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Signaling pathways regulating protein synthesis during ageing

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Abstract

Ageing in many organisms, including humans, is accompanied by marked alterations in both general and specific protein synthesis. Protein synthesis is normally under tight control by a broad array of regulatory factors, which facilitate appropriate rates of mRNA translation. Are the wide changes in protein synthesis simply a corollary of the ageing process or do they have a causative role in senescent decline? The jury is still out on this important question. Nevertheless, recent studies reveal an intimate interface between mechanisms that govern the translation of mRNA and molecular pathways implicated in ageing. In our manuscript we consider these links, which potentially underlie age-associated changes in protein synthesis.

Keywords: *Caenorhabditis elegans*; *Drosophila melanogaster*; IGF-1; Insulin; mRNA; TOR kinase; Translation factor; Yeast; P-bodies

1. Introduction

The process of protein synthesis involves three major, tightly regulated, events; initiation of mRNA translation, elongation of the polypeptide chain, and termination of mRNA translation. Initiation is the rate-limiting step in mRNA translation and is the most common target of translational control (Gingras et al., 1999). Several cellular signaling mechanisms converge to influence the rate of mRNA translation, in response to a variety of stimuli, by modulating the activity or the availability of important translational regulators (Proud, 2004). Alterations in protein synthesis occur during embryonic development, cell growth, cell differentiation, and ageing. Signal transduction cascades, such as the insulin/insulin growth factor 1 (IGF-1), the kinase *target of rapamycin* (TOR), and p38 mitogen-activated protein kinase (MAPK) pathways, play a key role in the global control of protein synthesis by targeting several components of the translation machinery (Proud, 2004; Wang et al., 1998). For example, a variety of agents that promote cell growth and proliferation, including hormones, growth factors and nutrients, have stimulatory effects on the initiation of protein synthesis (Gingras et al., 2004). Global control of protein synthesis is generally achieved by changes in the phosphorylation state of initiation factors or their regulators. The rate of protein synthesis is mainly determined by regulation of two discrete steps during mRNA translation initiation; recruitment of the 40S ribosomal subunit at the 5′ end of mRNA and loading of the 40S ribosomal subunit with the initiator methionyl-tRNA (Gebauer and Hentze, 2004). These events are coordinated by initiation factors eIF4E and eIF2/eIF2B respectively. Phosphorylation of the eIF2 α-subunit regulates dissociation of the eIF2B/eIF2 complex and eIF2 recycling (Gebauer and Hentze, 2004). Similarly, the availability of active eIF4E is controlled by phosphorylation of eIF4E binding proteins (4E-BPs; Richter and Sonenberg, 2005).

Global mRNA translation is reduced in response to most, if not all types of cellular stress. Studies of mRNA translation regulation under conditions of stress have focused on the formation and regeneration of the eIF2–methionyl-initiator tRNA-GTP ternary complex, and the recruitment of ribosomes on the mRNA (Holcik and Sonenberg, 2005). It is also well documented that protein synthesis rates and the activities of key mRNA translation

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factors decline with age in a variety of organisms (Rattan, 1996). For example, old mice tend to show an increase in small polysomes and a decrease in large polysomes, compared to young individuals, which is consistent with a reduction in the rate of translation (Makrides and Goldthwaite, 1984). Supporting evidence from *Drosophila melanogaster* shows that polyribosome levels exhibit a marked, age-related decrease (Webster and Webster, 1983). It is not clear whether such alterations are a consequence of the ageing process – for example, an adaptation to reduced mitochondrial function and energy production – or they contribute to senescent decline. The long-term effects of these alterations on maintenance, repair, and survival are still obscure. Early concepts such as the error-catastrophe hypothesis and the somatic mutation theory, suggested that erroneous synthesis is responsible for the progressive accumulation of damaged macromolecules within cells (Orgel, 1973). However, mRNA translation fidelity in cell-free extracts does not change with age, and no age-related increase in amino acid misincorporation in proteins was observed in human fibroblasts and in *Caenorhabditis elegans* (Johnson and McCaffrey, 1985; Rattan, 1996). In addition, there is very little change in the total RNA or rRNA content and only moderate change in the abundance of poly(A)$^+$ mRNA with increasing chronological age in worms (Fabian and Johnson, 1995). More recent, genome-wide studies in *C. elegans*, *Drosophila*, and mammals have revealed relative few changes in gene expression, with potential to influence protein synthesis, associated with ageing (Hamilton et al., 2005; Lund et al., 2002; Park and Prolla, 2005). It should be noted that while a drop of bulk protein turnover emerges as a common theme coupled with the ageing process, not all proteins are uniformly affected. Tissue-specific alterations have been reported, and individual protein levels do not always parallel the global trend (Park and Prolla, 2005). Here, we survey findings that link the ageing process with alterations in protein synthesis and discuss the molecular mechanisms underlying this association. Particularly, we elaborate on the regulation of key mRNA translational factors via insulin, nutrient and stress signaling pathways, and the effects of such integration on longevity.

2. Effects of the insulin/IGF-1 signaling on protein synthesis

The link between ageing and insulin/IGF-1 signaling was first discovered in *C. elegans* and has also been studied extensively in flies and rodents (Gems and Partridge, 2001). These elegant studies have demonstrated that the role of insulin/IGF-1 signaling in the regulation of lifespan is evolutionarily conserved (Tatar et al., 2003). In *C. elegans*, reduced function of the DAF-2 insulin/IGF-1 receptor and the phosphatidylinositol-3 (PI3) effector phosphatidylinositol-3 (PI3) kinase, AGE-1, compromises insulin/IGF-1 signaling and dramatically extends animal lifespan (Friedman and Johnson, 1988; Kenyon et al., 1993; Kimura et al., 1997). Longevity is dependent on the activity of DAF-16, a FOXO transcription factor. DAF-16 controls a wide-variety of downstream targets, affecting stress resistance, fat accumulation, fertility and metabolism (Gems and Partridge, 2001). In *Drosophila*, mutations in both the insulin-like receptor (*InR*) and the insulin-receptor substrate (*chico*) prolong lifespan in homozygous female flies, with evidence that dFOXO is also required for longevity (Clancy et al., 2001; Tatar et al., 2001). In the yeast *Saccharomyces cerevisiae* mutations in the protein kinase SCH9, which is homologous to the Akt/protein kinase B (PKB), increase lifespan and stress resistance (Fabrizio et al., 2001). Akt/PKB is implicated in the insulin/IGF-1 signaling and longevity in worms, flies, and mammals.

In mammals, separate receptors for insulin and IGF-1 mediate distinct signaling events in different tissues (Yang et al., 2005). Insulin coordinates cellular metabolism, whereas IGF-1 regulates growth and differentiation. Mutations in either receptor gene or in upstream genes that regulate insulin and IGF-1 levels extend lifespan (Bluher et al., 2003; Flurkey et al., 2001; Holzenberger et al., 2003). For example, the Ames and Snell dwarf mice, which have low levels of serum insulin, IGF-1 and growth hormone, live significantly longer than their normal siblings, as do dwarf mice mutant for the growth hormone receptor (Flurkey et al., 2001). These mice carry mutations in the *Prop*1 and *Pouf*I transcription factors and show pituitary gland defects (Yang et al., 2005). The underlying molecular mechanisms that mediate these effects on lifespan are not clear. Interestingly, the rate of protein synthesis is decreased significantly in long-lived Snell dwarf mice (Hsieh and Papaconstantinou, 2004). Downregulation of mRNA translation is the result of reduced insulin/IGF-1 signaling via the Akt/PKB and p38 MAPK kinases, which in turn control key translation regulators such as the mammalian target of rapamaycin (mTOR), the ribosomal S6 kinase (S6K), the eIF4E MAP-kinase interacting kinase 1 kinase (Mnk1), and the translation initiation factor eIF4E, either directly or via inhibitory interaction with the eIF4E binding protein 1 (4E-BP1; Hsieh and Papaconstantinou, 2004). A similar mechanism mediating protein synthesis reduction appears to operate in long-lived Ames dwarf mice, where PI3/Akt/mTOR signaling is attenuated (Sharp and Bartke, 2005). Therefore, low protein synthesis is a common denominator of both Ames and Snell long-lived, dwarf mice.

3. The TOR kinase: a multifaceted regulator of protein synthesis

The *target of rapamycin* (TOR), an evolutionary conserved Ser/Thr kinase, has emerged as a central regulator of cell growth and proliferation, development, metabolism, and ageing. TOR interfaces with several cellular processes such as DNA transcription, mRNA translation, protein turnover, autophagy and actin cytoskeletal organization among others (Martin and Hall, 2005). TOR activity is regulated by four major input signals: nutrient and energy availability, growth factors and stress (Fig. 1; Wullschleger

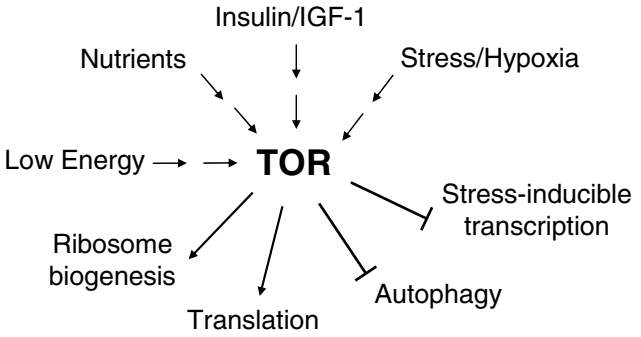

Fig. 1. TOR kinase integrates diverse inputs to modulate gene expression and protein turnover, under conditions of stress and during ageing.

et al., 2006). Genetic and biochemical studies in yeast, *C. elegans*, *Drosophila*, and mammalian cells have identified several upstream and downstream components of the TOR signaling pathway (Hay and Sonenberg, 2004). One of the best-characterized functions of mammalian TOR (mTOR) is the regulation of protein synthesis. Biochemical analysis has revealed an orchestrated sequence of events that promote efficient protein synthesis upon conditions that activate mTOR (Holz et al., 2005). Two key downstream targets of mTOR are the ribosomal S6 kinase (S6K) and the eIF4E binding protein 1 (4E-BP1, Table 1; Proud, 2004). S6K phosphorylates the 40S ribosomal protein S6. 4E-BP1 acts as a translational repressor since it competes with eIF4G for an overlapping binding site on eIF4E. Phosphorylation of 4E-BP promotes its dissociation from eIF4E, allowing for recruitment of eIF4G and eIF4A translational factors to the mRNA cap structure (Gingras et al., 1999). mTOR also regulates other translation initiation factors, such as eIF4GI and eIF4B, as well as elongation factors, such as eEF2 (Hay and Sonenberg, 2004). In addition to translation regulation, mTOR modulates autophagy, a lysosomal catabolic pathway for degradation and turnover of proteins and organelles. Under nutrient deprivation or environmental stresses (such as heat shock, osmotic shock, or UV irradiation) reduced mTOR activity generally reduces protein synthesis and induces autophagy in the cell (Lum et al., 2005).

Recent studies in yeast, *C. elegans* and *Drosophila* have implicated signaling via the TOR kinase in the regulation of ageing. In the following sections, we discuss the potential link between TOR effects on lifespan and the regulation of protein synthesis.

Table 1
Regulatory modifications on key translation factors, executed by effector kinases

Translation factor	Function	Phosphorylated by
eIF4E	Cap-binding/translation initiation	Mnk1
4E-BP	Inhibitor of eIF4E/translation initiation	TOR
S6K	Ribosome biosynthesis	TOR
eIF4B	mRNA translation initiation	S6K
eIF4GI	eIF4F assembly/translation initiation	TOR
eIF2B	eIF2-GTP exchange/translation initiation	Akt/PKB
eEF2K	Polypeptide chain elongation	S6K
S6	Ribosome biosynthesis	S6K

3.1. TOR signaling and ageing in yeast

Genetic screens in *S. cerevisiae* have implicated several genes encoding components of the TOR signaling pathway in the regulation of both replicative and chronological ageing (Kaeberlein et al., 2005; Powers et al., 2006). Replicative (or mitotic) lifespan is defined by the number of daughter cells produced by an individual mother cell before senescence, whereas chronological (or post-mitotic) lifespan represents the time a non-dividing cell population remains viable in liquid media. Pharmacological inhibition of the TOR pathway or removal of amino acids from the culture media significantly increases stationary phase survival (Powers et al., 2006). Given that TOR responds to nutrient availability, it has been proposed that reduced TOR extends yeast lifespan by simulating calorie restriction (CR; Kaeberlein et al., 2005). Indeed, superimposition of CR failed to further increase the replicative lifespan of cells lacking TOR (Kaeberlein et al., 2005). In yeast, TOR signaling regulates transcription of specific ribosomal RNA genes in response to nutrient and energy signals (Wullschleger et al., 2006). Ribosome biogenesis requires large amounts of cellular energy. Therefore, reduced TOR activity results in ample savings of energy that could be diverted to repair and maintenance mechanisms, having a beneficial effect on ageing.

3.2. TOR signaling and ageing in C. elegans

In *C. elegans*, deficiency of TOR and the associated regulatory protein Raptor, encoded by the *let-363* and *daf-15* genes respectively, causes developmental arrest and intestinal atrophy (Jia et al., 2004; Long et al., 2002). Interestingly, the lifespan of these arrested animals or adults subjected to RNAi with *let-363* is significantly increased (Jia et al., 2004; Meissner et al., 2004; Vellai et al., 2003). Although this lifespan extension is independent of DAF-16, the downstream effector of the insulin/IGF-1-like signaling pathway in worms, mutations in *let-363* and *daf-2* synergize to increase animal longevity (Vellai et al., 2003). Thus, cross-talk between the TOR and DAF-2/insulin signaling pathways appears to regulate development, metabolism and lifespan in worms (Jia et al., 2004). Recent experiments suggest that the TOR pathway is also be involved in mediating the life-extending effect of dietary restriction (DR) in *C. elegans*. Deletion of the intestinal peptide transporter, *pep-2*, enhances the *let-363* RNAi phenotype (Meissner et al., 2004). Therefore, the TOR and insulin/IGF-1-like signaling pathways may integrate nutrient sensing and nutrient uptake to influence adult nematode lifespan.

What are the targets of TOR signaling that mediate ageing effects in *C. elegans*? There is no apparent 4E-BP ortholog in the nematode genome (Long et al., 2002; P.S. and

N.T. unpublished observations), However, the *let-363* and the *Raptor* mutant phenotypes are phenocopied by general mRNA translational initiation factor deficiency. For example, elimination of the *C. elegans* eIF4G homolog or subunits of eIF2 yields phenotypes that resemble CeTOR deficiency, indicating that TOR functions as a regulator of protein synthesis in worms (Long et al., 2002). One additional effector of TOR signaling related to protein turnover, that influences ageing in *C. elegans* is autophagy. It is noteworthy that autophagy is increased in long-lived *daf-2* mutants, and *bec-1* (the worm homolog of mammalian beclin 1, a protein essential for autophagosome formation) is required for lifespan extension in these mutants (Melendez et al., 2003).

3.3. TOR signaling and ageing in Drosophila

Genetic studies in both *D. melanogaster*, and mammals, have established a functional link between the TOR and the insulin/IGF-1 signaling pathways in controlling cell growth and overall organ size. Deletion of the single dTOR or dS6K in *Drosophila* results in small cell size and severely reduced body size, similarly to loss-of-function mutations in positive regulators of the insulin/IGF-1 pathway (Martin and Hall, 2005). Both pathways interact to modulate adult lifespan by regulating reproductive and metabolic genes. This is likely accomplished via systemic, humoral mechanisms, since downregulation of the dTOR pathway or activation of dFOXO specifically in the fat body of *Drosophila* extends lifespan of the fly (Giannakou et al., 2004; Hwangbo et al., 2004; Kapahi et al., 2004). Interestingly, dTOR-deficient flies display phenotypes characteristic of amino acid-deprived animals, suggesting that effects of dietary restriction on lifespan results are mediated by TOR signaling (Kapahi et al., 2004).

The key mRNA translation regulators d4E-BP/Thor and dS6K are downstream targets of the insulin and TOR signal-transduction cascades in *Drosophila* (Miron et al., 2003). Both translation factors have been implicated in lifespan regulation and d4E-BP activity is critical for survival under DR and oxidative stress (Tettweiler et al., 2005). 4E-BP expression is induced under various stress conditions, and has been proposed to function as a 'metabolic brake' controlling fat metabolism through the levels of eIF4E (Teleman et al., 2005). Consistently, the *Drosophila* Mnk1/2 kinase homolog Lk6, which also modulates the activity of eIF4E, is required for normal growth under adverse food conditions (Reiling et al., 2005). Survival during starvation is also promoted by induction of autophagy in *Drosophila* larval fat body, a process normally inhibited by TOR signaling (Scott et al., 2004).

4. Localized mRNA translation regulation during ageing

mRNA translation is tightly linked to the process of mRNA decay. Recent findings suggest that upon exiting translation mRNAs enter a translationally repressed state via a transition into cytoplasmic structures, known as processing bodies or P-bodies (Sheth and Parker, 2003). P-bodies are sites of mRNA decapping and degradation, containing decapping enzymes and other proteins, such as key components of the RNA interference (RNAi) machinery (the RISC complex). P-bodies can also temporarily sequester mRNAs (naked or repressed by microRNAs; miRNAs) away from the translation machinery, probably for storage (Brengues et al., 2005; Ding et al., 2005). Therefore, P-bodies appear to play a direct role in the regulation of protein synthesis, by balancing two key events: active mRNA translation onto polysomes and active repression into P-bodies. Due to limiting activity of translation initiation factors, most mRNAs are distributed between an actively translated and a non-translated pool in the cytoplasm of cells, and changes in the activity of these limiting translation factors elicit changes in global protein synthesis. Interestingly, yeast cells lacking critical proteins for decapping/repressing translation of mRNAs that also facilitate formation of P-bodies, are not capable of turning-off protein synthesis under conditions, where it would normally be repressed by the TOR pathway (for example, glucose deprivation or amino acid starvation; Coller and Parker, 2005). In mammalian cells, repression of translation and targeting of mRNAs to P-bodies occurs through the interaction of eIF4E with the 4E-transporter (4E-T), via a conserved eIF4E-recognition motif, also found in eIF4G and 4E-BP (Ferraiuolo et al., 2005). Colocalization of 4E-T with eIF4E and decapping factors appear to control mRNA stability and the transition from translation to decay (Ferraiuolo et al., 2005).

In addition to their important role in the global control of protein synthesis, P-bodies also facilitate mRNA-specific control by storing miRNA-repressed mRNAs. Interestingly, studies in *C. elegans* implicate miRNAs in lifespan regulation. The *lin-4* miRNA, which controls the timing of larval development by inhibiting translation of the *lin-14* mRNA, also regulates adult lifespan (Boehm and Slack, 2005). The *lin-4* miRNA may slow down senescent decline through repression of *lin-14* mRNA translation during adulthood (Boehm and Slack, 2005).

5. Concluding remarks

One of the major hallmarks of ageing is the progressive accumulation of molecular damage in nucleic acids, proteins, lipids, and other macromolecules (Tavernarakis and Driscoll, 2002). This, in turn, leads to inexorable deterioration of essential cellular functions and consequently, to senescence. The limited capacity of maintenance, repair, and turnover pathways is the main cause of age-related accumulation of damage. Protein turnover determines the rate at which a protein pool is getting refreshed with protein synthesis providing fresh proteins and protein degradation removing the existing and likely damaged protein molecules. A decline in turnover rates would delay the removal and replacement of damaged proteins thus

contributing to senescence. This notion supports the simplistic assumption that conditions, which favor maintenance of high protein turnover rates, could have a beneficial effect on longevity. However, this scenario bears significant caveats. Protein synthesis is one of the most energy-consuming cellular processes, devouring an estimated 50% of the total cellular energy, depending on the organism and cell growth state. For example, mRNA and ribosome biosynthesis, two processes controlled by insulin/IGF-1 and TOR signaling are highly energy-consuming (Hay and Sonenberg, 2004). Under favorable conditions, yeast cells synthesize ~2000 ribosomes per minute, in order to maintain robust growth (Martin and Hall, 2005). Therefore, reduction of protein synthesis rates under unfavorable, stress conditions would result in notable energy savings. This energy could then be diverted to cellular repair and maintenance processes, thus contributing to longevity. In addition, the reduction in mRNA translation may prevent the synthesis of unwanted proteins that could interfere with the cellular stress response. Remarkably, the stress-induced attenuation of global translation is often accompanied by a switch to the selective translation of proteins that are required for cell survival under stress. However, the mechanisms that govern preferential translation of specific mRNAs under stress are not understood.

Is the reduction of protein synthesis an integral component of a broader response to stress? We hypothesize that hormesis, a phenomenon where mild stress stimulates maintenance and repair mechanisms, may in part depend on lowering mRNA translation to levels that increase energy availability but allow essential protein production. Hormesis is associated with reduced accumulation of damaged proteins, stimulation of proteasomal activity, increased cellular resistance to toxic agents and often prolongs lifespan (Cypser and Johnson, 2002; Rattan, 2004). It remains to be seen whether moderating protein synthesis is an integral component of hormetic effects on ageing.

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