *cis*-regulatory elements among different mRNA can affect how efficiently they are recruited to the ribosome. The first well-documented example of this was discovered in the yeast gene *GCN4*, which is activated in response to nitrogen-limiting conditions [95]. During normal levels of translation, *GCN4* is poorly translated due to the presence of multiple upstream ORFs. When translation is reduced from stress-responsive kinases targeting eIF2α (see Sect. 13.2.2), the ORF encoding GCN4 is able to efficiently compete with normally dominant upstream ORFs to selectively increase synthesis of *GCN4* [17]. The importance of differential translation for increased lifespan in this model was demonstrated when GCN4 was found to be required for the full lifespan increase associated with translational suppression via depletion of 60S ribosome subunits [96]. Because GCN4 is a highly conserved nutrient-responsive transcription factor (a.k.a., ATF4), results suggest that differential translation plays an important role in longevity effects associated with attenuating translation by removing ribosome complex subunits.

As a direct regulator of protein synthesis, translation initiation factor eIF4G has also been shown to respond to nutrient availability. However, unlike GCN4 which is translationally induced, eIF4G decreases in response to nutrient limitation in *C. elegans* (where it is referred to as IFG-1) [83], as well as to a lack of nutrient and

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420 TOR signalling in yeast [97, 98]. This factor acts as a scaffold to bring together
421 mRNA and ribosomal subunits and is linked to cancer [99–102] and Parkinson's
422 disease [103, 104] in humans. Previous studies showed that genetic suppression of
423 eIF4G increases lifespan in yeast [105] and *C. elegans* [6, 9, 62, 106]. In yeast, the
424 level of eIF4G was found to be negatively correlated with translational preference
425 based on mRNA length [107]. This correlation was shown to be preserved in *C.*
426 *elegans*, where a combination of polysome profiling and microarray analysis
427 showed differential translation associated with eIF4G/*ifg-1* knockdown that was
428 biased towards longer transcripts [83]. Certain translationally upregulated genes
429 involved in maintaining cellular homeostasis and responding to stress were required
430 for fully increased lifespan under this condition [83]. Another study showed that
431 depleting eIF4G, while diminishing overall protein synthesis, led to a widespread
432 effect on translational efficiency in yeast [108]. Together, results in both yeast and
433 *C. elegans* show that eIF4G differentially regulates mRNA translation, and further-
434 more, differentially regulated mRNAs are functionally connected in a manner con-
435 sistent with effects on longevity.
436 As indicated earlier, TOR is a nutrient-responsive kinase with inputs to transla-
437 tion and other cellular processes that increases lifespan when inhibited [24]. One
438 study showed that translational reporters of the pro-longevity transcription factor
439 genes *daf-16* and *skn-1* accumulated under TOR inhibition in *C. elegans* in a man-
440 ner consistent with increased translation and were required for longevity through
441 this pathway [109]. Interestingly, *daf-16* and *skn-1* were also among the translation-
442 ally upregulated genes in response to suppression of eIF4G/*ifg-1* [83]. This is con-
443 sistent with what is known about TOR in systems outside *C. elegans*, where it
444 regulates cap-mediated translation, of which eIF4G is a part [23]. In addition, TOR
445 regulates differential translation in yeast in a manner that was shown to be depen-
446 dent on eIF4G [110]. The links between eIF4G and the TOR pathway suggest that
447 differential translation mediated by the level of eIF4G may be a key player in lon-
448 gevity regulation through this translation factor.

449 13.3.3.3 Energy Allocation

450 The effect of translation on longevity may extend beyond protein abundance,
451 translation error rates, and differential translation. As translation is an energeti-
452 cally expensive process, its decrease could theoretically increase the energetic
453 resources for somatic maintenance (Fig. 13.1b). It is conceivable that some of
454 this energy could be redirected to ameliorate oxidative stress and DNA damage
455 that may contribute to the ageing process [111]. DNA repair and scavenging of
456 free radicals are both energy dependent processes [112, 113]. Some evidence of
457 enhanced DNA repair during lowered translation has been documented in *C.*
458 *elegans* eIF4E (*ife-1*/*2*) loss-of-function mutants and with cycloheximide treat-
459 ment [114]. eIF4E acts in physical association with eIF4G as a factor that binds

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to the 5′ methylated cap of mRNA to help initiate translation. When eIF4E mutant worms were subject to ionizing radiation, they showed reduced levels of induced germ-cell apoptosis [114]. As apoptosis is triggered by the accumulation of DNA damage, this result could be due to enhanced DNA repair due to resources freed from reduced translation. However, additional studies are required to determine if this effect was due to enhanced DNA repair or reduced apoptotic signalling.

13.4 Concluding Remarks

The ability of reduced translation to extend longevity has been considered as an example of the antagonistic pleiotropy theory of ageing. This theory proposes that beneficial gene function early in life can become detrimental after growth is complete [115]. However, the gene products that regulate translation rates; i.e., ribosome subunits, translation factors, and rRNA polymerases are necessary even in adulthood. In light of this, the relationship between translation and ageing might best be described as an example of hyperfunction. Hyperfunction is a modern theory in the ageing field introduced by M.V. Blagosklonny which proposes that ageing is caused by the over activity of biosynthetic processes necessary for development and reproduction [116]. With this view, translation rates are left on high due to lack of selection of programmes to turn them down effectively post-reproduction. When growth and development pathways like TOR and ILS are inhibited, they reduce translation, which may help offset the effects of hyperfunction. Despite the lack of selection pressure to reduce translation post-reproduction, other mechanisms have evolved to reduce translation in response to the environment. Understanding both the molecular mechanisms and the evolutionary context of how protein translation is regulated will be crucial in developing anti-ageing therapies in humans.

There are several unanswered questions remaining in the field of translational regulation of ageing. Is the reduction in translation that occurs in response to nutrient stress directly proportional to the decrease in available resources or is it reduced to an even greater extent so that remaining resources may be reallocated? Why are ribosomal subunits differentially regulated under stress [12]? Does altering ribosome composition modulate translation in a way conducive to increasing longevity? If so, do some remodelled ribosomes alter global translation rates while others differentially translate specific genes? Indeed, many of the mechanisms that support differential translation have yet to be elucidated. Direct comparisons of polysome bound mRNAs upon knockdown of different translation factors and ribosomal subunits will help differentiate these effects. Dissecting the mechanisms that control translation and how they impinge on other pathways through the use of model organisms like *C. elegans* will play a pivotal role effectively translating protein translational research into human anti-ageing therapies.

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