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Review

Alterations of the translation apparatus during aging and stress response

Yulia Gonskikh[a,b], Norbert Polacek[a,*]

[a] Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland
[b] Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland

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ABSTRACT

Aging is a biological process characterized by the irreversible and time-dependent deterioration of cell functions, tissues, and organs. Accumulating studies in a wide range of species from yeast to human revealed changes associated with the aging process to be conserved throughout evolution. The main characteristics of aging are (i) genomic instability, (ii) loss of telomere function, (iii) epigenetic changes,(iv) increased cellular senescence, (v) depletion of the stem cell pool, (vi) altered intercellular communication and (vii) loss of protein homeostasis. Among the multiple molecular mechanisms underlying aging, alterations of the translation machinery affecting the rate and selectivity of protein biosynthesis seem to play a central role. At the very heart of translation is the ribosome, a multifaceted and universally conserved RNA-protein particle responsible for accurate polypeptide synthesis and co-translational protein folding. Here we summarize and discuss recent developments on the contribution of altered translation and age-dependent modifications on the ribosome structure to aging and cellular senescence.

1. Modulation of aging pathways regulating mRNA translation

Despite the complexity of aging, several signaling pathways were shown to be involved in healthy aging. Multiple genetic manipulations at different levels of the insulin–insulin growth factor 1 (Ins-IGF-1) pathway, the target of rapamycin (TOR) pathway, or the p38 mitogen-activated protein kinase (MAPK) pathway led to increased life-spans in multiple model organisms. Interestingly, all three mentioned pathways target and in the end modulate different components of the translation machinery affecting the global rate of protein biosynthesis.

1.1. TOR

The target of rapamycin (TOR) pathway is regulated by nutrient and energy supply, growth factors and stress. It has been shown that the inhibition of the TOR pathway by rapamycin treatment or by depleting different components of the pathway significantly extends life-spans in yeast, nematodes, fruit flies and mice (Johnson et al., 2013). This confirms that the TOR signaling pathway is a central and evolutionarily conserved regulator of longevity. Upon activation, TOR primarily regulates translation initiation, which is considered to be the primary rate-limiting step of protein biosynthesis (Sonenberg and Hinnebusch, 2009). Under normal conditions the eukaryal initiation factor eIF4E recognizes and binds to the 5′ cap structure of mRNAs and, together with eIF4G and eIF4A, forms the eIF4F complex (Fig. 1). Then eIF4G interacts with the poly(A)-binding protein (PABP) deposited at the mRNA 3'end. The interaction of eIF4G with eIF4E and PABP circularizes the mRNA by bringing together its 5'- and 3'-ends. eIF4G also recruits eIF4A to the cap for local unwinding of mRNA secondary structure elements. Unwinding of mRNA and interactions of eIF4G with eIF3, eIF5, or eIF1 facilitate the recruitment of 43S preinitiation complex. Thus, interaction between eIF4E and eIF4G is considered to be the pivotal step for the initiation of mRNA translation. Activation of the mTOR pathway leads to phosphorylation of eIF4E-binding proteins (4E-BP), which compete with eIF4G for binding to eIF4E. As a result, phosphorylation of 4E-BP leads to its release from eIF4E and consequently stimulates cap-dependent translation initiation (Gebauer and Hentze, 2004; Averous and Proud, 2006). Besides the regulation of translation initiation, the mTOR pathway also controls translation elongation through activation of elongation factor 2 (eEF2) (Fig. 1). In eukaryal cells eEF2 promotes the GTP-dependent translocation of the ribosome along the mRNA. eEF2 undergoes phosphorylation at Thr56 by its highly specific eEF2 protein kinase that leads to inactivation of eEF2. Insulin and a number of other molecules that activate protein synthesis cause rapid dephosphorylation of Thr56 thus increasing eEF2 activity and thereby accelerating translation elongation (Spriggs et al., 2010). Activation of mTOR promotes phosphorylation of the eEF2 kinase at least at three sites (Ser366, Ser359 and Ser78) which leads to its inactivation. As a consequence, mTOR activates eEF2 and thereby enhance translation elongation (Wang and Proud, 2006). Other known

* Corresponding author.
E-mail address: Norbert.poacek@dcb.unibe.ch (N. Polacek).

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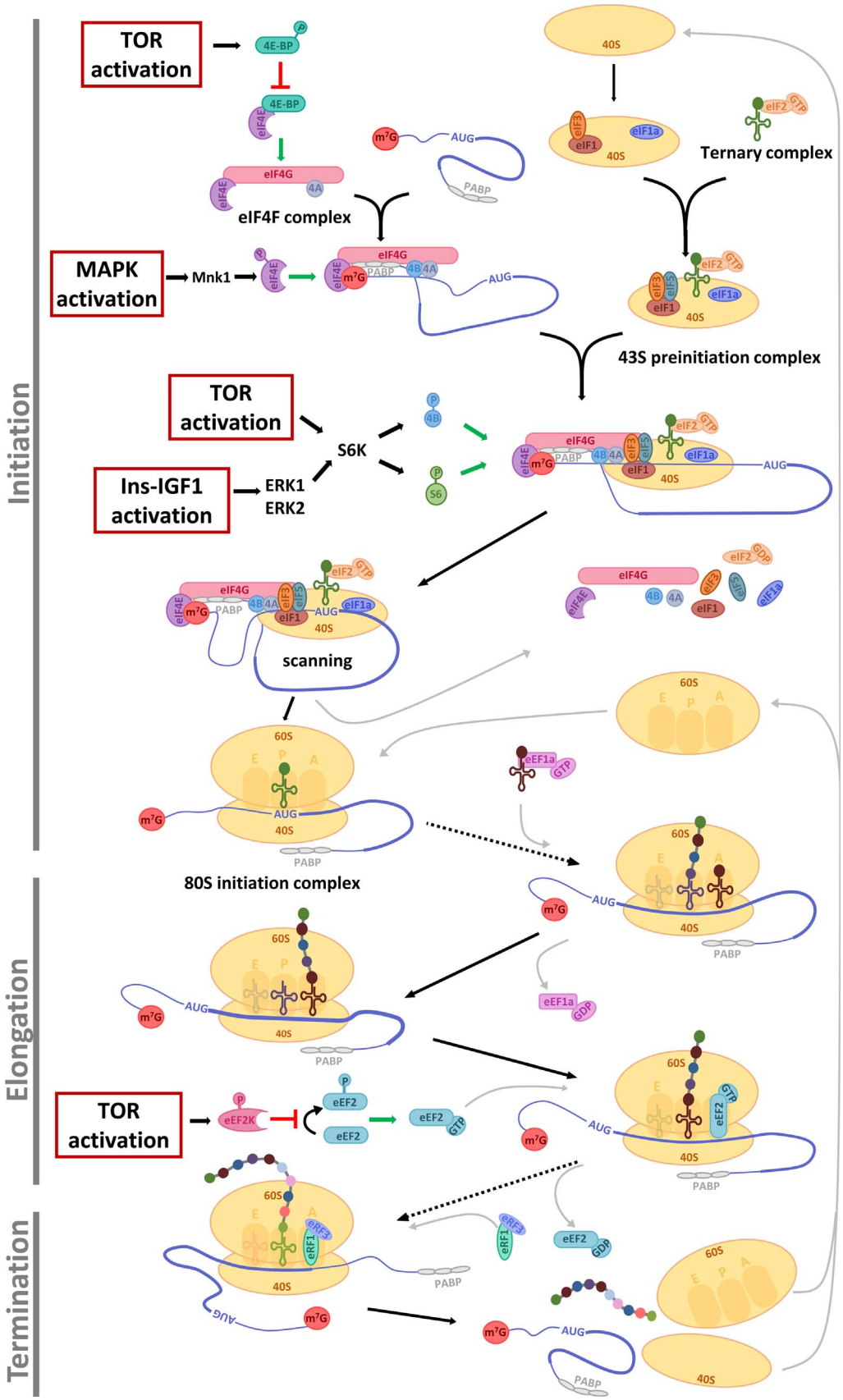

Fig. 1. Aging signaling pathways regulate eukaryotic mRNA translation. Schematic overview of the initiation, elongation and termination phases of eukaryotic protein biosynthesis. Green arrows indicate positive (stimulatory) regulation. Red bar lines indicate negative (inhibitory) regulation. TOR signaling stimulates translation initiation *via* phosphorylation of 4E binding protein that causes its dissociation from eIF4E and eIF4F complex formation. TOR phosphorylates and deactivates eEF2 kinase, and thus promotes the translation elongation. Activation of the extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) through Ins-IGF1 signaling together with TOR stimulate S6 kinases (S6K), which phosphorylate the small ribosomal subunit protein S6 and eIF4B enhancing translation initiation. Upon MAPK activation kinase Mnk1 promotes eIF4E phosphorylation and thus stimulates translation initiation. See text for more details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

targets of TOR signaling are the S6 kinases S6K1 and S6K2 (Fig. 1). Activation of TOR leads to activation of both kinases S6K1 and S6K2 which subsequently phosphorylate several components of the translation machinery including 40S ribosomal protein S6, eIF4 B and eEF2 kinase (Showkat et al., 2014).

1.2. MAPK

The p38 protein kinase is one of the four main sub-groups of the mitogen-activated protein (MAP) kinases. Like all MAP kinases, p38 kinases are activated by MAP kinase kinases (MKKs) such as MKK3 and MKK6. Several studies demonstrated that activation of MKK6 and MKK3 led to cell cycle arrest and premature senescence in p38 kinase-dependent manner, demonstrating involvement of the p38 MAP kinase pathway in aging (Zarubin and Han, 2005). Under stress conditions MAPK activates the mitogen-activated protein kinase-interacting kinase Mnk1. Mnk1 is a member of the eIF4F complex and it binds directly to eIF4G (Fig. 1). After activation through p38 kinase, Mnk1 phosphorylates eukaryotic initiation factor 4E (eIF4E) at S209 (Waskiewicz et al., 1999). The phosphorylation of eIF4E by Mnk1 might stabilize its interaction with eIF4G and 5′ cap of mRNA, enhancing in such a way translation initiation (Pyronnet, 2000).

1.3. Ins-IGF-1

Reduced insulin/IGF-1 signaling by mutation of different components consistently extended the lifespan of worms, flies, and mice (Johnson et al., 2013). Activation of insulin–insulin growth factor 1 pathway through the insulin receptor results in the activation of a downstream cascade of kinases. One of the activated kinases Akt activates the TOR signaling pathway. The extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2), activated in respond to Ins-IGF-1 signaling, stimulate the S6 kinase (S6K), which phosphorylates the small ribosomal subunit protein S6 (Fig. 1). Besides S6K phosphorylation, ERK1 and ERK2 together with p38 MAPK activate Mnk1 that stimulates translation initiation (Tavernarakis, 2008).

2. Translation inhibition and lifespan extension – is there a causal link?

The observed links between aging and translation control raises the question if down-regulation of protein biosynthesis is a causative agent of aging or if it is the consequence of physiological changes accompanying aging. A number of studies in different model organisms including yeast, nematodes and the fruit fly demonstrated that specific deletion of different components of the translational machinery and thus attenuation of protein biosynthesis significantly increase their lifespans.

2.1. Ribosomal proteins

Systematic analysis of yeast strains with single gene deletions identified several genes that significantly increase replicative life span. Among them were deletions of ribosomal proteins (r-proteins) rpl3, rpl6b, rpl10, rps6, and rps18 (Chiocchetti et al., 2007; Kaeberlein et al., 2005). Since the majority of ribosomal proteins in yeast are present in paralogs and to ensure that the phenotypes are not rescued by the paralogous gene copy, a set of yeast strains lacking both gene copies were constructed. In this study eleven new long-lived r-protein deletions were identified (Steffen et al., 2012). Similarly, in *C. elegens* depletion of six r-proteins of the small subunit and five of the large subunit by siRNA-mediated knockdown reduced the level of translation and concomitantly extended the lifespan (Hansen et al., 2007). Thus genetic evidence indeed supports the view that stripping the ribosome from particular r-proteins affects the rate of protein biosynthesis and modulates the lifespan.

2.2. Initiation factors

Screening of 2700 genes in the nematode *C. elegens* by siRNA-mediated knockdown revealed that inhibition of translation initiation factors increased lifespan up to 50% (Curran and Ruvkun, 2007). In *C. elegens* eIF4E is represented by five isoforms, termed IFE-1 – IFE-5. Three of them, IFE-1, IFE-3 and IFE-5, are expressed in the germline, whereas IFE-2 and IF-4 are expressed specifically in somatic cells (Rhoads et al., 2006). Depletion of the specific eIF4E isoform IFE-2, that functions in somatic tissues, reduces global protein synthesis and extends the lifespan (Hansen et al., 2007; Syntichaki et al., 2007). Inhibition of the translation initiation factors eIF2 and eIF4G significantly extended lifespans in nematodes (Chen et al., 2007; Hansen et al., 2007; Pan et al., 2007). In *D. melanogaster* overexpression of 4E-BP, the eukaryotic translation initiation factor 4E binding protein, significantly increased life span (Zid et al., 2009). Overexpression of the initiation factor of translation (eIF-4E) induces cellular senescence in mouse embryonic fibroblasts and in B cells (Ruggero et al., 2004).

2.3. Other factors

Depletion of other factors including factors involved in ribosomal biogenesis and tRNA synthesis (Chen et al., 2007), rsks-1 (the worm homologue of S6 kinase) results in lifespan extension in *Caenorhabditis elegans* (Pan et al., 2007). In *S. cerevisiae* it was found that depletion of 60S ribosomal subunits by deleting processing factors or by diazaborine treatment leads to increased replicative life span (Steffen et al., 2008). Depletion of the methyltransferase NSUN5 in worms, fly and its yeast homolog Rcm1, which methylates cytosine within conserved region of 28S/25S rRNA, increase lifespan (Schosserer et al., 2015).

2.4. Possible reasons of reduced protein synthesis promoting longevity

The observed phenomenon that inhibition of global protein synthesis increases lifespan across species suggests several mechanisms explaining how the rate of protein synthesis might influence the process of aging. Rapid protein biosynthesis is accompanied by the production of damaged proteins due to transcriptional or translational errors and co-translational misfolding. As a result, erroneous proteins are not able to maintain their native function and have the tendency to accumulate in the cell forming potentially toxic aggregates. Accumulation of cellular damage is considered to be one of the main indications and cause for cellular aging and aging-associated diseases (López-Otín et al., 2013). Attenuation of global protein synthesis induced by the depletion of components of the translation machinery decreases protein synthesis of normal as well as damaged proteins, which as a result might reduce the risk of accumulating toxic proteins (Hipkiss, 2007).

In this context the contribution of the protein quality control system should be pointed out. Under normal conditions damaged or misfolded proteins are recognized by the protein quality control systems and are either targeted to degradation or to assisted protein folding. It is a known fact that activities of the proteolytic system implicated in protein quality control decline with age. Hence in aged cells the proteolytic system cannot deal with all the damaged proteins thus leading to their cellular accumulation (López-Otín et al., 2013). Slowdown of translation prevents production of excessive amounts of misfolded proteins and, as a result, overloading the protein quality control system. This idea is also supported by the observation that upregulation of several of chaperones in different model organism counteracts protein aggregation/misfolding and indeed extending the lifespan (Hipkiss, 2007).

Protein synthesis is one of the most energy-consuming cellular processes and it requires ~75% of the total cell's energy (Lane and Martin, 2010). Consequently reduction of mRNA translation increases overall energy availability. It may allow diversion of critical resources that can be mobilized towards cellular maintenance and repair pro-

cesses, thus promoting organism longevity (Tavernarakis, 2007). This theory is supported by the fact that depletion of ribosomal proteins and translational factors in several model organisms decreases protein synthesis and increase stress resistance and extends lifespan (Tavernarakis, 2008)

3. Translation during aging

Bulk protein synthesis slows down during aging in a wide variety of cells, tissues, organs and organisms. This was confirmed by a number of studies where protein synthesis was monitored by the *in vitro* incorporation of radioactively labeled amino acids using cell-free extracts derived from different organisms. In the majority of these studies protein synthesis decreased significantly (from 20 to 75%) with increasing age (Ward and Richardson, 1991). Moreover several groups could confirm a significant (around 50%) decrease in protein synthesis using suspensions of mice and rat hepatocytes (Ward and Richardson, 1991). Purified liver ribosomes from old *versus* young mice showed lower poly (U) translational activity (Mori et al., 1979). Up to two fold decrease in the elongation rate was also observed *in vivo* in rodent liver and in the brain cortex with age (Rattan, 1996). The *in vitro* assay with natural mRNA confirmed previously observed data that the activity of mouse liver ribosomes decreased with age in cell-free translation assays. Particularly, the activity for formation of the initiation complex of 40S ribosomal subunits in the livers of old mice was found to be 15–20% lower compared to young ones (Nakazawa et al., 1984). The amount and activity of translation factors is known to decline with age, which also contributes to the reduction of protein synthesis rates (Takahashi et al., 1985; Kimball et al., 1992)

Experimental data furthermore revealed change in amino acid misincorporation into proteins with age. Using mRNA of the tobacco mosaic virus coat protein (CcTMV) for translation by cell extracts prepared from young or old human fibroblasts, a sevenfold increase in cysteine misincorporation during cellular aging was observed. Furthermore, the aminoglycoside antibiotic paromomycin, a drug known to reduce ribosomal accuracy during decoding, induced more errors during the translation of CcTMV coat protein mRNA by cell extracts prepared from senescent human fibroblasts than those from young human fibroblasts (Luce and Bunn, 1989).

4. Translation under oxidative stress

According to the free radical theory of aging postulated by Denham Harman in 1956, aging and age-related diseases are the result of the accumulation of oxidative damage over time. Reactive oxygen species are produced during normal metabolism as by-product of aerobic metabolism. Oxidative stress occurs as a result of an imbalance between reactive oxygen species production and intracellular antioxidant factors. As oxidative stress arises from many unavoidable sources and is harmful to all biological components, cells have developed strategies to disable reactive oxygen species (ROS) by both enzymes and small molecules. Oxidation of DNA has been studied with increasing molecular insight over the past 40 years (Cooke et al., 2003). On the other hand, research on RNA oxidative damage has only gained attention more recently (reviewed in Küpfer and Leumann, 2014). Especially oxidative damage by ROS on transcripts with longer half-lives, such as tRNAs and rRNAs (t1/2 in the range of several hours to several days), appear to be an attractive field of research. Oxidative damage contributes to many age-related diseases including cancer, diabetes, Alzheimer's disease and cardiovascular disease. Accumulating evidence links oxidized RNA to a variety of diseases, particularly age-related degeneration such as Alzheimer's disease (AD), Parkinson disesase and amyotropic lateral sclerosis (ALS) (Poulsen et al., 2012). For example, oxidative stress in the brain leads to a decline in protein biosynthesis in early AD stages. Examination of AD patients brain revealed unaltered amounts of polyribosomes compared to control subjects, but their translation activity was dramatically reduced (Ding et al., 2005). Thus it can be hypothesized that weakening of protein synthesis, mediated by oxidative stress obstructing the ribosome by damaging rRNA (and likely also mRNA), contributes to impaired neuronal function and the neuropathology of AD and other age-related diseases. Indeed, the overall contribution of oxidation on dimming the speed of translation observed in age-associated diseases was confirmed by *in vitro* translation experiments with oxidized ribosomes from rabbit reticulocyte lysates (Wickens, 2001). Exposure of *S. cerevisiae* to H_2O_2 also reduces general protein biosynthesis through inhibition of translation initiation. However microarray analysis demonstrated that certain mRNAs such as those coding for stress response were enriched in polysomal fractions after oxidative stress (Shenton et al., 2006). While the possible causal link between oxidative stress, translation inhibition and the onset of age-related diseases is intriguing, the molecular consequences of rRNA oxidation on ribosome functions and protein synthesis have yet to be uncovered in detail.

5. Selective translation

Decline of global translation is often accompanied by a switch to the selective translation of mRNAs coding for proteins that are required for cellular maintenance, repair and turnover pathways. Similarly age-related translation attenuation does not mean inhibition of the production of each and every protein, since some proteins are needed to be upregulated for damage repair and cellular maintenance. Several studies have shown association of increased life-span with enhanced translation of specific mRNAs despite the decreased global protein synthesis (Zid et al., 2009; Rogers et al., 2011). Also several proteome studies revealed differences in protein composition with age and showed upregulation of age-associated proteins including proteins involved in energy metabolism, proteostasis, cell cycle, response to stress signal transduction, and apoptosis (Li et al., 2007; Yang et al., 2008; Waldera-Lupa et al., 2014). Moreover expression of 77% of the age-associated proteins were not linked to expression of the corresponding transcripts demonstrated that the changes of age-associated proteins detected at the proteome level are caused by post-transcriptional mechanisms, including selective translation (Waldera-Lupa et al., 2014). The most intensely studied example of selective translation during aging is the overproduction of the transcription factor Atf4 in mammals and its yeast orthologue Gcn4. Levels of both proteins were found to be altered in model organisms with age, in mutants with increased life-span including deletions of ribosomal proteins (Steffen et al., 2008), and under conditions increasing life-span such as rapamycin treatment and dietary restriction (Li et al., 2014). Repression of Gcn4/Atf4 under basal condition and upregulation under stress/age is mediated by structural features of the four small upstream open reading frames in the 5′ region of the GCN4/ATF4 mRNA (Spriggs et al., 2010). Elevation of Atf4 results in activation of Atf4-dependent genes, which are involved in stress resistance and life-span extension (Li et al., 2014). Another example of selective translation occurring during aging is the translation of at least two specific subsets of mRNAs under TOR regulation. Since mTOR regulates phosphorylation of 4E-BP and, as a consequence, triggers the release of eIF4E, inhibition of mTOR promotes translation of "eIF4E-sensitive" mRNAs. Those mRNAs typically have long and structured 5′ UTRs and they are usually implicated in cell survival and proliferation. The second known subset of TOR-regulated mRNAs originates from genes containing a specific *cis*-regulatory element in their 5′ UTR, called 5′ terminal oligopyrimidine tract (5′ TOP). These mRNAs encode for components of the translational machinery including all ribosomal proteins, elongation factors and a few other translational regulators. Translation of 5′ TOP mRNA is repressed under normal condition and is upregulated under stress and growth conditions through mTOR signaling (Nandagopal and Roux, 2015). Selective translation was also shown to be implicated in *Drosophila* development (Marr et al., 2007; Ferguson et al., 2012).

Under nutrition limitation, when cap-dependent translation is blocked as a result of eIF4E inactivation by 4E binding protein, a certain population of mRNAs, evidently involving IRES elements in the 5′ UTRs, is selectively translated to ensure correct development.

6. Age-related translation regulation by non-coding RNAs

Recent studies on the parallel quantification of transcriptomes and proteomes in mammalian cells came to quite opposite conclusions (Li and Biggin, 2015). Some reports provide evidence that protein levels are primarily regulated by mRNA abundance and thus by the rate of transcription (Battle et al., 2015; Jovanovic et al., 2015) while others came to the exact opposite conclusion (de Souza, 2011; Schwanhäusser et al., 2011; Zhang et al., 2014). The latter studies demonstrated a poor correlation between the concentration of the majority of proteins and abundances of their corresponding mRNA. While being a currently controversially debated field of molecular biology, the majority of scientists probably agree that regulation of gene expression at the post-transcriptional level significantly contributes to protein homeostasis and stress response.

In the last decade non-protein-coding RNAs (ncRNAs) have been increasingly associated in various reports with translation regulation. In fact it has been speculated that the increased complexity of multi-cellular organisms rely, at least in part, on the establishment of sophisticated regulatory gene expression networks utilizing ncRNAs (Mattick, 2004). In particular microRNAs (miRNA) have emerged as potent regulators of gene expression at different levels during aging and in age-associated diseases. miRNA base pairing with their target mRNA at the 3′UTR leading to mRNA degradation and/or translational repression (Bartel, 2004). Level of age-associated miRNA (gero-miRNA) vary with age from nematode to human. Gero-miRNA target mRNAs of different signaling pathways implicated in aging and a number of them were shown to modulate longevity in several model organisms (Garg and Cohen, 2014). Another class of ncRNAs, called long non-coding RNAs (lncRNAs) is represented by transcripts heterogeneous in their size, origin and functions (Grammatikakis et al., 2014). LncRNA appear to play important role in regulation of gene expression at transcriptional, post-transcriptional and post-translational levels. Thus lncRNA are emerging candidates for modulating a variety of cellular processes underlying the aging and development of age-associated diseases (Kim et al., 2016).

7. The specialized ribosome concept

Regulation of gene expression at the post-transcriptional level has mostly been attributed to the mRNA features or a variety of factors that target mRNA including miRNA and lncRNA. The ribosome has been traditionally viewed as unchangeable molecular machine that has to be constantly equipped with the entire complement of r-proteins, rRNA or r-protein modifications. Furthermore it has been strongly assumed that each ribosome in the cell carry the exact same set of rRNA molecules to precisely accomplish protein translation and thus the ribosome itself has for a long time never been implicated in contributing to selective translation regulation. This concept was challenged over the last few years by studies that suggest the formation of distinct ribosomal subpopulations with unique molecular compositions, properties and functions (Xue and Barna, 2012). According to the concept of specialized ribosomes, the translational apparatus can adapt its molecular composition and three dimensional structure in response to various stimuli allowing these “specialized” ribosomes to selectively translate a specific set of mRNAs. This concept would neatly explain how the proteome could be modulated according to the cellular needs. Sources for ribosome heterogeneity might originate from modulating the composition of r-proteins paralogs, post-synthetic modification of r-proteins or rRNAs, or by the binding to distinct ribosome-associated factors (Xue and Barna, 2012; Filipovska and Rackham, 2013).

While the concept of specialized ribosomes contributing to proteome modulation and stress response is tempting, conclusive experimental evidence is still sparse. Probably the most convincing case on the existence of specialized ribosomes contributing to specialized protein production has been reported in *E. coli*. It has been shown that the endonuclease MazF from the MazE-MazF toxin-anti toxin pair cleaves off a 43 nucleotide fragment from the 3′end of 16S rRNA within the 30S ribosomal subunit (Vesper et al., 2011). This truncated 16S rRNA lacks the anti-Shine-Dalgarno sequence typically required for bacterial translation initiation on canonical mRNAs. These “stressed ribosomes” formed upon MazF-mediated cleavage selectively translate leaderless mRNA that lack the canonical Shine-Dalgarno mRNA sequences (Vesper et al., 2011; Moll and Engelberg-Kulka, 2012). Whether or not such truncated and thereby “specialized ribosomes” contribute to proteome modulation during physiological conditions in *E. coli* still awaits experimental support (Sauert et al., 2016).

Proteomic analysis of ribosome in different model organisms revealed ribosome heterogeneity within the r-protein complement, suggesting their possible role in modulation of ribosome function (Deusser, 1972; Deusser and Wittmann, 1972; Bickle et al., 1973; Giavalisco et al., 2005; Komili et al., 2007; Lopes et al., 2010). Deletion or mutation of Rpl38, a protein of large ribosomal subunit, leads to a pronounced phenotype in the mouse exhibiting skeletal patterning defects. Rpl38 depletion does not change global protein synthesis, however it suppresses the translation of a specific set of mRNAs, namely the Hox mRNA (Kondrashov et al., 2011). Another example uncovers the contribution of rRNA methylations in ribosome heterogeneity. It was reported that upregulation of the methyltransferase fibrillarin in a p53-dependent manner alters the pattern of rRNA methylation. This consequently leads to the formation of a distinct group of ribosomes with decreased translational fidelity and selectively increased translation of the IRES-containing mRNA (Marcel et al., 2013).

If specialized ribosomes are also at work during aging and cellular stress in mammalian cells and organisms is still unclear. However first reports emerged suggesting that “specialized ribosomes” might indeed be involved in shaping the proteome in senescent cells. A recent study by Schosserer et al. showed that the lack of a single conserved C5 methylation of 25S rRNA at position C2278 (*S. cerevisiae* nomenclature) extends the lifespan and stress resistance of yeast, worms and flies (Schosserer et al., 2015). The lack of this particular methylation at C2278 locally alters the ribosome structure which has so far unexplained molecular consequences on translational fidelity and selective mRNA recruitment. It has been suggested in that study that the lack of C2278 modification results in a ‘reprogramming’ of the ribosome towards translation of mRNAs involved in cellular stress-response and aging (Gigova et al., 2014; Schosserer et al., 2015).

8. RancRNA

Besides the canonical components of the translational machinery, ribosomes can also be directly regulated by ncRNA. Several studies showed that numerous small and long ncRNAs are bound to the ribosome (Pircher et al., 2014b; van Heesch et al., 2014). These ribosome-associated ncRNAs, also referred to as rancRNAs, have been identified as novel class of translation regulators capable of modulating protein biosynthesis on a global level in a stress-dependent manner (Gebetsberger et al., 2012, 2016; Pircher et al., 2014a). In these studies it was shown that tRNA- or mRNA-derived fragments bind the small or large ribosomal subunits, respectively, and regulate translation in an mRNA independent manner. As a consequence of this direct ncRNA-ribosome interaction the global rates of protein synthesis decreased *in vitro* and *in vivo* during particular stress conditions in an archaeal (*H. volcanii*) or eukaryal (*S. cerevisiae*) model system. RancRNAs do in principle also have the potential of stimulating protein synthesis of specific mRNAs, however so far conclusive experimental evidence is missing. However, we have recently identified specific tRNA half

molecules that seem to stimulate protein production in yeast cells and in *Trypanosoma brucei* (unpublished data). Based on the findings that global translation attenuation can be mediated by short rancRNAs, it is possible, but by no means proven, that rancRNAs can also contribute to age-related changes on the rate of translation. Since rancRNAs appear to be abundant and present in all three domains of life (Zywicki et al., 2012; Pircher et al., 2014b) it is tempting to speculate that this emerging class of ncRNA regulators is also involved in either generating "specialized ribosomes" or in globally attenuating protein biosynthesis during aging or stress response.

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