Ageing Research Reviews 23 (2015) 75–89

Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review

Protein synthesis as an integral quality control mechanism during ageing

Nikolaos Charmpilas[a,c,1], Ioanna Daskalaki[a,c,1], Margarita Elena Papandreou[a,b,1], Nektarios Tavernarakis[a,b,*]

[a] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion 70013, Crete, Greece
[b] Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion 70013, Crete, Greece
[c] Department of Biology, University of Crete, Heraklion 70013, Crete, Greece

ARTICLE INFO

Article history:
Received 7 November 2014
Received in revised form
18 December 2014
Accepted 22 December 2014
Available online 30 December 2014

Keywords:
Insulin/IGF-1 signalling
mRNA translation
Non-coding RNAs
Ribosomes
RNA binding proteins
TOR signalling

ABSTRACT

Ageing is manifested as functional and structural deterioration that affects cell and tissue physiology. mRNA translation is a central cellular process, supplying cells with newly synthesized proteins. Accumulating evidence suggests that alterations in protein synthesis are not merely a corollary but rather a critical factor for the progression of ageing. Here, we survey protein synthesis regulatory mechanisms and focus on the pre-translational regulation of the process exerted by non-coding RNA species, RNA binding proteins and alterations of intrinsic RNA properties. In addition, we discuss the tight relationship between mRNA translation and two central pathways that modulate ageing, namely the insulin/IGF-1 and TOR signalling cascades. A thorough understanding of the complex interplay between protein synthesis regulation and ageing will provide critical insights into the pathogenesis of age-related disorders, associated with impaired proteostasis and protein quality control.

Contents

* Corresponding author at: Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion 70013, Crete, Greece. Tel.: +30 2810 391066; fax: +30 2810 391067.
E-mail address: tavernarakis@imbb.forth.gr (N. Tavernarakis).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.arr.2014.12.008
1568-1637/

1. Introduction

Proteins have been selected as the main executor biomolecules utilized by the cellular arsenal in diverse organisms, ranging from unicellular prokaryotes to complex multicellular eukaryotes. Approximately half of the total energy output of the cells is consumed for the translation process while specialized systems, for instance, the methionine sulfoxide reduction system (Kim, 2013) and the thioredoxin antioxidant system (Lu and Holmgren, 2014), have evolved to remove oxidative damage existing in polypeptides. Additively, autophagy and the proteasome machinery constantly survey for aggregated or dysfunctional proteins, subsequently eliminating them. Hence, a milieu of specialized factors is dedicated to the preservation of proteostasis per se, illustrating once again the importance of maintaining the protein repertoire intact, devoid of any sort of damage. In parallel, the need for maintaining cellular homeostasis dictates that at any given time the protein synthesis rate should be coupled with protein degradation rhythms. When this balance is perturbed, defective or damaged proteins may accumulate progressively, ultimately leading to a detrimental state and even cell death (Hipp et al., 2014). Considering the importance of the proteome integrity for cellular functions, it is not surprising that the loss of proteostasis is tightly linked with the onset of ageing and neurodegenerative disease and is classified as a typical hallmark of ageing (López-Otín et al., 2013).mRNA translation is a cytoplasmic cellular process which is affected by age. It involves a series of complex reactions, namely the initiation which involves multiple steps followed by the elongation and the termination phase. Elucidating the mechanisms by which translational components function will allow a more profound understanding of their contribution to the ageing process. Here, we review the regulatory mechanisms of the translation initiation phase as well as the role of ribosomal proteins, ribosome biogenesis and structure in whole organism longevity. Dysfunction of mitochondria including altered structure, mitochondrial gene expression and metabolism is a characteristic of ageing (Gomes et al., 2013). However, interestingly enough, the communication between cytoplasmic translation and this organelle is also crucial; thus the interaction between mitochondrial function and cytosolic protein synthesis will also be discussed in the context of the ageing process.

Even at the pre-translation level multiple regulatory mechanisms are utilized so that the process of protein synthesis is tightly controlled both spatially and temporally. Dysregulation of protein synthesis surveillance mechanisms at either the pre-translational or translational level has been related to ageing and disease onset (Tavernarakis, 2007; Yoon et al., 2006). From DNA replication to functional protein folding, a pleiad of molecules with distinct functions cooperate in multiple regulatory networks. Studies so far have partially shed light on several of these factors. Specifically, after DNA transcription and before translation initiation, non-coding RNAs and RNA binding proteins (RBPs) function to regulate translation. Intriguingly, recent studies highlight that the endogenous properties of RNA molecules change with age, raising the question of whether artificial intervention on intrinsic RNA properties could either delay or accelerate ageing.

Genomic studies show that in contrast to the high percentage of the human genome that is being transcribed, fewer than 2% of the produced RNA molecules are finally translated, producing functional proteins (Kapranov et al., 2007). Relative to their coding potential, RNA molecules are divided into two broad categories: the coding and the non-coding RNAs. Non-coding RNAs are further subdivided into the small and long non-coding RNAs. Both are exponentially attracting the interest of the scientific society as they have been shown to play key roles in fundamental cellular processes such as DNA and mRNA regulation as well as protein synthesis control, thus affecting a variety of complex biological processes such as tissue differentiation, development, cellular senescence, ageing and disease onset (Abdelmohsen et al., 2013; Gong and Maquat, 2011; Kretz et al., 2013; Lovat et al., 2011; Smith-Vikos and Slack, 2012; Wang et al., 2008; Wolfson et al., 2009). Some of their endogenous properties such as 2-D and 3-D folding, modified forms; such as methylated or oxidized ones, are implicated in disease onset and cell physiology disruption (Halvorsen et al., 2010; Poulsen et al., 2012; Squires et al., 2012; Wang et al., 2014). The RBPs in concert with non-coding RNAs play a significant role in the regulation of protein synthesis at a post-transcriptional level and more specifically in mRNA homeostasis. This multi-layered regulation of protein synthesis is of high importance for the regulation of the ageing process (Rattan, 2010; Schimanski and Barnes, 2010). RBPs are capable of either repressing or activating protein synthesis by binding to specific mRNAs, while some of them execute both. Moreover, it is evident that each one of them can bind to multiple targets (Lebedeva et al., 2011). Besides mRNAs, their targets include transcription factors and other RBPs or proteins that modulate ageing, even if knowledge in this field is still constrained.

Ageing is manifested by the progressive decline in cellular functions, compromising tissue functionality and finally causing fatality. The prevailing theory was that ageing is a stochastic and passive process accelerated by the accumulation of irreversible damage in vital macromolecules. However, it has nowadays become evident that the onset of ageing is tightly regulated by conserved signalling pathways, mainly the TOR and the insulin/IGF-1 (Gems and Partridge, 2013). Protein synthesis, and especially translation initiation, is a rather reasonable node of interaction with ageing-determining pathways, as it essentially enables the replenishment of defective or damaged proteins with fresh, intact ones. Intriguingly, it is the attenuation of protein synthesis, instead of its enhancement, that has beneficial effects on lifespan probably via shifting valuable energy resources from anabolism to repair and the activation of stress-response mechanisms. Concomitantly, depletion of specific amino acids extends chronological lifespan of yeast cells and delays senescence of mammalian cell cultures (Johnson and Johnson, 2014; Kozieł et al., 2014). These findings are consistent with the theory of antagonistic pleiotropy, which postulates that genes which accelerate ageing are maintained in the population because of their requirement in early developmental stages (Parsons, 2007). In this review, we survey the link between protein synthesis quality control mechanisms and the ageing process.

2. Protein synthesis quality control

2.1. mRNA translation initiation

The most tightly controlled step of translation is the initiation phase when the ribosomes initiate translation of the messenger RNA (mRNA) in the 5′ to 3′ direction by pairing of the mRNA initiation codon with the anticodon loop of initiator tRNA. Specifically, the 43S pre-initiation complex, comprised of 40S ribosomal subunit and several initiation factors, is formed to unwind the RNA secondary structure; this is a scanning mechanism invoking several core and auxiliary initiation factors. Alternatively, translation of certain mRNAs can start from the internal ribosome entry sites (Jackson et al., 2010). The pre-initiation complex then attaches to the mRNA and scans the 5′ UTR, followed by recognition of the initiation codon. Initiation is modulated by mechanisms which affect either the eukaryotic initiation factors (eIFs) and the ribosomes or the mRNA itself through ribosome binding proteins (RBPs) and microRNAs (miRs).

Translation initiation control has been directly linked to the ageing process. It has been well established that reduced protein synthesis, albeit reducing growth rate and development, extends

adult lifespan in *Caenorhabditis elegans*. Firstly, a homologue of the somatic eukaryotic initiation factor 4E (eIF4E) in *C. elegans*, IFE-2 isoform, a major modulator of translation, binds the 7-monomethyl guanosine cap at the 5′ end of nuclear mRNAs and protects against oxidative stress. Genetic inhibition of *ife-2* can extend lifespan (Syntichaki et al., 2007). Under hypoxia, this capping is repressed by eIF4E sequestration, and translation initiation at the internal ribosome entry site cannot explain the translation levels detected. Recently, an alternative oxygen-regulated translation initiation complex has been proposed. eIF4E2 together with RBM4 and hypoxia-inducible factor 2alpha complex are recruited by RNA hypoxia response element and capture the 5′ cap of a pleiad of mRNAs to polysomes for translation (Uniacke et al., 2012).

Eukaryotic translation initiation factor 4G (eIf4G), which is downregulated during starvation, alters the expression of developmental and lifespan genes. Moreover, suppression of *ifg-1* which encodes the *C. elegans* orthologue of eIF4G triggered ribosomal loading increase and expression of stress response genes required for longevity (Rogers et al., 2011). Translational inhibition due to stress has mainly been described during the initiation phase, through the phosphorylation of the alpha subunit of eIF2 at serine 51. This allows cells to protect themselves from oxidative stress and reactive oxygen species (ROS) while simultaneously avoiding senescence (Rajesh et al., 2013). On the contrary, when the mechanism of translational inhibition is defective, specifically in the absence of phosphorylated eIF2α, namely eIF2αP-deficient tumour cells, it causes increased sensitivity to DNA damage and increased ROS production leading to premature senescence (Rajesh et al., 2013).

2.2. Ribosome quality control

Ribosomes are not merely ribozymes but they themselves can act as a protein quality control gate. Ribosomes can be composed of different subunits which include different rRNAs, ribosomal proteins which are differentially translated and are prone to post-translational modifications, as well as different ribosome-associated proteins (Xue and Barna, 2012). Their role in the ageing process can only be delineated under the scope of their aforementioned specificity and their protein interaction variation. One of the first intriguing observations was that reduced translation by suppressing 60S ribosomal subunit expression or nutrient deprivation delay the ageing process in yeast, *C. elegans* and *Drosophila melanogaster* (Hansen et al., 2007; Steffen et al., 2008). This was found to be triggered by a nutrient-responsive transcription factor, GCN-4.

At the transcriptional level, budding yeast ribosomal protein expression is regulated by the transcriptional coactivator Ifhp1 together with which the expression of a battery of other genes such as GCN-4 and translation elongation factors is activated (Cai et al., 2013). This coactivator is regulated according to the growth state of the cells by acetylation in response to intracellular acetyl-CoA levels, and phosphorylation due to TORC1 signalling. These nutrients sensing mechanisms do not seem to alter cell growth but instead the former regulates the stability of proteins while the latter, the organism's replicative lifespan. Concerning ribosomal chaperones, emerging evidence has demonstrated their importance both in prokaryotes and eukaryotes in regulating translational activity especially under cellular stress conditions. Proteotoxic insults trigger a negative feedback loop specifically by post-translationally regulating ribosome function. A significant reduction of protein synthesis has been observed during ageing and in response to proteotoxic stress in *C. elegans*. Specifically, a ribosome-associated chaperone complex which contributes to this fine-tuning is the nascent polypeptide-associated complex, NAC, which links the translational machinery, the ribosomes, with folding of newly synthesized proteins (Kirstein-Miles et al., 2013; Preissler and Deuerling, 2012). Most importantly, NAC is able to reversibly dissociate from the ribosomes, reducing the amount of polysomes and thus translational activity, while simultaneously supporting the resolubilization of misfolded proteins under acute stress conditions. Thus, ribosomes themselves are central for protein synthesis quality control in terms of sensing the nature of the polypeptide chain and engaging transport and protein folding components. However, during ageing, chronic stress conditions or constitutive expression of protein aggregates such as Aβ and polyQ, there is irreversible sequestration of these chaperones leading to a halt in ribosomal activity (Ben-Zvi et al., 2009; Kirstein-Miles et al., 2013). These studies underscore that mRNA translation is restricted to a specific age time frame, and thus it can compensate for accumulation of misfolded proteins after a certain age.

On a similar note, acute proteotoxic stress or heat shock has been recently proposed to cause ribosomal pausing during elongation. Specifically ribosomes pause early during the elongation phase where nascent polypeptide chains exit the ribosomal tunnel by detecting intracellular protein folding and homeostasis (Liu et al., 2013). Translocation from the interior of ribosomes to the cytoplasm is significantly aided by ribosome-associated chaperones, namely Hsc/Hsp70, whose availability becomes limited during proteotoxic stress, pausing ribosomes on the first fifty codons of the transcript, thus reducing translational rate (Liu et al., 2013; Shalgi et al., 2013). It is further implicated that ribosomal pausing during elongation occurs before attenuation of translational initiation which has been considered to be the main modulator stage for protein synthesis. The mechanism of ribosomal pausing after initiation and before the end of translation was recently verified under physiological conditions (Han et al., 2014). Specifically, ribosomes were shown to pause after initiation independently of a specific sequence, but rather due to the geometry of the loop region of the exit tunnel. Indeed, mutations in this loop region cause this ribosome variant to abandon translation. Thus, post-initiation ribosomal pausing could be an intrinsic regulatory mechanism to ensure proper translation.

Furthermore, the ribosomal exit site has another quality control function in order to prevent proteotoxicity due to secretory protein misfolding. Specifically, defective protein mRNA translation is prevented by the regulation of aberrant protein production (RAPP) mechanism when the signal recognition particle (SRP) at the ribosomal exit site is unable to associate with mutant N-terminal signal sequences. Instead, the mutant nascent chain comes in close proximity to the ribosome-interacting protein Argonaute 2 (Ago2), which in turn directs the respective transcripts for degradation (Karamyshev et al., 2014). Importantly, SRP is essential for this process as its absence causes mRNA degradation of otherwise wild type secreted proteins.

Other mechanisms which affect ribosome functioning include the circadian clock, which affects temporal transcription and subsequent translation of genes involved in ribosome biogenesis regulation (Jouffe et al., 2013). This is achieved by modulating the expression and activation of initiation factor of the translational machinery as well as activation of signalling pathways regulating these factors. In parallel, disrupting the circadian rhythm has been shown to accelerate ageing (Dubrovsky et al., 2010). There are also mechanisms which are implicated in the temporal and spatial restriction of translation, such as the localization of the ribosomes (Besse and Ephrussi, 2008). For instance, local mRNA translation in the axons of neurons affects axon guidance, survival, regeneration; while its dysregulation has been recently suggested to cause neurological disease (Shigeoka et al., 2013). Recently, tissue-specific brain ageing, neurodegeneration due to affected ribosomes and translational efficiency, has been revealed. Ablation of a CNS-specific nuclear encoded tRNA caused ribosomal pausing

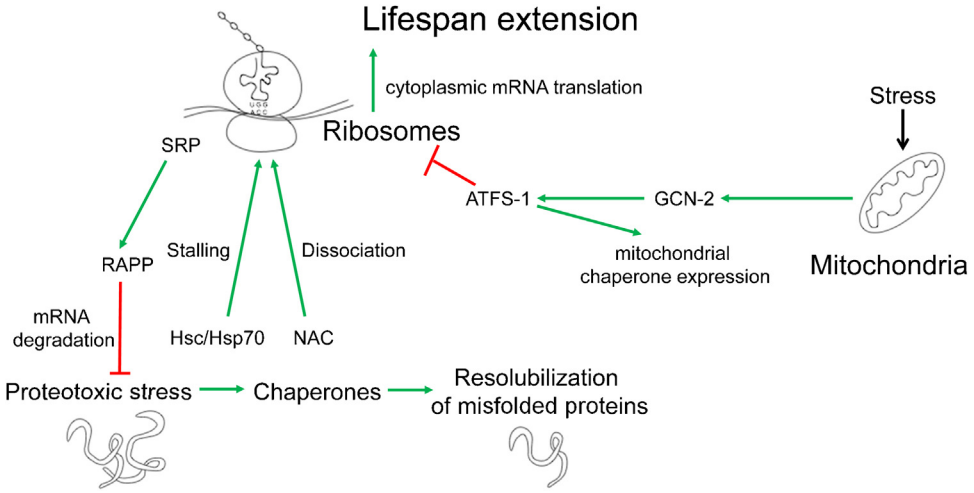

Fig. 1. The ribosome is a major protein quality control hub during mRNA translation. The signal recognition particle at the ribosomal exit site is unable to interact with mutant N-terminal sequences and initiates the RAPP (regulation of protein production) mechanism allowing the mutant nascent chain to be recognized by Argonaute which recruits the respective mRNAs for degradation, preventing proteotoxic stress. If proteotoxic stress cannot be avoided, a ribosome-associated chaperone complex, the nascent polypetide-associated complex (NAC), dissociates from the ribosomes, reducing the amount of polysomes while aiding the resolubilization of misfolded proteins. Moreover, upon proteotoxicity, ribosome-associated chaperones Hsc/Hsp70 availability becomes limited; these proteins aid the polypeptide transition from the interior of the ribosomes to the cytoplasm. This causes ribosomal pausing reducing the overall translational rate, thereby extending lifespan. GCN-2 functions in a complementary fashion to the ATFS-1 response pathway during mitochondrial dysfunction, mitochondrial unfolded protein response, via triggering expression of mitochondrial chaperone genes.

and neurodegeneration whose effect was only rescued by overexpression of GTBP2 which associates with the ribosomal protein Pelota (Ishimura et al., 2014).

2.3. Mitochondrial function and cytosolic mRNA translation

Although mRNA translation is cytoplasmic, mitochondrial proteins have also been implicated in the modulation of this process. More specifically, a recent study which categorized long-lived yeast strains based on their oxidative, thermal, ER, DNA damage stress response expression profile, indicated that yeast mutants for mitochondrial protease Afg3 display a similar expression profile with large ribosomal subunit protein mutants (Delaney et al., 2013). Interestingly enough, these mutants depicted lower levels of mRNA translation and Gcn-4-dependent lifespan extension similar to the long-lived large ribosomal subunit gene deletion mutants. In *C. elegans*, GCN-2 is the eIF2alpha kinase homologue controlling cytoplasmic translation. This protein functions in a complementary fashion to ATFS-1-dependent response pathway both during development and upon mitochondria dysfunction (Baker et al., 2012). ATFS-1 controls the transcription of mitochondria chaperone genes when the mitochondrial unfolded protein response is triggered (Baker et al., 2012). GCN-2 induces this pathway during mitochondrial stress and further extends lifespan of mutants with perturbed mitochondria function causing increased expression of mitochondrial chaperones. This study firstly underlies the concerted protective action of cytoplasmic protein synthesis and mitochondria chaperone expression against mitochondrial dysfunction and secondly could imply that translational attenuation by GCN-2 increases the lifespan of the nematode by providing stress resistance as indicated in Fig. 1. Thus, the interplay between cytosolic mRNA translation and mitochondrial function is crucial both under stress conditions and during ageing.

The interplay between mitochondria and cytoplasmic protein synthesis has been implicated in neurodegenerative disease. Recent evidence implicates PINK1 and Parkin proteins in mitochondrial quality control, namely in mitochondrial transport and mitophagy. Mutations in both have been linked to Parkinson's disease. Reduction of mRNA translation as well as induction of autophagy protect against neurodegeneration triggered by PINK and Parkin mutations, in *D. melanogaster* (Liu et al., 2012). On the one hand, increasing translation by S6K activation leads to aggravation of the phenotype while on the other hand, suppression led to alleviation of the symptoms. Hence, once again dampening the translational mechanism under conditions where mitochondria are dysfunctional acts as a protective compensatory mechanism.

3. Non-coding RNAs

3.1. Small non-coding RNAs

The aim of ageing research is to investigate both the molecular and genetic pathways underlying the deteriorating effect of the ageing process. Protein synthesis rates drop significantly with age and the energy left is consumed in surveying mechanisms, with the notion that functional and unerring gene products would keep the cell healthy much longer (Tavernarakis, 2007). In this context, considering the causative relationship between alteration in protein synthesis and ageing, as well as the role of non-coding RNAs in translation regulation, one could characterize them as "ageing coordinators".

The biosynthetic pathway of small non-coding RNAs such as miRNAs, piRNAs and endo-siRNAs among others, is well established and extensively reviewed over the last years. Briefly, DNA is transcribed by RNA polymerases in the nucleus and gives rise to pre-miRNA molecules that are further processed by Drosha and cleaved into pre-miRNA molecules. These translocate from the nucleus to the cytoplasm with the aid of protein complexes including exportin. In the cytoplasm, upon binding by the Dicer protein complex, they are further cleaved and their maturation is completed by the time they become single-stranded. Following, they are loaded on the RISC complex which is anchored by Argonaute proteins to carry out their main function of translational repression (Cullen, 2005; Hodzic et al., 2013).

Supporting their effect on longevity, the first characterized miRNA in *C. elegans*, *lin-4*, seems to shorten lifespan when genetically inhibited or mutated. Also, overexpression of its target, *lin-14* which is a developmental regulator, shortens lifespan by inhibiting DAF-16 expression (Boehm and Slack, 2005). Additional evidence for the role of miR *lin-4* in lifespan determination is provided in

studies showing that *lin-4* deletion results in elevated ROS levels and reduced fat accumulation in accordance (Oh et al., 2006; Zhu et al., 2010). Recently, it was shown that even when general miRNA expression appears significantly decreased with age, specific members of the miR-35-41 miRNA cluster are increased in a gonad-dependent manner in *C. elegans* (Li et al., 2011; Lucanic et al., 2013; Maes et al., 2008). Still, these miRNAs do not seem to play important roles in the regulation of longevity with the exception of miR-71 which appears to be essential for lifespan extension in an insulin/IGF-1 signalling pathway-independent manner. Further analysis showed that miR-71 is responsible for the lifespan extension of germ line-deficient animals acting cell non-autonomously through the nervous system ultimately affecting DAF-16 intestinal function and localization. This recently suggested mechanism highlights the significance of spatial regulation of gene expression, implemented by non-coding RNAs (Boulias and Horvitz, 2012). However, the effect of miR-71 deletion or overexpression on longevity is partially DAF-16-dependent (de Lencastre et al., 2010; Pincus et al., 2011). Moreover, miR-71 deletion leads to increased levels of DNA checkpoint-control proteins, implicating a possible role of DNA damage response pathways in the process. MiR-71 together with miR-246 and miR-238 are found to be significantly upregulated during ageing and needed for insulin pathway-mediated lifespan extension. The effects of the first two miRNAs on ageing appear so profound that they were characterized as biomarkers of ageing (Pincus et al., 2011). Other results of this study suggest that miR-239 affects lifespan negatively in *C. elegans* in a DAF-16 dependent manner.

The *Drosophila* miR-14 is a defined cell death suppressor, whose deletion causes abnormal fat accumulation; an outcome mediated through its target, *sugarbabe* (Varghese et al., 2010; Xu et al., 2003). This effect depends on the insulin/IGF-1 signalling pathway. Another miR that was shown to control body fat by intervening with the IGF-signalling pathway is miR-8/-200. When miR-8 is highly expressed, it blocks the expression of ush/FOG2 mRNA. Upon miR-8 shortage, ush/FOG2 binds to p85a enabling the formation of the IRS-1/p85a/p110 complex, thus allowing downstream signalling (Teleman, 2010). Importantly, the role of miR-470, -669b and -681 in the regulation of genes involved in the IGF-1 signalling pathway in the brain of Ames dwarf mice has recently been elucidated (Liang et al., 2011). In the same mouse model, it was shown that miR-27a in the liver controls the levels of several other miRNAs implicated in intermediate metabolism and detoxification control during ageing, which causes lifespan extension without severe metabolic side effects (Bates et al., 2010). Three more miRNAs, miR-4929, miR-4930 and miR-4934, identified in *C. elegans* are insulin/IGF-1 pathway-dependent. These miRs are expressed only in the *daf-2* mutants probably contributing to the profound lifespan extension of these animals, a hypothesis that should be further elucidated (de Lencastre et al., 2010). Further, miR-34 shows an age- and gonad-dependent upregulation, while its role in ageing seems to be conserved (Kato et al., 2011). What is being proposed is that its deletion in *D. melanogaster* causes lifespan shortening most probably stemming from the accumulation of misfolded proteins. A congruent study supports the neuroprotective role of miR-34 during ageing in *Drosophila*. This protective effect is related to its ability to inhibit the formation of polyQ inclusions, thereby extending lifespan (Bilen et al., 2006). Finally, reduction in the levels of miR-34a, miR-30e and miR-181a leads to elevated levels of the anti-apoptotic protein Bcl-2, a direct target of these miRNAs, further maintaining the levels of BAX and caspase 9 proteins low enough to resist apoptosis and provoke neuronal survival in caloric restricted mice (Khanna et al., 2011; Rippo et al., 2014).

Several miRNAs with functions discrete from the insulin/IGF-1 signalling pathway still retain the capacity to significantly affect ageing. MiR-100 was denoted as an mTOR signalling pathway inhibitor in ovarian cancer cells. Its inhibitory effect is fulfilled by down regulating the levels of phosphorylated 4EBP1 and p70 S6 kinase (Nagaraja et al., 2010). The question that arises is whether this inhibition is direct or indirect and also whether this effect is conserved or detectable in normally proliferating cells. miRNAs are not only participants in the regulation of the ageing process, but are themselves reciprocally regulated by it. This is evident from the fact that the percentage of different miRNA isoforms change with age, such as the 2′-O-methylated miRs. Additionally, it should be pointed out that the Ago1 versus Ago2 binding seems to affect the ageing process (Abe et al., 2014). The information about the small non-coding RNAs is summarized in Table 1.

3.2. Long non-coding RNAs

In contrast to the 2% of the genome that is both transcribed and translated into functional protein, it was recently shown that the majority, represented by the 75% of the genome, is only transcribed (Djebali et al., 2012). DNA transcription gives rise to both coding and non-coding transcripts. The non-coding transcripts encompass the well-known small RNAs that where described earlier and the upcoming and lately discovered long non-coding RNAs (Rinn and Chang, 2012). Long non-coding RNAs have become the subject of many recently published research articles as they are implicated in several human pathologies by epigenetically regulating gene expression in several ways (Guttman et al., 2009; Sanchez and Huarte, 2013; Troy and Sharpless, 2012). Their discovery, mapping and function elucidation was facilitated by the utilization of techniques such as tiling microarrays, SAGE, CAGE, chromatin markers, RNA-deep sequencing technologies and expression correlation analysis, among others (Guttman et al., 2009; Rinn and Chang, 2012). Long non-coding RNAs as their name implicates, are transcripts that exceed the 200 nucleotides in length and are devoid of open reading frames (ORFs), even if that is still ambiguous (Bazzini et al., 2014; Boerner and McGinnis, 2012; Derrien et al., 2012). Rather, they represent polyadenylated products of intergenic regions (Troy and Sharpless, 2012). Their expression pattern is well-coordinated, both spatially and temporally, adding a collateral level of genome regulation, hence explaining part of its complex nature (Rinn and Chang, 2012; van de Vondervoort et al., 2013).

Even if there are many studies related to the functional elucidation of long non-coding RNAs, much remains to be answered or clarified. Due to their function both in the nucleus and the cytoplasm, where they are found to be anchored on ribosomes, they are established as gene expression regulators operating in a multimodal way (van Heesch et al., 2014). The mechanism used in order to achieve transcriptional repression is not very well elucidated yet, but differs from the one that has been characterized for miRNAs. The diversification noted, is that for long non-coding RNAs, the epigenetic chromatin alterations are both permanent and heritable playing significant roles in genetic imprinting, in contrast to those imposed by miRs (Lee and Bartolomei, 2013; Troy and Sharpless, 2012).

One key characteristic that distinguishes small from long non-coding RNAs, except from their size, is their dependence not only on their primary sequence but also on the 2-D and 3-D structures they are able to form in order to accomplish their function, adding extra complexity to gene expression regulation (Novikova et al., 2013; Rinn and Chang, 2012). Furthermore, long non-coding RNAs operate both *in cis* and *in trans*. They regulate gene expression mainly by affecting transcription; by repressing RNA polymerase II binding on DNA, by altering chromatin structure or by provoking epigenetic modifications on histones. Moreover, it has been reported that they affect spliceosome recognition ability, thus changing the final sequence of the transcripts produced. In other cases they bind to

Table 1
miRs influencing physiology and lifespan. Summary of miR effects on lifespan and animal physiology. n/d stands for: no data, DAF-16 dependence refers to lifespan effects. The "−" in the lifespan column signifies lifespan shortening and the "+" lifespan extension.

Name	Organism	Genetic intervention	DAF-16 dependence	Implicated mechanism	Effect on lifespan	References
lin-4	*C. elegans*	Inhibited/mutated	Yes	Elevated ROS levels and reduced fat accumulation	−	Boehm and Slack (2005), Oh et al. (2006), Zhu et al. (2010)
miR-35-41	*C. elegans*	Deletion	n/d	Enhanced RNAi sensitivity	n/d (higher levels in aged animals compared to younger ones)	Li et al. (2011), Lucanic et al. (2013), Maes et al. (2008), Massirer et al. (2012)
miR-71	*C. elegans*	Overexpression	Partial	DNA damage response pathways (needs verification)	+	Boulias and Horvitz (2012), de Lencastre et al. (2010), Pincus et al. (2011)
miR-246	*C. elegans*	Deletion	Yes	Heat and oxidative stress sensitive	−	de Lencastre et al. (2010), Pincus et al. (2011)
miR-238	*C. elegans*	Deletion	No	Heat and oxidative stress sensitive	−	de Lencastre et al. (2010), Pincus et al. (2011)
miR-239	*C. elegans*	Deletion	Yes	Heat and oxidative stress resistant	+	de Lencastre et al. (2010), Pincus et al. (2011)
miR-14	*D. melanogaster*	Deletion	Yes	Abnormal fat accumulation	−	Varghese et al. (2010), Xu et al. (2003)
miR-8/-200	*D. melanogaster*	Deletion	Yes	Growth, body size and fat accumulation	n/d	Hyun et al. (2009), Teleman (2010)
miR-470	*M. musculus*	Overexpression	Yes	IGF1R repression	+	Liang et al. (2011)
miR-669b	*M. musculus*	Overexpression	Yes	IGF1R repression	+	Liang et al. (2011)
miR-681	*M. musculus*	Overexpression	Yes	IGF1R repression	+	Liang et al. (2011)
miR-27a	*M. musculus*	Overexpression	Yes	Intermediate metabolism and detoxification, polyamine biosynthesis	+	Bates et al. (2010)
miR-4929	*C. elegans*	Normally expressed in *daf-2* mutants only	Yes	n/d	+ (further verification needed)	de Lencastre et al. (2010)
miR-4930	*C. elegans*	Normally expressed in *daf-2* mutants only	Yes	n/d	+ (further verification needed)	de Lencastre et al. (2010)
miR-4934	*C. elegans*	Normally expressed in *daf-2* mutants only	Yes	n/d	+ (further verification needed)	de Lencastre et al. (2010)
miR-34	*D. melanogaster*	Deletion	Yes	Misfolded protein accumulation	−	Bilen et al. (2006), Kato et al. (2011), Liu et al. (2012)
miR-34a	*M. musculus*	Downregulation	Yes	Antiapoptotic effect	n/d	Khanna et al. (2011)
miR-30e	*M. musculus*	Downregulation	Yes	Antiapoptotic effect	n/d	Khanna et al. (2011)
miR-181a	*M. musculus*	Downregulation	Yes	Antiapoptotic effect	n/d	Khanna et al. (2011)
miR-100	Ovarian cancer cells	Overexpression	No	4EBP1 and p70 S6 kinase inhibitor	n/d	Nagaraja et al. (2010)

RISC-related proteins such as Dicer leading to endo-siRNA production. Related to this, it should be pointed out that their sequence per se can trigger small RNA (such as miRNAs, piRNAs, etc.) production. Finally, their ability to form secondary structures gives them the opportunity to tether proteins, thus affecting or even changing their function, localization and physiology. In addition, their ability to bind both to DNA and protein molecules enables them not only to regulate the physiology of these molecules but also affect their endogenous functional interactions. In consequence, long non-coding RNAs have been characterized as "signals", "decoys", "enhancers", "scaffolds" or "guides" according to their mode of function (Derrien and Guigo, 2011; Engreitz et al., 2013; Guenther et al., 2007; Guttman et al., 2009; Hung and Chang, 2010; Ng et al., 2012; Salmena et al., 2011; Spitale et al., 2011; Troy and Sharpless, 2012; Tsai et al., 2010; Wang et al., 2011). Lately, it has been reported that long non-coding RNAs are also capable of binding to specific miRNAs, thus antagonizing them, making gene expression regulation a much more intricate procedure than it was previously thought (Kallen et al., 2013).

Long non-coding RNAs have only recently been studied, thus there is little evidence about their effect on ageing. Additionally, recent findings associate long non-coding RNAs with neurodegenerative disorders (Faghihi et al., 2008; Seong et al., 2010). Most long non-coding RNAs with known roles have been related to transcriptional and post-transcriptional RNA surveillance pathways as well as mRNA processing. There are several ways by which long non-coding RNAs affect transcription. First of all, non-coding RNAs such as XIST, HOTAIR, KCNQ1OT1 and ANRIL repress chromatin epigenetically by binding to and regulating chromatin-modifying complexes (Batista and Chang, 2013; Cloutier et al., 2013; Pandey et al., 2008; Tsai et al., 2010; Yap et al., 2010; Zhao et al., 2010), not excluding the possibility that other long non coding RNAs can activate chromatin by using the same mechanism. Otherwise, long non-coding RNAs can either directly bind to RNA polymerase II in order to activate or repress transcription by affecting its function or, indirectly affect the binding of secondary regulatory molecules needed for the transcription process, either *in cis* or *in trans* (Batista and Chang, 2013). Molecules that adopt such mechanisms of function are the ICR1, PWR1, SRG1, fbp1 and the Alu repeat-containing RNA among others (Bumgarner et al., 2012; Hainer et al., 2011; Hirota et al., 2008; Mariner et al., 2008; Martianov et al., 2007; Ng et al., 2012). Because of their ability to form 2-D and 3-D structures,

long non-coding RNAs are able to form structures very relevant to ones formed by endogenous DNA molecules and in this way antagonize the binding of either transcription-regulatory molecules or transport molecules that bring the second at the sites of transcription. Such molecules are: GAS5, EVF2, CCND1 and NRON among others (Bond et al., 2009; Kino et al., 2010; Wang et al., 2008; Willingham et al., 2005).

A second level of regulation is accomplished after the mRNA molecules are synthesized. It has been shown that, before the mRNA molecules exit the nucleus, long non-coding RNAs can either regulate part of the splicing process, leading to an altered spliced isoform (Tollervey et al., 2011) or mediate direct mRNA modifications accomplished by ADAR molecules (Chawla and Sokol, 2014; Ota et al., 2013). Outside the nucleus, they can either bind miRNA/RISC-target mRNAs, impeding their binding on the 5′ UTR of specific mRNAs, thus leading to mRNA translation upregulation. This binding is facilitated by the SINEB2 element embedded in the sequence of the long non-coding RNA (Carrieri et al., 2012; Faghihi et al., 2010).

4. RNA binding proteins

RNA binding proteins facilitate the translation of newly transcribed mRNAs, functioning in numerous pre-translation pathways. Their role includes the stabilization and surveillance of RNA molecules among others, rendering them meritorious of studying. Most published data derived from human tissues and cell cultures can be directly linked to human disease and ageing. To begin with, HuR protein has been characterized as an RNA binding protein that enhances translation of target mRNAs (Mansfield and Keene, 2012), by competing with miRs for binding to them (Simone and Keene, 2013). HuR is a member of the Hu protein family and has three isoforms. The levels of HuR, TIA-1 and AUF-1, two other RBPs, were tested in a variety of human tissues with advancing age as well as in human diploid fibroblasts, a good model for testing replicative senescence. The results of such studies show that ageing differentially affects the levels of RNA binding proteins within the same or different tissues. More specifically, in senescent human fibroblasts it seems that the levels of HuR, AUF-1, and TIA-1 decrease. The opposite was shown for these proteins in specific tested tissues. For example, HuR protein's levels were sustained or even elevated with advancing age in many tissues such as the respiratory system or the gastrointestinal tract with the only exception of reduction in the nervous system. On the other hand, artificial HuR depletion did not cause senescence in various cancer cell lines (Basu et al., 2011; Kakuguchi et al., 2010). These results suggest an increased need for HuR in the ageing tissues, posing a need for further investigation of HuR's role during senescence (Baker et al., 2011). Similar upregulation was observed for AUF-1, suggesting a functional role in ageing tissues. TIA-1 is less upregulated compared to the other two (Masuda et al., 2009; Pang et al., 2013). Recently it was shown that AUF-1 blocks senescence and mediates normal ageing by activating the transcription of the telomerase catalytic subunit (TERT), thereby preserving telomere length. The way this activation is accomplished needs further investigation (Pont et al., 2012). The hTERT levels in aged humans compared to younger ones are lower even if the mRNA levels are unaltered, suggesting that post-transcriptional or post-translational mechanisms may account for these altered protein levels (Shervington and Patel, 2008). Similarly to AUF-1, TIA-1 binds mainly to the 3′ UTR of its targets in order to repress their translation. These findings are summarized in Table 2.

Tristetrapolin (TTP) is an RNA binding protein, member of the TIS11 family. In contrast to TIA-1, AUF-1 and HuR, its levels were highly elevated in senescent fibroblasts and downregulated in most tissues during ageing with the exception of the reproductive system (Masuda et al., 2009). In addition, its levels are increased in cells after addition of both growth factors and insulin into their medium (Cao et al., 2008). This effect has been also connected to the formation of P-bodies which are non-membranous structures comprised of only mRNAs and RNA binding proteins (Kedersha and Anderson, 2007). They are implicated in the quality control of mRNA transcripts representing "decision centers" where mRNAs stall and are further driven to stress granules upon stress, to be degraded or return back to the translation pathway, if needed (Jakymiw et al., 2007; Kulkarni et al., 2010). The identified close association between P-bodies and ribosomes implies mRNA exchange at different time points (Cougot et al., 2013). This association is probably very complex as it is also reported that P-bodies do not form unless mRNA is present (Teixeira et al., 2005). Which kind of mRNAs manage to induce P-body formation and which is the threshold of cytoplasmic mRNA abundance under which P-bodies do not form even if small quantities are present to the cytoplasm need to be elucidated. As it is implicated by their function, P-bodies have the ability to control translation especially of specific mRNAs (Weil et al., 2012). It has not yet become clear whether certain types of mRNAs harbour to P-bodies or if mRNAs gather to these formations indiscriminately (Anderson and Kedersha, 2006; Sheth and Parker, 2006). Despite their obvious connection with translation, it is not yet clear whether their disruption or malfunction could in any way affect the ageing process. The first time that a pathway related to the ageing process is proposed to be connected with P-body formation was this year (Blanco et al., 2014). This study suggests that induction of TGF-β/Smad pathway leads to TTP upregulation. TTP then binds on ARE-containing mRNAs mediating their deadenylation and delivery to P-bodies, increasing their abundance (Blanco et al., 2014; Fabian et al., 2013).

P-body formation is needed for cell survival in yeast, implying a functional role during ageing. This is explained by the fact that highly expressed mRNAs need to be tightly regulated by P-bodies, as upon their appearance cells die prematurely (Lavut and Raveh, 2012). This finding raises the possibility that P-bodies deregulate during ageing playing important role in the ageing process, but this remains a hypothesis that needs to be verified. Last but not least is Nuclear factor 90 (NF90), another RNA binding protein that binds to AU-rich regions on target mRNAs suppressing their translation, or stabilizing them by preventing their degradation (Masuda et al., 2013).

5. RNA modifications

RNA shares some common physicochemical properties with DNA but it simultaneously retains unique properties that attract the scientific interest. Importantly, unlike DNA molecules, RNA molecules are able to form secondary and tertiary structures. These structures are not dispensable for their function; conversely, studies show that their proper formation and conservation delivers important messages or implement functions vital for cell viability. On the other hand, disruption of such structures has been correlated with ageing and the development of neurodegenerative disorders (Ramos and Laederach, 2014), mainly in the context of the huge effect RNA molecules have on protein synthesis regulation.

For example, highly structured regions around the translation initiation codon have been correlated with translation efficiency (Kudla et al., 2009) and highly structured regions inside the coding sequence with ribosome pausing and protein region folding (Dana and Tuller, 2012; Han et al., 2012). In addition, folding within the coding sequences in *Saccharomyces cerevisiae* has been correlated

Table 2
Tissue-specific levels of RNA binding proteins during ageing. Findings suggesting a functional role of HuR, TIA-1, AUF-1, hTERT and TTP in different tissues and age groups.

Name	Tested in	Levels	State	Tissue with highest expression in specific age groups	Comments		References
HuR	Human diploid fibroblast	Decreased	Senescent	Colon (Middle-aged) Fallopian Tube (Young)	Role in senescence unclear	Increased in the ageing tissues/Especially observed in gastrointestinal and reproductive organs	Basu et al. (2011), Baker et al. (2011), Kakuguchi et al. (2010), Kawagishi et al. (2013), Masuda et al. (2009), Pang et al. (2013)
	Mouse embryonic Fibroblasts	Decreased	Senescent				
	Respiratory system	Sustained/elevated	Aged				
	Gastrointestinal tract	Sustained/elevated	Aged				
	Nervous system	Sustained/elevated	Aged				
TIA-1	Human diploid fibroblast	Decreased	Senescent	Eye (Old) Tongue (Foetal) Stomach (Foetal) Small intestine (Middle-aged, Old) Colon (Foetal, Middle-aged, Old) Bladder (Foetal, Young, Middle-aged) Fallopian Tube (Young) Uterus-Cervix (Middle-aged) Thymus (Foetal, Young)	Upregulated in the ageing tissues but less than HuR and AUF-1		Masuda et al. (2009)
AUF-1	Immune System	Increased		Eye (Old)	Suppression of senescence by activation of telomerase transcription/How is this activation accomplished is not clear yet		Masuda et al. (2009), Pont et al. (2012)
	Human diploid fibroblast	Decreased	Senescent				
	auf-1 deficient mice	Absent			Increased cellular senescence		
hTERT					Reduced levels in aged human tissues		Shervington and Patel (2008)
TTP	Human fibroblasts	Elevated	Senescent	Cerebellum (Foetal) Cerebral Cortex (Foetal) Lung (Young) Tongue (Foetal, Young) Stomach (Foetal, Young) Small intestine (Old) Colon (Foetal, Young, Middle-aged, Old) Liver (Foetal) Bladder (Foetal, Young) Spleen (Foetal) Thymus (Foetal) Skin (Foetal) Striated muscle (Young)	Reduced levels during ageing in all tissues apart from the reproductive system		Masuda et al. (2009)

with the control of both spatial and temporal presence of specific RNA transcripts, a master regulator mechanism of protein synthesis important for cell viability and function (Kertesz et al., 2010). Contrary to the above-mentioned, it has been recently shown that in human cells, RNA secondary structures are more often formed in the untranslated regions (UTRs) compared to the coding region. In the same study, the importance and functionality of these 2-D structures is pointed out and additional emphasis is placed on the fact that RNA secondary structure is sequence-dependent. It is also explained how 2-D RNA structures can control fundamental processes such as RNA splicing, degradation, through AGO protein harbouring, and altered gene expression while being evolutionarily conserved (Wan et al., 2014). In this context, it would be very interesting to check whether the occurrence of specific single nucleotide variants (SNVs) that are able to alter RNA 2-D structures is age-dependent and vice versa. Many questions arise relative to potential interplay between alterations in RNA sequence, 2-D structure, gene expression and ageing.

Table 3
RNA modifications associated with ageing. RNA modifications and their association with ageing and neurodegeneration highlight the importance of secondary and tertiary RNA structures.

RNA properties/modifications				State/characteristic	Effect
Secondary and tertiary structure				Proper formation and conservation	Cell viability
				Disruption	Ageing and neurodegenerative disorders
Highly structured regions near the translation initiation codon					Translational efficiency
Highly structured regions inside the coding sequence					Ribosome pausing, protein region folding
Oxidation				Non-selective	Ribosome stalling, premature termination of protein synthesis, misfolded protein products, malfunctioning protein products
8-hydroxyguanosine	8-hydroxyadenosine	5-hydroxycytidine	5-hydroxyuridine		Positively correlated with: Age-related muscle atrophy, Parkinson's disease, Lewy bodies, dementia and Alzheimer's disease Highly present in: The ageing brain and neurons

Oxidation is another mechanism which alters the characteristics of RNA molecules, thus affecting translation. Their single-stranded nature and topology nearby mitochondria makes them vulnerable to oxidative damage. 8-Hydroxyguanosine (8-OHG), 8-hydroxyadenosine, 5-hydroxycytidine and 5-hydroxyuridine are the known RNA oxidized forms, with the first one being the more frequent one. Such types of oxidized bases either form on the RNA molecules after RNA synthesis, or pre-exist in the oxidized form and are subsequently incorporated in the RNA chain during the transcription process. The effects of such RNA alterations on translation are multidimensional. First of all, they are able to cause ribosome stalling leading to delayed translation or even premature termination of protein synthesis, finally causing accumulation of non-functional peptide chains. In other cases, the protein product is produced, but the protein is misfolded leading either to protein aggregates known to be implicated in the onset of many neurodegenerative diseases (Weidner et al., 2011) and the ageing process itself or to malfunctioning proteins which also negatively affect the proper cellular function (Shan et al., 2007; Tanaka et al., 2007). RNA oxidation is not selective and can affect all types of produced RNAs such as mRNAs, rRNAs etc.

Whether the percentage of oxidative RNA damage is age-dependent is a question tried to be answered by experiments implemented on post-mortem human brains. It was shown that 8-OHG is positively correlated with ageing (Nunomura et al., 2012). The same correlation was observed by using human urine (Andreoli et al., 2011). Confirmatory results indicate that the age-related muscle atrophy underlies increased RNA oxidation levels and decreased protein levels in experiments implemented on mice gastrocnemius muscles (Hofer et al., 2008). Furthermore, the high occurrence of oxidative RNA damage observed in both ageing neurons and the brain and its high correlation with neurodegenerative diseases such as Parkinson's disease, Lewy bodies, dementia and the Alzheimer's disease unveils a possible new ageing biomarker and therapeutic target for neurodegenerative syndromes (Bai et al., 2012). RNA properties and modifications are summarized in Table 3.

6. Protein synthesis: a common nexus for pathways regulating ageing

Recent findings suggest that ageing onset is tightly regulated by signalling pathways. TOR and insulin/IGF-1 are the major cellular nutrient-sensing signalling cascades. Their inhibition, either genetically or pharmacologically, is sufficient to extend lifespan in several model organisms. Below, we discuss the intricate relationship between pathways influencing lifespan and mRNA translation, focusing mainly on studies in invertebrate models.

6.1. TOR signalling and caloric restriction

The two distinct target of rapamycin (TOR) complexes, TORC1 and TORC2, lie at the centre of the metabolic state-sensing pathways of eukaryotic cells. TOR, which belongs to the serine/threonine kinase family, becomes activated when nutrients (such as amino acids) and growth factors are plentiful in the cellular environment and responds by shifting the balance towards anabolic processes at the expense of catabolic ones. Specifically TOR activation triggers protein and lipid biosynthesis, while it inhibits autophagy and lysosomal biogenesis (Laplante and Sabatini, 2012). Not surprisingly considering the wide range of processes where the pathway is involved, TOR genetic inhibition results in significant lifespan extension in several model organisms (Kapahi et al., 2010). Similarly, either knockout or knockdown of factors lying downstream of TOR, such as the ribosomal protein kinase (S6K) increase lifespan in *C. elegans* (Hansen et al., 2007; Pan et al., 2007; Syntichaki et al., 2007), *Drosophila* (Kapahi et al., 2004) and mice (Selman et al., 2009). Additionally, rapamycin, a specific TORC1 inhibitor, is reported to have a beneficial effect on lifespan and this can be completely abolished upon overexpression of a constitutively active form of S6K, proving that reduction of protein synthesis rates is pivotal for the manifestation of the enhanced survival phenotype (Bjedov et al., 2010). Activation of transcription factors such as HSF-1 and PHA-4/FOXA in combination with inhibition of HIF-1 are required for longevity emerging from TOR or S6K inhibition (Chen et al., 2009; Sheaffer et al., 2008).

TOR is believed to increase general mRNA translation rate, affecting a variety of molecular players. For example, it directly phosphorylates S6K, eukaryotic elongation factor 2 kinase (EEF2K) and eIF4E binding protein (4E-BP), a negative regulator of eIF4E cap-binding protein, thus allowing the formation of functional eIF4F complexes prone to cap-dependent translation initiation (Ma and Blenis, 2009). TORC1 can also enhance transcription of tRNA and 5S rRNA species by RNA polymerase III (pol III) through the direct phosphorylation and inactivation of Maf1, a pol III repressor (Kantidakis et al., 2010). Among translation initiation factors, only

DRR-2/eIF4H is proposed to act as a direct downstream effector of LET-363/TOR in the nematodes, tightly linking nutrient availability with protein synthesis (Ching et al., 2010). Remarkably, recent findings suggest that TORC1 activation can also augment protein degradation, through enhancing the expression of proteasomal subunits via the activation of nuclear factor erythroid-derived 2-related factor 1 (NRF-1) (Zhang et al., 2014). The above-mentioned mechanism, apart from obviously acting as a protein quality control process, can potentially provide raw materials essential for the mRNA translation machinery.

On the other hand, caloric restriction (CR), accomplished by reducing calorie intake but simultaneously avoiding harmful malnutrition, is a conserved lifespan-extending intervention (Anderson and Weindruch, 2012; Colman et al., 2009; Fontana et al., 2010; Mattison et al., 2012; Stein et al., 2012) that, to a great extent, intertwines with TORC1 complex function, hence with mRNA translation. In yeast, epistasis analyses indicate that dietary restriction conditions, mimicked when feeding with glucose-poor medium, cannot further extend replicative lifespan, calculated by the total number of divisions of a single mother cell (Longo et al., 2012), of *tor1* mutants (Kaeberlein et al., 2005). Likewise, the lifespan of *eat-2 C. elegans* mutants that genetically phenocopy CR due to slower pharyngeal pumping, is not affected by LET-363/TOR knock down (Hansen et al., 2007). In contrast, IFE-2/eIF4E depletion in the nematode seems to act in an additive manner with LET-363/TOR inhibition, pointing to a severe, nevertheless complicated, cross talk between TOR, protein synthesis and ageing (Hansen et al., 2007; Syntichaki et al., 2007). Autophagy, a central catabolic process which provides raw materials (amino acids, among others) and removes damaged, dysfunctional or superfluous organelles, might be the missing denominator in the TOR/eIF longevity-extending fraction (Boya et al., 2013).

Notably, several studies have introduced the novel perception that although general protein synthesis rate is dramatically reduced under CR conditions, specific transcripts exempt the rule and their translation is instead reinforced. In *S. cerevisiae*, deletion mutants for ribosomal proteins constituting either the small or the large subunit display an extended replicative lifespan regulated by a mechanism that shares common features with CR. The privileged translation of the versatile transcriptional activator GCN-4 is essential for the positive outcome on lifespan, as ribosomal protein deletion mutants exhibit increased levels of GCN-4 (Steffen et al., 2008). In *D. melanogaster*, 4E-BP is induced and mediates the effects of CR by reducing general protein synthesis, while in parallel translation of transcripts mainly involved in proper mitochondrial function is enhanced (i.e. respiratory chain components). Interestingly, intrinsic mRNA properties account for the biased translation of this specific subset of transcripts upon CR, with lower GC content 5′ UTRs which are less structured favouring translation (Zid et al., 2009). In *C. elegans*, IFG-1/eIF4G genetic inhibition extends lifespan through the preferential translation of transcripts participating in the execution of stress responses. In that case, 3′ UTR length is positively correlated with the probability of mRNA translation when *ifg-1* is knocked-down (Rogers et al., 2011). Collectively, these studies propose that specific transcripts remain unresponsive to the prevailing trend which dictates translation attenuation under CR conditions and this is indeed crucial for their beneficial effect with regard to whole organism's longevity.

6.2. Insulin/IGF-1 signalling

Milestone studies in the ageing field have highlighted that genetic inhibition of the insulin/IGF-1 signalling pathway can attenuate the rate of normal ageing and substantially extend lifespan, delay the onset of age-related diseases and confer resistance against stress factors in a wide range of model organisms (Kaletsky and Murphy, 2010; Kenyon, 2010; Slack et al., 2011). Loss of function mutations affecting the *daf-2* and *age-1* genes, which encode the *C. elegans* orthologues of the insulin/IGF receptor (IGFR) and phosphatidiylinositol 3-kinase (PI3K) respectively, more than double the worm's lifespan. DAF-2/IGFR is a membrane receptor belonging to the tyrosine kinase family, which upon ligand binding is activated and triggers a cascade of serial phosphorylation events sequestering AGE-1/PI3K, 3-phosphoinositide-dependent kinase 1 (PDK-1), AKT-1/2 (also known as protein kinase B, PKB) and serine/threonine-protein kinase 1 (SGK-1) (Lapierre and Hansen, 2012). With respect to lifespan modulation, the crucial target of the pathway is DAF-16, a homologue of the mammalian highly polymorphic FOXO transcription factors (Flachsbart et al., 2009; Willcox et al., 2008), which when phosphorylated is preferentially maintained at the cytoplasm and excluded from the nucleus, thus being incapable of binding to the promoters of its numerous target genes. However, when upstream components of the pathway are perturbed (as in the case of *daf-2* and *age-1* mutants) the phosphorylation status of DAF-16/FOXO is modified, enabling its subsequent translocation to the nucleus. DAF-16/FOXO repertoire of transcriptional targets includes genes implicated in diverse cellular processes, such as metabolism, resistance to oxidative stress, detoxification and immunity (Dong et al., 2007; McElwee et al., 2003; Murphy et al., 2003). Along with DAF-16/FOXO, SKN-1 (the mammalian NRF-1/2 homologue) (Tullet et al., 2008) and HSF-1 (Hsu et al., 2003; Morley and Morimoto, 2004) are essential for the lifespan extension arising from insulin/IGF-1 signalling inhibition.

Even if the importance of insulin/IGF-1 pathway in lifespan determination is widely appreciated, its putative cross-talk with the translation process still remains ambiguous. Radioactive ^{35}S incorporation experiments comparing wild type with *daf-2* mutant worms demonstrated that inhibition of insulin/IGF-1 signalling is not marked by a decrease in global translation rates (Hansen et al., 2007). Furthermore, the lifespan extension following genetic inhibition of translation initiation factors eIF2β, 4G and 4E is completely abolished when DAF-16/FOXO abundance is reduced either by RNAi feeding or mutation, suggesting that the longevity effect of some translation components is mediated by DAF-16/FOXO (Hansen et al., 2007). In contrast, IFE-2/eIF4E or IFG-1/eIF4G knockdown significantly extend lifespan of both *daf-16* and *daf-2* mutant worms, thus placing translation attenuation and the insulin/IGF-1 signalling in discrete longevity pathways (Pan et al., 2007; Syntichaki et al., 2007). However, lifespan extension by knockdown of translation initiation factors requires the activity of both DAF-16/FOXO and SKN-1/NRF-1,2 (Wang et al., 2010). It is also important to note that DAF-16/FOXO as well as SKN-1/NRF-1,2 transcriptional programs execution is critical for TOR inhibition or rapamycin-mediated longevity (Robida-Stubbs et al., 2012). Moreover, a direct link between FOXO activation and 4E-BP was established in the fruit fly *D. melanogaster*. Specifically, overexpression of *dFoxo* in the muscles can increase the relative *4E-BP* mRNA levels, thus indicating a direct inhibitory relationship between insulin/IGF-1 signalling and protein synthesis (Demontis and Perrimon, 2010). Importantly, *4E-BP* overexpression is necessary and sufficient to ameliorate the accumulation of polyubiquitin aggregates (the same stands for *dFoxo* overexpression) thereby efficiently preserving muscles from age-associated functional deterioration. A reminiscent protective role in organ and organism decline was established in the case of proper cardiac function maintenance following overexpression of *4E-BP* (Wessells et al., 2009).

Reduced insulin/IGF-1 signalling is also linked with increased tolerance to elevated temperatures (Akerfelt et al., 2010). In *C. elegans*, HSF-1 activation and nuclear translocation is central in the process (Kourtis and Tavernarakis, 2011), orchestrating a salvation transcriptional response mainly depending on the expression of

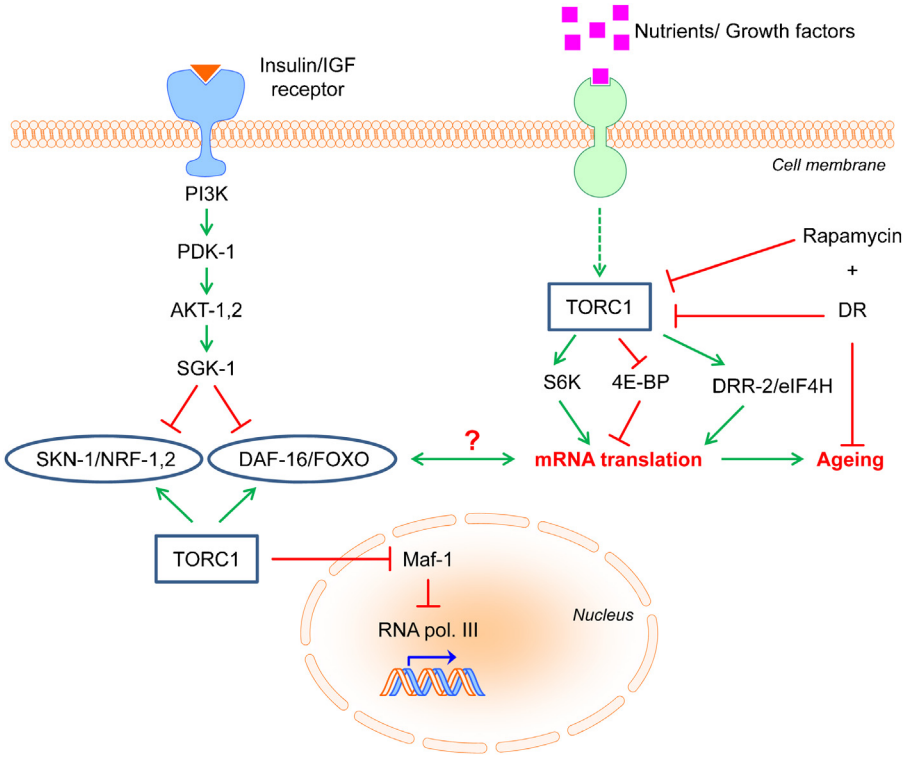

Fig. 2. mRNA translation lies at the core of pathways regulating longevity. TORC1 is thought to affect mRNA translation either directly, through the phosphorylation of S6K or 4E-BP, or indirectly, releasing the inhibition of RNA pol III-driven transcription of tRNA and rRNA species by Maf-1. Rapamycin, which is considered to be a specific inhibitor of TORC1, as well as dietary restriction which also decreases TORC1 activity, are proposed to have a beneficial effect on lifespan relying (at least partially) on protein synthesis attenuation. The nematode's eIF4H homologue is proposed to act immediately downstream of TORC1, linking nutrient availability with translation initiation and vice versa. On the other hand, recent findings suggest that the insulin/IGF-1 pathway, initially thought to act in a mode independent of protein synthesis, in a yet unidentified way, intertwines with the mRNA translation efficiency. Moreover, DAF-16 and SKN-1 transcriptional programs seem vital for the beneficial effect of TOR signalling inhibition and rapamycin treatment. Collectively, a unifying model is emerging, where mRNA translation senses the metabolic state of the cell through its interaction with well-known longevity pathways.

heat shock protein (HSP) chaperones, that fortifies cells against protein toxicity caused by excessive misfolding (Ben-Zvi et al., 2009). Surprisingly, HSF-1 seems to partially limit the acquired thermotolerance (that is resistance to an acute stress dose gained after an initial non-toxic stress stimulus) in *C. elegans*, but is dispensable for the intrinsic thermotolerance of insulin/IGF-1 mutants. This effect depends on the preferential de novo translation events for a specific set of mRNAs, contributing proteins with a protective role (such as members of the hsp chaperones) in the insulin/IGF-1 mutants upon acute stress (McColl et al., 2010). Hence, the notion of selective mRNA translation seems to apply in different cases, unifying insulin/IGF-1 inhibition with CR.

Intriguingly, recent studies exploiting high-throughput proteomic approaches propose that the extended longevity phenotype associated with inhibition of the insulin/IGF-1 signalling is accompanied by reduced general protein synthesis rates. *C. elegans* mutants for the *daf-2*/IGFR display decreased amounts of protein compounds belonging to the small (40S) and large (60S) ribosomal subunits, as well as numerous tRNA synthetases participating in the formation of the translational machinery. Interestingly, this is not a consequence of the reduction in transcription of ribosomal genes per se, highlighting that post-transcriptional mechanisms are responsible for the aforementioned changes (Depuydt et al., 2013). Apart from ribosomal subunits, translation initiation and elongation factors seem to be significantly underrepresented in the proteome of long-lived *daf-2* mutant worms. Polysome profiling further confirmed that de novo protein synthesis is compromised and this is dependent on the presence of functional DAF-16/FOXO (Stout et al., 2013). Additively, these studies recommend a unifying model where dietary restriction is not the only longevity-promoting intervention relying on the reduction of protein synthesis to exert its beneficial effect on lifespan. The insulin/IGF-1 pathway is known to be capable of modulating the activity of S6K through the action of PDK and AKT kinases lying upstream of DAF-16 (Vellai and Takacz Vellai, 2010). However, since the amelioration of their transcription does not account for the concomitant reduced abundance of factors involved in protein synthesis (ribosomal components etc.), the exact molecular mechanisms leading to these alterations at the post-transcriptional level (maybe some sort of decreased stability or enhanced degradation) need to be fully delineated. A simplified view of the main pathways associated with ageing and their links with mRNA translation is depicted in Fig. 2.

7. Concluding remarks

Translation initiation is the most tightly controlled step and consequently is the major hub for protein control that could decay during ageing. Moreover, during the elongation phase, ribosomes and their associated partners are the main translation components affected by proteotoxic or mitochondrial stress which contribute to the degenerative process of ageing. Importantly, what is chosen to be expressed and what not, also varies during ageing. The high throughput technology nowadays, including RNA-sequencing and proteomics will allow for a more profound analysis of the alterations in gene expression, which could be used as markers of ageing (Harries et al., 2011). For instance, decreased expression of components of the proteasome has been shown to be directly linked to ageing. Thus, translation of these proteins required for protein quality control is reduced during ageing, causing reduction of proper

protein recycling and increased probability of protein aggregation which can result in disease (Huber et al., 2009).

However, protein synthesis regulation is not limited to the mRNA translation process. A great variety of regulators such as non-coding RNAs and RBPs function before translation. It has been shown that the impact of their function on protein synthesis control is indispensable even if much remains to be elucidated. Significantly, small and especially long non-coding RNAs are thought to possess novel functions. Their physical characteristics favour their participation in protein complexes (Gong and Maquat, 2015). Some non-coding RNAs have already been characterized as biomarkers of ageing. Specifically their expression pattern, their targets and their function during ageing remains to be elucidated. Another key question is whether specific non-coding RNAs have common targets, thus redundant functions with RBPs. Answering such questions would only bring us one step closer to understand and possibly manipulate the ageing process and prevent its detrimental effects. The understanding of the mechanisms underlying RNA structure and modification during ageing is very intriguing. Primary studies on primates (*Microcebus murinus*) indicate that ageing could be delayed by pharmacological manipulation of these RNAs (Marchal et al., 2013a, 2013b). Still, a clear correlation between RNA modifications, protein synthesis and ageing is required to advance knowledge in this field.

Although milestone studies conjugating mRNA translation with the onset of ageing have been recently published, much remains undescribed. First of all, what is the molecular signature of long-lived animals due to attenuated protein synthesis? Which are the intrinsic features of specific mRNAs which allow them to evade the general trend of translation inhibition and confer resistance against stress insults? Treatment with rapamycin extends lifespan in numerous model organisms tested. It relies on the inhibition of TORC1 activity which in turn reduces translation rhythms and induces catabolism through autophagy. Are the quality control mechanisms, especially degradation through the proteasome and autophagy, enhanced upon translation inhibition? Interestingly, recent studies indicate that insulin/IGF-1 signalling, a pathway which universally affects ageing, interconnects with the process of translation. Is this a corollary of altered cellular metabolism or a mutual, leading force delaying the onset of ageing? If this is the case, a unifying axis emerges where ageing-determining pathways converge on translation to influence ageing in a beneficial manner.

Acknowledgements

We thank Maria Markaki for discussions and comments on the manuscript. Work in the authors' laboratory is funded by grants from the European Research Council (ERC), the European Commission Framework Programmes, and the Greek Ministry of Education.

References

Abdelmohsen, K., Panda, A., Kang, M.J., Xu, J., Selimyan, R., Yoon, J.H., Martindale, J.L., De, S., Wood 3rd, W.H., Becker, K.G., Gorospe, M., 2013. Senescence-associated lncRNAs: senescence-associated long noncoding RNAs. Aging Cell 12, 890–900.

Abe, M., Naqvi, A., Hendriks, G.J., Feltzin, V., Zhu, Y., Grigoriev, A., Bonini, N.M., 2014. Impact of age-associated increase in 2′-O-methylation of miRNAs on aging and neurodegeneration in Drosophila. Genes Dev. 28, 44–57.

Akerfelt, M., Morimoto, R.I., Sistonen, L., 2010. Heat shock factors: integrators of cell stress, development and lifespan. Nat. Rev. Mol. Cell Biol. 11, 545–555.

Anderson, P., Kedersha, N., 2006. RNA granules. J. Cell Biol. 172, 803–808.

Anderson, R.M., Weindruch, R., 2012. The caloric restriction paradigm: implications for healthy human aging. Am. J. Human Biol. 24, 101–106.

Andreoli, R., Mutti, A., Goldoni, M., Manini, P., Apostoli, P., De Palma, G., 2011. Reference ranges of urinary biomarkers of oxidized guanine in (2′-deoxy)ribonucleotides and nucleic acids. Free Radic. Biol. Med. 50, 254–261.

Bai, H., Kang, P., Tatar, M., 2012. Drosophila insulin-like peptide-6 (dilp6) expression from fat body extends lifespan and represses secretion of Drosophila insulin-like peptide-2 from the brain. Aging Cell 11, 978–985.

Baker, B.M., Nargund, A.M., Sun, T., Haynes, C.M., 2012. Protective coupling of mitochondrial function and protein synthesis via the eIF2α kinase GCN-2. PLoS Genet. 8, e1002760.

Baker, D.J., Wijshake, T., Tchkonia, T., LeBrasseur, N.K., Childs, B.G., van de Sluis, B., Kirkland, J.L., van Deursen, J.M., 2011. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479, 232–236.

Basu, S.K., Malik, R., Huggins, C.J., Lee, S., Sebastian, T., Sakchaisri, K., Quinones, O.A., Alvord, W.G., Johnson, P.F., 2011. 3′ UTR elements inhibit Ras-induced C/EBPbeta post-translational activation and senescence in tumour cells. EMBO J. 30, 3714–3728.

Bates, D.J., Li, N., Liang, R., Sarojini, H., An, J., Masternak, M.M., Bartke, A., Wang, E., 2010. MicroRNA regulation in Ames dwarf mouse liver may contribute to delayed aging. Aging Cell 9, 1–18.

Batista, P.J., Chang, H.Y., 2013. Long noncoding RNAs: cellular address codes in development and disease. Cell 152, 1298–1307.

Bazzini, A.A., Johnstone, T.G., Christiano, R., Mackowiak, S.D., Obermayer, B., Fleming, E.S., Vejnar, C.E., Lee, M.T., Rajewsky, N., Walther, T.C., Giraldez, A.J., 2014. Identification of small ORFs in vertebrates using ribosome footprinting and evolutionary conservation. EMBO J. 33, 981–993.

Ben-Zvi, A., Miller, E.A., Morimoto, R.I., 2009. Collapse of proteostasis represents an early molecular event in *Caenorhabditis elegans* aging. Proc. Natl. Acad. Sci. U. S. A. 106, 14914–14919.

Besse, F., Ephrussi, A., 2008. Translational control of localized mRNAs: restricting protein synthesis in space and time. Nat. Rev. Mol. Cell Biol. 9, 971–980.

Bilen, J., Liu, N., Burnett, B.G., Pittman, R.N., Bonini, N.M., 2006. MicroRNA pathways modulate polyglutamine-induced neurodegeneration. Mol. Cell 24, 157–163.

Bjedov, I., Toivonen, J.M., Kerr, F., Slack, C., Jacobson, J., Foley, A., Partridge, L., 2010. Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. Cell Metab. 11, 35–46.

Blanco, F.F., Sanduja, S., Deane, N.G., Blackshear, P.J., Dixon, D.A., 2014. Transforming growth factor beta regulates P-body formation through induction of the mRNA decay factor tristetraprolin. Mol. Cell. Biol. 34, 180–195.

Boehm, M., Slack, F., 2005. A developmental timing microRNA and its target regulate life span in *C. elegans*. Science 310, 1954–1957.

Boerner, S., McGinnis, K.M., 2012. Computational identification and functional predictions of long noncoding RNA in *Zea mays*. PLoS ONE 7, e43047.

Bond, A.M., Vangompel, M.J., Sametsky, E.A., Clark, M.F., Savage, J.C., Disterhoft, J.F., Kohtz, J.D., 2009. Balanced gene regulation by an embryonic brain ncRNA is critical for adult hippocampal GABA circuitry. Nat. Neurosci. 12, 1020–1027.

Boulias, K., Horvitz, H.R., 2012. The *C. elegans* microRNA mir-71 acts in neurons to promote germline-mediated longevity through regulation of DAF-16/FOXO. Cell Metab. 15, 439–450.

Boya, P., Reggiori, F., Codogno, P., 2013. Emerging regulation and functions of autophagy. Nat. Cell Biol. 15, 713–720.

Bumgarner, S.L., Neuert, G., Voight, B.F., Symbor-Nagrabska, A., Grisafi, P., van Oudenaarden, A., Fink, G.R., 2012. Single-cell analysis reveals that noncoding RNAs contribute to clonal heterogeneity by modulating transcription factor recruitment. Mol. Cell 45, 470–482.

Cai, L., McCormick, Mark, A., Kennedy, Brian, K. Tu, Benjamin, P., 2013. Integration of multiple nutrient cues and regulation of lifespan by ribosomal transcription factor Ifh1. Cell Rep. 4, 1063–1071.

Cao, H., Urban Jr., J.F., Anderson, R.A., 2008. Insulin increases tristetraprolin and decreases VEGF gene expression in mouse 3T3-L1 adipocytes. Obesity (Silver Spring) 16, 1208–1218.

Carrieri, C., Cimatti, L., Biagioli, M., Beugnet, A., Zucchelli, S., Fedele, S., Pesce, E., Ferrer, I., Collavin, L., Santoro, C., Forrest, A.R., Carninci, P., Biffo, S., Stupka, E., Gustincich, S., 2012. Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature 491, 454–457.

Chawla, G., Sokol, N.S., 2014. ADAR mediates differential expression of polycistronic microRNAs. Nucleic Acids Res. 42, 5245–5255.

Chen, D., Thomas, E.L., Kapahi, P., 2009. HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in *Caenorhabditis elegans*. PLoS Genet. 5, e1000486.

Ching, T.-T., Paal, A.B., Mehta, A., Zhong, L., Hsu, A.-L., 2010. drr-2 encodes an eIF4H that acts downstream of TOR in diet-restriction-induced longevity of *C. elegans*. Aging Cell 9, 545–557.

Cloutier, S.C., Wang, S., Ma, W.K., Petell, C.J., Tran, E.J., 2013. Long noncoding RNAs promote transcriptional poising of inducible genes. PLoS Biol. 11, e1001715.

Colman, R.J., Anderson, R.M., Johnson, S.C., Kastman, E.K., Kosmatka, K.J., Beasley, T.M., Allison, D.B., Cruzen, C., Simmons, H.A., Kemnitz, J.W., Weindruch, R., 2009. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325, 201–204.

Cougot, N., Molza, A.E., Giudice, E., Cavalier, A., Thomas, D., Gillet, R., 2013. Structural organization of the polysomes adjacent to mammalian processing bodies (P-bodies). RNA Biol. 10, 314–320.

Cullen, B.R., 2005. RNAi the natural way. Nat. Genet. 37, 1163–1165.

Dana, A., Tuller, T., 2012. Determinants of translation elongation speed and ribosomal profiling biases in mouse embryonic stem cells. PLoS Comput. Biol. 8, e1002755.

de Lencastre, A., Pincus, Z., Zhou, K., Kato, M., Lee, S.S., Slack, F.J., 2010. MicroRNAs both promote and antagonize longevity in *C. elegans*. Curr. Biol. 20, 2159–2168.

Delaney, J.R., Ahmed, U., Chou, A., Sim, S., Carr, D., Murakami, C.J., Schleit, J., Sutphin, G.L., An, E.H., Castanza, A., Fletcher, M., Higgins, S., Jelic, M., Klum, S., Muller, B., Peng, Z.J., Rai, D., Ros, V., Singh, M., Wende, H.V., Kennedy, B.K., Kaeberlein,

M., 2013. Stress profiling of longevity mutants identifies Afg3 as a mitochondrial determinant of cytoplasmic mRNA translation and aging. Aging Cell 12, 156–166.
Demontis, F., Perrimon, N., 2010. FOXO/4E-BP signaling in drosophila muscles regulates organism-wide proteostasis during aging. Cell 143, 813–825.
Depuydt, G., Xie, F., Petyuk, V.A., Shanmugam, N., Smolders, A., Dhondt, I., Brewer, H.M., Camp, D.G., Smith, R.D., Braeckman, B.P., 2013. Reduced insulin/insulin-like growth factor-1 signaling and dietary restriction inhibit translation but preserve muscle mass in *Caenorhabditis elegans*. Mol. Cell. Proteomics 12, 3624–3639.
Derrien, T., Guigo, R., 2011. Long non-coding RNAs with enhancer-like function in human cells. Med. Sci. (Paris) 27, 359–361.
Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Djebali, S., Tilgner, H., Guernec, G., Martin, D., Merkel, A., Knowles, D.G., Lagarde, J., Veeravalli, L., Ruan, X., Ruan, Y., Lassmann, T., Carninci, P., Brown, J.B., Lipovich, L., Gonzalez, J.M., Thomas, M., Davis, C.A., Shiekhattar, R., Gingeras, T.R., Hubbard, T.J., Notredame, C., Harrow, J., Guigo, R., 2012. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 22, 1775–1789.
Djebali, S., Davis, C.A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., Tanzer, A., Lagarde, J., Lin, W., Schlesinger, F., Xue, C., Marinov, G.K., Khatun, J., Williams, B.A., Zaleski, C., Rozowsky, J., Roder, M., Kokocinski, F., Abdelhamid, R.F., Alioto, T., Antoshechkin, I., Baer, M.T., Bar, N.S., Batut, P., Bell, K., Bell, I., Chakrabortty, S., Chen, X., Chrast, J., Curado, J., Derrien, T., Drenkow, J., Dumais, E., Dumais, J., Duttagupta, R., Falconnet, E., Fastuca, M., Fejes-Toth, K., Ferreira, P., Foissac, S., Fullwood, M.J., Gao, H., Gonzalez, D., Gordon, A., Gunawardena, H., Howald, C., Jha, S., Johnson, R., Kapranov, P., King, B., Kingswood, C., Luo, O.J., Park, E., Persaud, K., Preall, J.B., Ribeca, P., Risk, B., Robyr, D., Sammeth, M., Schaffer, L., See, L.H., Shahab, A., Skancke, J., Suzuki, A.M., Takahashi, H., Tilgner, H., Trout, D., Walters, N., Wang, H., Wrobel, J., Yu, Y., Ruan, X., Hayashizaki, Y., Harrow, J., Gerstein, M., Hubbard, T., Reymond, A., Antonarakis, S.E., Hannon, G., Giddings, M.C., Ruan, Y., Wold, B., Carninci, P., Guigo, R., Gingeras, T.R., 2012. Landscape of transcription in human cells. Nature 489, 101–108.
Dong, M.Q., Venable, J.D., Au, N., Xu, T., Park, S.K., Cociorva, D., Johnson, J.R., Dillin, A., Yates 3rd, J.R., 2007. Quantitative mass spectrometry identifies insulin signaling targets in *C. elegans*. Science 317, 660–663.
Dubrovsky, Y.V., Samsa, W.E., Kondratov, R.V., 2010. Deficiency of circadian protein CLOCK reduces lifespan and increases age-related cataract development in mice. Aging (Albany NY) 2, 936–944.
Engreitz, J.M., Pandya-Jones, A., McDonel, P., Shishkin, A., Sirokman, K., Surka, C., Kadri, S., Xing, J., Goren, A., Lander, E.S., Plath, K., Guttman, M., 2013. The Xist lncRNA exploits three-dimensional genome architecture to spread across the X chromosome. Science 341, 1237973.
Fabian, M.R., Frank, F., Rouya, C., Siddiqui, N., Lai, W.S., Karetnikov, A., Blackshear, P.J., Nagar, B., Sonenberg, N., 2013. Structural basis for the recruitment of the human CCR4-NOT deadenylase complex by tristetraprolin. Nat. Struct. Mol. Biol. 20, 735–739.
Faghihi, M.A., Modarresi, F., Khalil, A.M., Wood, D.E., Sahagan, B.G., Morgan, T.E., Finch, C.E., St Laurent, G. 3rd, Kenny, P.J., Wahlestedt, C., 2008. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. Nat. Med. 14, 723–730.
Faghihi, M.A., Zhang, M., Huang, J., Modarresi, F., Van der Brug, M.P., Nalls, M.A., Cookson, M.R., St-Laurent, G. 3rd, Wahlestedt, C., 2010. Evidence for natural antisense transcript-mediated inhibition of microRNA function. Genome Biol. 11, R56.
Flachsbart, F., Caliebe, A., Kleindorp, R., Blanché, H., von Eller-Eberstein, H., Nikolaus, S., Schreiber, S., Nebel, A., 2009. Association of FOXO3A variation with human longevity confirmed in German centenarians. Proc. Natl. Acad. Sci. U. S. A. 106, 2700–2705.
Fontana, L., Partridge, L., Longo, V.D., 2010. Extending healthy life span—from yeast to humans. Science 328, 321–326.
Gems, D., Partridge, L., 2013. Genetics of longevity in model organisms: debates and paradigm shifts. Annu. Rev. Physiol. 75, 621–644.
Gomes, A.P., Price, N.L., Ling, A.J., Moslehi, J.J., Montgomery, M.K., Rajman, L., White, J.P., Teodoro, J.S., Wrann, C.D., Hubbard, B.P., Mercken, E.M., Palmeira, C.M., de Cabo, R., Rolo, A.P., Turner, N., Bell, E.L., Sinclair, D.A., 2013. Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell 155, 1624–1638.
Gong, C., Maquat, L.E., 2011. lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3′ UTRs via Alu elements. Nature 470, 284–288.
Gong, C., Maquat, L.E., 2015. Affinity purification of long noncoding RNA–protein complexes from formaldehyde cross-linked mammalian cells. Methods Mol. Biol. 1206, 81–86.
Guenther, M.G., Levine, S.S., Boyer, L.A., Jaenisch, R., Young, R.A., 2007. A chromatin landmark and transcription initiation at most promoters in human cells. Cell 130, 77–88.
Guttman, M., Amit, I., Garber, M., French, C., Lin, M.F., Feldser, D., Huarte, M., Zuk, O., Carey, B.W., Cassady, J.P., Cabili, M.N., Jaenisch, R., Mikkelsen, T.S., Jacks, T., Hacohen, N., Bernstein, B.E., Kellis, M., Regev, A., Rinn, J.L., Lander, E.S., 2009. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458, 223–227.
Hainer, S.J., Pruneski, J.A., Mitchell, R.D., Monteverde, R.M., Martens, J.A., 2011. Intergenic transcription causes repression by directing nucleosome assembly. Genes Dev. 25, 29–40.
Halvorsen, M., Martin, J.S., Broadaway, S., Laederach, A., 2010. Disease-associated mutations that alter the RNA structural ensemble. PLoS Genet. 6, e1001074.
Han, Y., David, A., Liu, B., Magadan, J.G., Bennink, J.R., Yewdell, J.W., Qian, S.B., 2012. Monitoring cotranslational protein folding in mammalian cells at codon resolution. Proc. Natl. Acad. Sci. U. S. A. 109, 12467–12472.
Han, Y., Gao, X., Liu, B., Wan, J., Zhang, X., Qian, S.B., 2014. Ribosome profiling reveals sequence-independent post-initiation pausing as a signature of translation. Cell Res. 24, 842–851.
Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.-J., Kenyon, C., 2007. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. Aging Cell 6, 95–110.
Harries, L.W., Hernandez, D., Henley, W., Wood, A.R., Holly, A.C., Bradley-Smith, R.M., Yaghootkar, H., Dutta, A., Murray, A., Frayling, T.M., Guralnik, J.M., Bandinelli, S., Singleton, A., Ferrucci, L., Melzer, D., 2011. Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. Aging Cell 10, 868–878.
Hipp, M.S., Park, S.-H., Hartl, F.U., 2014. Proteostasis impairment in protein-misfolding and -aggregation diseases. Trends Cell Biol. 24, 506–514.
Hirota, K., Miyoshi, T., Kugou, K., Hoffman, C.S., Shibata, T., Ohta, K., 2008. Stepwise chromatin remodelling by a cascade of transcription initiation of non-coding RNAs. Nature 456, 130–134.
Hodzic, M., Naaldijk, Y., Stolzing, A., 2013. Regulating aging in adult stem cells with microRNA. Z. Gerontol. Geriatr. 46, 629–634.
Hofer, T., Marzetti, E., Xu, J., Seo, A.Y., Gulec, S., Knutson, M.D., Leeuwenburgh, C., Dupont-Versteegden, E.E., 2008. Increased iron content and RNA oxidative damage in skeletal muscle with aging and disuse atrophy. Exp. Gerontol. 43, 563–570.
Hsu, A.-L., Murphy, C.T., Kenyon, C., 2003. Regulation of aging and age-related disease by DAF-16 and heat-shock factor. Science 300, 1142–1145.
Huber, N., Sakai, N., Eismann, T., Shin, T., Kuboki, S., Blanchard, J., Schuster, R., Edwards, M.J., Wong, H.R., Lentsch, A.B., 2009. Age-related decrease in proteasome expression contributes to defective nuclear factor-kappaB activation during hepatic ischemia/reperfusion. Hepatology 49, 1718–1728.
Hung, T., Chang, H.Y., 2010. Long noncoding RNA in genome regulation: prospects and mechanisms. RNA Biol. 7, 582–585.
Hyun, S., Lee, J.H., Jin, H., Nam, J., Namkoong, B., Lee, G., Chung, J., Kim, V.N., 2009. Conserved microRNA miR-8/miR-200 and its target USH/FOG2 control growth by regulating PI3K. Cell 139, 1096–1108.
Ishimura, R., Nagy, G., Dotu, I., Zhou, H., Yang, X.L., Schimmel, P., Senju, S., Nishimura, Y., Chuang, J.H., Ackerman, S.L., 2014. RNA function. Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. Science 345, 455–459.
Jackson, R.J., Hellen, C.U., Pestova, T.V., 2010. The mechanism of eukaryotic translation initiation and principles of its regulation. Nat. Rev. Mol. Cell Biol. 11, 113–127.
Jakymiw, A., Pauley, K.M., Li, S., Ikeda, K., Lian, S., Eystathioy, T., Satoh, M., Fritzler, M.J., Chan, E.K., 2007. The role of GW/P-bodies in RNA processing and silencing. J. Cell Sci. 120, 1317–1323.
Johnson, J.E., Johnson, F.B., 2014. Methionine restriction activates the retrograde response and confers both stress tolerance and lifespan extension to yeast, mouse and human cells. PLOS ONE 9, e97729.
Jouffe, C., Cretenet, G., Symul, L., Martin, E., Atger, F., Naef, F., Gachon, F., 2013. The circadian clock coordinates ribosome biogenesis. PLoS Biol. 11, e1001455.
Kaeberlein, M., Powers, R.W., Steffen, K.K., Westman, E.A., Hu, D., Dang, N., Kerr, E.O., Kirkland, K.T., Fields, S., Kennedy, B.K., 2005. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science 310, 1193–1196.
Kakuguchi, W., Kitamura, T., Kuroshima, T., Ishikawa, M., Kitagawa, Y., Totsuka, Y., Shindoh, M., Higashino, F., 2010. HuR knockdown changes the oncogenic potential of oral cancer cells. Mol. Cancer Res. 8, 520–528.
Kaletsky, R., Murphy, C.T., 2010. The role of insulin/IGF-like signaling in *C. elegans* longevity and aging. Dis. Models Mech. 3, 415–419.
Kallen, A.N., Zhou, X.B., Xu, J., Qiao, C., Ma, J., Yan, L., Lu, L., Liu, C., Yi, J.S., Zhang, H., Min, W., Bennett, A.M., Gregory, R.I., Ding, Y., Huang, Y., 2013. The imprinted H19 lncRNA antagonizes let-7 microRNAs. Mol. Cell 52, 101–112.
Kantidakis, T., Ramsbottom, B.A., Birch, J.L., Dowding, S.N., White, R.J., 2010. mTOR associates with TFIIIC, is found at tRNA and 5S rRNA genes, and targets their repressor Maf1. Proc. Natl. Acad. Sci. U. S. A. 107, 11823–11828.
Kapahi, P., Chen, D., Rogers, A.N., Katewa, S.D., Li, P.W.-L., Thomas, E.L., Kockel, L., 2010. With TOR, less is more: a key role for the conserved nutrient-sensing TOR PATHWAY IN AGINg. Cell Metab. 11, 453–465.
Kapahi, P., Zid, B.M., Harper, T., Koslover, D., Sapin, V., Benzer, S., 2004. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Curr. Biol. 14, 885–890.
Kapranov, P., Cheng, J., Dike, S., Nix, D.A., Duttagupta, R., Willingham, A.T., Stadler, P.F., Hertel, J., Hackermuller, J., Hofacker, I.L., Bell, I., Cheung, E., Drenkow, J., Dumais, E., Patel, S., Helt, G., Ganesh, M., Ghosh, S., Piccolboni, A., Sementchenko, V., Tammana, H., Gingeras, T.R., 2007. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 316, 1484–1488.
Karamyshev, A.L., Patrick, A.E., Karamysheva, Z.N., Griesemer, D.S., Hudson, H., Tjon-Kon-Sang, S., Nilsson, I., Otto, H., Liu, Q., Rospert, S., von Heijne, G., Johnson, A.E., Thomas, P.J., 2014. Inefficient SRP interaction with a nascent chain triggers a mRNA quality control pathway. Cell 156, 146–157.
Kato, M., Chen, X., Inukai, S., Zhao, H., Slack, F.J., 2011. Age-associated changes in expression of small, noncoding RNAs, including microRNAs, in *C. elegans*. RNA 17, 1804–1820.

Kawagishi, H., Hashimoto, M., Nakamura, H., Tsugawa, T., Watanabe, A., Kontoyiannis, D.L., Sugimoto, M., 2013. HuR maintains a replicative life span by repressing the ARF tumor suppressor. Mol. Cell. Biol. 33, 1886–1900.
Kedersha, N., Anderson, P., 2007. Mammalian stress granules and processing bodies. Methods Enzymol. 431, 61–81.
Kenyon, C.J., 2010. The genetics of ageing. Nature 464, 504–512.
Kertesz, M., Wan, Y., Mazor, E., Rinn, J.L., Nutter, R.C., Chang, H.Y., Segal, E., 2010. Genome-wide measurement of RNA secondary structure in yeast. Nature 467, 103–107.
Khanna, A., Muthusamy, S., Liang, R., Sarojini, H., Wang, E., 2011. Gain of survival signaling by down-regulation of three key miRNAs in brain of calorie-restricted mice. Aging (Albany NY) 3, 223–236.
Kim, H.-Y., 2013. The methionine sulfoxide reduction system: selenium utilization and methionine sulfoxide reductase enzymes and their functions. Antioxid. Redox Signal. 19, 958–969.
Kino, T., Hurt, D.E., Ichijo, T., Nader, N., Chrousos, G.P., 2010. Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. Sci. Signal. 3, ra8.
Kirstein-Miles, J., Scior, A., Deuerling, E., Morimoto, R.I., 2013. The nascent polypeptide-associated complex is a key regulator of proteostasis. EMBO J. 32, 1451–1468.
Kourtis, N., Tavernarakis, N., 2011. Cellular stress response pathways and ageing: intricate molecular relationships. EMBO J. 30, 2520–2531.
Kozieł, R., Ruckenstuhl, C., Albertini, E., Neuhaus, M., Netzberger, C., Bust, M., Madeo, F., Wiesner, R.J., Jansen-Dürr, P., 2014. Methionine restriction slows down senescence in human diploid fibroblasts. Aging Cell 13, 1038–1048.
Kretz, M., Siprashvili, Z., Chu, C., Webster, D.E., Zehnder, A., Qu, K., Lee, C.S., Flockhart, R.J., Groff, A.F., Chow, J., Johnston, D., Kim, G.E., Spitale, R.C., Flynn, R.A., Zheng, G.X., Aiyer, S., Raj, A., Rinn, J.L., Chang, H.Y., Khavari, P.A., 2013. Control of somatic tissue differentiation by the long non-coding RNA TINCR. Nature 493, 231–235.
Kudla, G., Murray, A.W., Tollervey, D., Plotkin, J.B., 2009. Coding-sequence determinants of gene expression in *Escherichia coli*. Science 324, 255–258.
Kulkarni, M., Ozgur, S., Stoecklin, G., 2010. On track with P-bodies. Biochem. Soc. Trans. 38, 242–251.
Lapierre, L.R., Hansen, M., 2012. Lessons from *C. elegans*: signaling pathways for longevity. Trends Endocrinol. Metab. 23, 637–644.
Laplante, M., Sabatini, D.M., 2012. mTOR signaling in growth control and disease. Cell 149, 274–293.
Lavut, A., Raveh, D., 2012. Sequestration of highly expressed mRNAs in cytoplasmic granules, P-bodies, and stress granules enhances cell viability. PLoS Genet. 8, e1002527.
Lebedeva, S., Jens, M., Theil, K., Schwanhausser, B., Selbach, M., Landthaler, M., Rajewsky, N., 2011. Transcriptome-wide analysis of regulatory interactions of the RNA-binding protein HuR. Mol. Cell 43, 340–352.
Lee, J.T., Bartolomei, M.S., 2013. X-inactivation, imprinting, and long noncoding RNAs in health and disease. Cell 152, 1308–1323.
Li, N., Bates, D.J., An, J., Terry, D.A., Wang, E., 2011. Up-regulation of key microRNAs, and inverse down-regulation of their predicted oxidative phosphorylation target genes, during aging in mouse brain. Neurobiol. Aging 32, 944–955.
Liang, R., Khanna, A., Muthusamy, S., Li, N., Sarojini, H., Kopchick, J.J., Masternak, M.M., Bartke, A., Wang, E., 2011. Post-transcriptional regulation of IGF1R by key microRNAs in long-lived mutant mice. Aging Cell 10, 1080–1088.
Liu, B., Han, Y., Qian, S.B., 2013. Cotranslational response to proteotoxic stress by elongation pausing of ribosomes. Mol. Cell 49, 453–463.
Liu, S., Sawada, T., Lee, S., Yu, W., Silverio, G., Alapatt, P., Millan, I., Shen, A., Saxton, W., Kanao, T., Takahashi, R., Hattori, N., Imai, Y., Lu, B., 2012. Parkinson's disease-associated kinase PINK1 regulates Miro protein level and axonal transport of mitochondria. PLoS Genet. 8, e1002537.
Longo, Valter, D., Shadel, Gerald, S., Kaeberlein, M., Kennedy, B., 2012. Replicative and chronological aging in *Saccharomyces cerevisiae*. Cell Metab. 16, 18–31.
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217.
Lovat, F., Valeri, N., Croce, C.M., 2011. MicroRNAs in the pathogenesis of cancer. Semin. Oncol. 38, 724–733.
Lu, J., Holmgren, A., 2014. The thioredoxin antioxidant system. Free Radic. Biol. Med. 66, 75–87.
Lucanic, M., Graham, J., Scott, G., Bhaumik, D., Benz, C.C., Hubbard, A., Lithgow, G.J., Melov, S., 2013. Age-related micro-RNA abundance in individual *C. elegans*. Aging (Albany NY) 5, 394–411.
Ma, X.M., Blenis, J., 2009. Molecular mechanisms of mTOR-mediated translational control. Nat. Rev. Mol. Cell Biol. 10, 307–318.
Maes, O.C., An, J., Sarojini, H., Wang, E., 2008. Murine microRNAs implicated in liver functions and aging process. Mech. Ageing Dev. 129, 534–541.
Mansfield, K.D., Keene, J.D., 2012. Neuron-specific ELAV/Hu proteins suppress HuR mRNA during neuronal differentiation by alternative polyadenylation. Nucleic Acids Res. 40, 2734–2746.
Marchal, J., Dal-Pan, A., Epelbaum, J., Blanc, S., Mueller, S., Wittig Kieffer, M., Metzger, F., Aujard, F., Consortium, R., 2013a. Calorie restriction and resveratrol supplementation prevent age-related DNA and RNA oxidative damage in a non-human primate. Exp. Gerontol. 48, 992–1000.
Marchal, J., Pifferi, F., Aujard, F., 2013b. Resveratrol in mammals: effects on aging biomarkers, age-related diseases, and life span. Ann. N.Y. Acad. Sci. 1290, 67–73.
Mariner, P.D., Walters, R.D., Espinoza, C.A., Drullinger, L.F., Wagner, S.D., Kugel, J.F., Goodrich, J.A., 2008. Human Alu RNA is a modular transacting repressor of mRNA transcription during heat shock. Mol. Cell 29, 499–509.
Martianov, I., Ramadass, A., Serra Barros, A., Chow, N., Akoulitchev, A., 2007. Repression of the human dihydrofolate reductase gene by a non-coding interfering transcript. Nature 445, 666–670.
Massirer, K.B., Perez, S.G., Mondol, V., Pasquinelli, A.E., 2012. The miR-35-41 family of microRNAs regulates RNAi sensitivity in *Caenorhabditis elegans*. PLoS Genet. 8, e1002536.
Masuda, K., Kuwano, Y., Nishida, K., Rokutan, K., Imoto, I., 2013. NF90 in post-transcriptional gene regulation and microRNA biogenesis. Int. J. Mol. Sci. 14, 17111–17121.
Masuda, K., Marasa, B., Martindale, J.L., Halushka, M.K., Gorospe, M., 2009. Tissue- and age-dependent expression of RNA-binding proteins that influence mRNA turnover and translation. Aging (Albany NY) 1, 681–698.
Mattison, J.A., Roth, G.S., Beasley, T.M., Tilmont, E.M., Handy, A.M., Herbert, R.L., Longo, D.L., Allison, D.B., Young, J.E., Bryant, M., Barnard, D., Ward, W.F., Qi, W., Ingram, D.K., de Cabo, R., 2012. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature 489, 318–321.
McColl, G., Rogers, A.N., Alavez, S., Hubbard, A.E., Melov, S., Link, C.D., Bush, A.I., Kapahi, P., Lithgow, G.J., 2010. Insulin-like signaling determines survival during stress via posttranscriptional mechanisms in *C. elegans*. Cell Metab. 12, 260–272.
McElwee, J., Bubb, K., Thomas, J.H., 2003. Transcriptional outputs of the *Caenorhabditis elegans* forkhead protein DAF-16. Aging Cell 2, 111–121.
Morley, J.F., Morimoto, R.I., 2004. Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. Mol. Biol. Cell 15, 657–664.
Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., Kenyon, C., 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. Nature 424, 277–283.
Nagaraja, A.K., Creighton, C.J., Yu, Z., Zhu, H., Gunaratne, P.H., Reid, J.G., Olokpa, E., Itamochi, H., Ueno, N.T., Hawkins, S.M., Anderson, M.L., Matzuk, M.M., 2010. A link between mir-100 and FRAP1/mTOR in clear cell ovarian cancer. Mol. Endocrinol. 24, 447–463.
Ng, S.Y., Johnson, R., Stanton, L.W., 2012. Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. EMBO J. 31, 522–533.
Novikova, I.V., Dharap, A., Hennelly, S.P., Sanbonmatsu, K.Y., 2013. 3S: shotgun secondary structure determination of long non-coding RNAs. Methods 63, 170–177.
Nunomura, A., Tamaoki, T., Motohashi, N., Nakamura, M., McKeel Jr., D.W., Tabaton, M., Lee, H.G., Smith, M.A., Perry, G., Zhu, X., 2012. The earliest stage of cognitive impairment in transition from normal aging to Alzheimer disease is marked by prominent RNA oxidation in vulnerable neurons. J. Neuropathol. Exp. Neurol. 71, 233–241.
Oh, S.W., Mukhopadhyay, A., Dixit, B.L., Raha, T., Green, M.R., Tissenbaum, H.A., 2006. Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. Nat. Genet. 38, 251–257.
Ota, H., Sakurai, M., Gupta, R., Valente, L., Wulff, B.E., Ariyoshi, K., Iizasa, H., Davuluri, R.V., Nishikura, K., 2013. ADAR1 forms a complex with Dicer to promote microRNA processing and RNA-induced gene silencing. Cell 153, 575–589.
Pan, K.Z., Palter, J.E., Rogers, A.N., Olsen, A., Chen, D., Lithgow, G.J., Kapahi, P., 2007. Inhibition of mRNA translation extends lifespan in *Caenorhabditis elegans*. Aging Cell 6, 111–119.
Pandey, R.R., Mondal, T., Mohammad, F., Enroth, S., Redrup, L., Komorowski, J., Nagano, T., Mancini-Dinardo, D., Kanduri, C., 2008. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. Mol. Cell 32, 232–246.
Pang, L., Tian, H., Chang, N., Yi, J., Xue, L., Jiang, B., Gorospe, M., Zhang, X., Wang, W., 2013. Loss of CARM1 is linked to reduced HuR function in replicative senescence. BMC Mol. Biol. 14, 15.
Parsons, P., 2007. Antagonistic pleiotropy and the stress theory of aging. Biogerontology 8, 613–617.
Pincus, Z., Smith-Vikos, T., Slack, F.J., 2011. MicroRNA predictors of longevity in *Caenorhabditis elegans*. PLoS Genet. 7, e1002306.
Pont, A.R., Sadri, N., Hsiao, S.J., Smith, S., Schneider, R.J., 2012. mRNA decay factor AUF1 maintains normal aging, telomere maintenance, and suppression of senescence by activation of telomerase transcription. Mol. Cell 47, 5–15.
Poulsen, H.E., Specht, E., Broedbaek, K., Henriksen, T., Ellervik, C., Mandrup-Poulsen, T., Tonnesen, M., Nielsen, P.E., Andersen, H.U., Weimann, A., 2012. RNA modifications by oxidation: a novel disease mechanism? Free Radic. Biol. Med. 52, 1353–1361.
Preissler, S., Deuerling, E., 2012. Ribosome-associated chaperones as key players in proteostasis. Trends Biochem. Sci. 37, 274–283.
Rajesh, K., Papadakis, A.I., Kazimierczak, U., Peidis, P., Wang, S., Ferbeyre, G., Kaufman, R.J., Koromilas, A.E., 2013. eIF2alpha phosphorylation bypasses premature senescence caused by oxidative stress and pro-oxidant antitumor therapies. Aging (Albany NY) 5, 884–901.
Ramos, S.B., Laederach, A., 2014. Molecular biology: a second layer of information in RNA. Nature 505, 621–622.
Rattan, S.I., 2010. Synthesis, modification and turnover of proteins during aging. Adv. Exp. Med. Biol. 694, 1–13.
Rinn, J.L., Chang, H.Y., 2012. Genome regulation by long noncoding RNAs. Annu. Rev. Biochem 81, 145–166.
Rippo, M.R., Olivieri, F., Monsurro, V., Prattichizzo, F., Albertini, M.C., Procopio, A.D., 2014. MitomiRs in human inflamm-aging: a hypothesis involving miR-181a, miR-34a and miR-146a. Exp. Gerontol. 56, 154–163.

Robida-Stubbs, S., Glover-Cutter, K., Lamming, Dudley, W., Mizunuma, M., Narasimhan, Sri, D., Neumann-Haefelin, E., Sabatini, David, M., Blackwell, T.K., 2012. TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab. 15, 713–724.

Rogers, Aric, N., Chen, D., McColl, G., Czerwieniec, G., Felkey, K., Gibson, Bradford, W., Hubbard, A., Melov, S., Lithgow, Gordon, J., Kapahi, P., 2011. Life span extension via eIF4G inhibition is mediated by posttranscriptional remodeling of stress response gene expression in *C. elegans*. Cell Metab. 14, 55–66.

Salmena, L., Poliseno, L., Tay, Y., Kats, L., Pandolfi, P.P., 2011. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 146, 353–358.

Sanchez, Y., Huarte, M., 2013. Long non-coding RNAs: challenges for diagnosis and therapies. Nucleic Acid Ther. 23, 15–20.

Schimanski, L.A., Barnes, C.A., 2010. Neural protein synthesis during aging: effects on plasticity and memory. Front. Aging Neurosci., 2.

Selman, C., Tullet, J.M.A., Wieser, D., Irvine, E., Lingard, S.J., Choudhury, A.I., Claret, M., Al-Qassab, H., Carmignac, D., Ramadani, F., Woods, A., Robinson, I.C.A., Schuster, E., Batterham, R.L., Kozma, S.C., Thomas, G., Carling, D., Okkenhaug, K., Thornton, J.M., Partridge, L., Gems, D., Withers, D.J., 2009. Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science 326, 140–144.

Seong, I.S., Woda, J.M., Song, J.J., Lloret, A., Abeyrathne, P.D., Woo, C.J., Gregory, G., Lee, J.M., Wheeler, V.C., Walz, T., Kingston, R.E., Gusella, J.F., Conlon, R.A., MacDonald, M.E., 2010. Huntingtin facilitates polycomb repressive complex 2. Hum. Mol. Genet. 19, 573–583.

Shalgi, R., Hurt, J.A., Krykbaeva, I., Taipale, M., Lindquist, S., Burge, C.B., 2013. Widespread regulation of translation by elongation pausing in heat shock. Mol. Cell 49, 439–452.

Shan, X., Chang, Y., Lin, C.L., 2007. Messenger RNA oxidation is an early event preceding cell death and causes reduced protein expression. FASEB J. 21, 2753–2764.

Sheaffer, K.L., Updike, D.L., Mango, S.E., 2008. The target of rapamycin pathway antagonizes pha-4/FoxA to control development and aging. Curr. Biol. 18, 1355–1364.

Shervington, A., Patel, R., 2008. Differential hTERT mRNA processing between young and older glioma patients. FEBS Lett. 582, 1707–1710.

Sheth, U., Parker, R., 2006. Targeting of aberrant mRNAs to cytoplasmic processing bodies. Cell 125, 1095–1109.

Shigeoka, T., Lu, B., Holt, C.E., 2013. Cell biology in neuroscience: RNA-based mechanisms underlying axon guidance. J. Cell Biol. 202, 991–999.

Simone, L.E., Keene, J.D., 2013. Mechanisms coordinating ELAV/Hu mRNA regulons. Curr. Opin. Genet. Dev. 23, 35–43.

Slack, C., Giannakou, M.E., Foley, A., Goss, M., Partridge, L., 2011. dFOXO-independent effects of reduced insulin-like signaling in Drosophila. Aging Cell 10, 735–748.

Smith-Vikos, T., Slack, F.J., 2012. MicroRNAs and their roles in aging. J. Cell Sci. 125, 7–17.

Spitale, R.C., Tsai, M.C., Chang, H.Y., 2011. RNA templating the epigenome: long noncoding RNAs as molecular scaffolds. Epigenetics 6, 539–543.

Squires, J.E., Patel, H.R., Nousch, M., Sibbritt, T., Humphreys, D.T., Parker, B.J., Suter, C.M., Preiss, T., 2012. Widespread occurrence of 5-methylcytosine in human coding and non-coding RNA. Nucleic Acids Res. 40, 5023–5033.

Steffen, K.K., MacKay, V.L., Kerr, E.O., Tsuchiya, M., Hu, D., Fox, L.A., Dang, N., Johnston, E.D., Oakes, J.A., Tchao, B.N., Pak, D.N., Fields, S., Kennedy, B.K., Kaeberlein, M., 2008. Yeast life span extension by depletion of 60S ribosomal subunits is mediated by Gcn4. Cell 133, 292–302.

Stein, P.K., Soare, A., Meyer, T.E., Cangemi, R., Holloszy, J.O., Fontana, L., 2012. Caloric restriction may reverse age-related autonomic decline in humans. Aging Cell 11, 644–650.

Stout, G.J., Stigter, E.C.A., Essers, P.B., Mulder, K.W., Kolkman, A., Snijders, D.S., van den Broek, N.J.F., Betist, M.C., Korswagen, H.C., MacInnes, A.W., Brenkman, A.B., 2013. Insulin/IGF-1-mediated longevity is marked by reduced protein metabolism. Mol. Syst. Biol., 9.

Syntichaki, P., Troulinaki, K., Tavernarakis, N., 2007. eIF4E function in somatic cells modulates ageing in *Caenorhabditis elegans*. Nature 445, 922–926.

Tanaka, M., Chock, P.B., Stadtman, E.R., 2007. Oxidized messenger RNA induces translation errors. Proc. Natl. Acad. Sci. U. S. A. 104, 66–71.

Tavernarakis, N., 2007. Protein synthesis and aging: eIF4E and the soma vs. germline distinction. Cell Cycle 6, 1168–1171.

Teixeira, D., Sheth, U., Valencia-Sanchez, M.A., Brengues, M., Parker, R., 2005. Processing bodies require RNA for assembly and contain nontranslating mRNAs. RNA 11, 371–382.

Teleman, A.A., 2010. miR-200 de-FOGs insulin signaling. Cell Metab. 11, 8–9.

Tollervey, J.R., Curk, T., Rogelj, B., Briese, M., Cereda, M., Kayikci, M., Konig, J., Hortobagyi, T., Nishimura, A.L., Zupunski, V., Patani, R., Chandran, S., Rot, G., Zupan, B., Shaw, C.E., Ule, J., 2011. Characterizing the RNA targets and position-dependent splicing regulation by TDP-43. Nat. Neurosci. 14, 452–458.

Troy, A., Sharpless, N.E., 2012. Genetic "lnc"-age of noncoding RNAs to human disease. J. Clin. Invest. 122, 3837–3840.

Tsai, M.C., Manor, O., Wan, Y., Mosammaparast, N., Wang, J.K., Lan, F., Shi, Y., Segal, E., Chang, H.Y., 2010. Long noncoding RNA as modular scaffold of histone modification complexes. Science 329, 689–693.

Tullet, J.M.A., Hertweck, M., An, J.H., Baker, J., Hwang, J.Y., Liu, S., Oliveira, R.P., Baumeister, R., Blackwell, T.K., 2008. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in *C. elegans*. Cell 132, 1025–1038.

Uniacke, J., Holterman, C.E., Lachance, G., Franovic, A., Jacob, M.D., Fabian, M.R., Payette, J., Holcik, M., Pause, A., Lee, S., 2012. An oxygen-regulated switch in the protein synthesis machinery. Nature 486, 126–129.

van de Vondervoort, I.I.G.M., Gordebeke, P.M., Khoshab, N., Tiesinga, P.H.E., Buitelaar, J.K., Kozicz, T., Aschrafi, A., Glennon, J.C., 2013. Long non-coding RNAs in neurodevelopmental disorders. Front. Mol. Neurosci. 6, 53.

van Heesch, S., van Iterson, M., Jacobi, J., Boymans, S., Essers, P.B., de Bruijn, E., Hao, W., MacInnes, A.W., Cuppen, E., Simonis, M., 2014. Extensive localization of long noncoding RNAs to the cytosol and mono- and polyribosomal complexes. Genome Biol. 15, R6.

Varghese, J., Lim, S.F., Cohen, S.M., 2010. Drosophila miR-14 regulates insulin production and metabolism through its target, sugarbabe. Genes Dev. 24, 2748–2753.

Vellai, T., Takacz Vellai, K., 2010. Regulation of protein turnover by longevity pathways. Adv. Exp. Med. Biol. 694, 69–80.

Wan, Y., Qu, K., Zhang, Q.C., Flynn, R.A., Manor, O., Ouyang, Z., Zhang, J., Spitale, R.C., Snyder, M.P., Segal, E., Chang, H.Y., 2014. Landscape and variation of RNA secondary structure across the human transcriptome. Nature 505, 706–709.

Wang, J., Robida-Stubbs, S., Tullet, J.M.A., Rual, J.-F., Vidal, M., Blackwell, T.K., 2010. RNAi screening implicates a SKN-1-dependent transcriptional response in stress resistance and longevity deriving from translation inhibition. PLoS Genet. 6, e1001048.

Wang, K.C., Yang, Y.W., Liu, B., Sanyal, A., Corces-Zimmerman, R., Chen, Y., Lajoie, B.R., Protacio, A., Flynn, R.A., Gupta, R.A., Wysocka, J., Lei, M., Dekker, J., Helms, J.A., Chang, H.Y., 2011. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature 472, 120–124.

Wang, X., Arai, S., Song, X., Reichart, D., Du, K., Pascual, G., Tempst, P., Rosenfeld, M.G., Glass, C.K., Kurokawa, R., 2008. Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. Nature 454, 126–130.

Wang, Y., Li, Y., Toth, J.I., Petroski, M.D., Zhang, Z., Zhao, J.C., 2014. N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat. Cell Biol. 16, 191–198.

Weidner, A.M., Bradley, M.A., Beckett, T.L., Niedowicz, D.M., Dowling, A.L., Matveev, S.V., LeVine, H. 3rd, Lovell, M.A., Murphy, M.P., 2011. RNA oxidation adducts 8-OHG and 8-OHA change with Abeta42 levels in late-stage Alzheimer's disease. PLoS ONE 6, e24930.

Weil, T.T., Parton, R.M., Herpers, B., Soetaert, J., Veenendaal, T., Xanthakis, D., Dobbie, I.M., Halstead, J.M., Hayashi, R., Rabouille, C., Davis, I., 2012. Drosophila patterning is established by differential association of mRNAs with P bodies. Nat. Cell Biol. 14, 1305–1313.

Wessells, R., Fitzgerald, E., Piazza, N., Ocorr, K., Morley, S., Davies, C., Lim, H.-Y., Elmén, L., Hayes, M., Oldham, S., Bodmer, R., 2009. d4eBP acts downstream of both dTOR and dFoxo to modulate cardiac functional aging in Drosophila. Aging Cell 8, 542–552.

Willcox, B.J., Donlon, T.A., He, Q., Chen, R., Grove, J.S., Yano, K., Masaki, K.H., Willcox, D.C., Rodriguez, B., Curb, J.D., 2008. FOXO3A genotype is strongly associated with human longevity. Proc. Natl. Acad. Sci. U. S. A. 105, 13987–13992.

Willingham, A.T., Orth, A.P., Batalov, S., Peters, E.C., Wen, B.G., Aza-Blanc, P., Hogenesch, J.B., Schultz, P.G., 2005. A strategy for probing the function of non-coding RNAs finds a repressor of NFAT. Science 309, 1570–1573.

Wolfson, M., Budovsky, A., Tacutu, R., Fraifeld, V., 2009. The signaling hubs at the crossroad of longevity and age-related disease networks. Int. J. Biochem. Cell Biol. 41, 516–520.

Xu, P., Vernooy, S.Y., Guo, M., Hay, B.A., 2003. The Drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Curr. Biol. 13, 790–795.

Xue, S., Barna, M., 2012. Specialized ribosomes: a new frontier in gene regulation and organismal biology. Nat. Rev. Mol. Cell Biol. 13, 355–369.

Yap, K.L., Li, S., Munoz-Cabello, A.M., Raguz, S., Zeng, L., Mujtaba, S., Gil, J., Walsh, M.J., Zhou, M.M., 2010. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. Mol. Cell 38, 662–674.

Yoon, A., Peng, G., Brandenburger, Y., Zollo, O., Xu, W., Rego, E., Ruggero, D., 2006. Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita. Science 312, 902–906.

Zhang, Y., Nicholatos, J., Dreier, J.R., Ricoult, S.J.H., Widenmaier, S.B., Hotamisligil, G.S., Kwiatkowski, D.J., Manning, B.D., 2014. Coordinated regulation of protein synthesis and degradation by mTORC1. Nature 513, 440–443.

Zhao, J., Ohsumi, T.K., Kung, J.T., Ogawa, Y., Grau, D.J., Sarma, K., Song, J.J., Kingston, R.E., Borowsky, M., Lee, J.T., 2010. Genome-wide identification of polycomb-associated RNAs by RIP-seq. Mol. Cell 40, 939–953.

Zhu, C., Ji, C.B., Zhang, C.M., Gao, C.L., Zhu, J.G., Qin, D.N., Kou, C.Z., Zhu, G.Z., Shi, C.M., Guo, X.R., 2010. The lin-4 gene controls fat accumulation and longevity in *Caenorhabditis elegans*. Int. J. Mol. Sci. 11, 4814–4825.

Zid, B.M., Rogers, A.N., Katewa, S.D., Vargas, M.A., Kolipinski, M.C., Lu, T.A., Benzer, S., Kapahi, P., 2009. 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in Drosophila. Cell 139, 149–160.