Protein Synthesis and Ageing

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Running title: **Linking protein synthesis to ageing**

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Abstract

Protein synthesis is an essential cellular process affecting growth, reproduction and survival in response to both intrinsic and extrinsic cues such as nutrient availability and energy levels. Studies in many organisms, including humans, have revealed that during ageing, the rate of global protein synthesis declines, indicating a link between the regulation of protein synthesis and the ageing process. Recent studies in *C. elegans* demonstrate that depletion of specific translation initiation factors, such as eIF4G, eIF2B and eIF4E increases lifespan. Similarly, depletion of specific ribosomal proteins increases lifespan both in yeast and worms. In all cases, these manipulations reduce the rate of general protein synthesis. Why does attenuation of protein synthesis promote longevity? The process of mRNA translation is one of the most energy consuming cellular processes, requiring, depending on growth conditions, up to 50% of the total energy generated by the cell. A reduction of protein synthesis would moderate this energy load, generating an energy surplus, which can be channeled to mechanisms of damage repair and cellular maintenance, thus, extending lifespan. In addition, lowering protein synthesis may be beneficial during ageing by reducing the accumulation of altered, misfolded, aggregated or damaged proteins, as it occurs in many age-related pathologies, such as Alzheimer's and Parkinson's disease. The recent experimental findings reveal a key role for protein synthesis in ageing and suggest that perturbation of mRNA translation provides an effective approach for interventions aiming to modulate ageing and senescent decline.

Key Words: Ageing, *Caenorhabditis elegans*, Lifespan, mRNA translation regulation, Protein modification

Introduction

To maintain their homeostasis and function, cells must continuously synthesize and degrade proteins in a highly regulated manner. The mechanisms of transcription and mRNA translation regulate the synthesis of new proteins. Damaged or aggregated proteins are removed by specialized and selective protein degradation mechanisms (Ding et al., 2007). Studies in various organisms, ranging from yeast to humans, revealed that both protein synthesis and protein degradation rates change during ageing (Makrides, 1983; Partridge and Gems, 2002; Rattan, 1996). The activity of key translation factors appears to decline with age, resulting in reduction of protein synthesis rates (Kimball et al., 1992; Moldave et al., 1979; Takahashi et al., 1985; Vargas and Castaneda, 1981, 1983; Webster and Webster, 1983). However, it was unclear whether these changes were simply a consequence of the general deterioration of the cellular functions that characterize ageing or they had a causative role in the process. Recent studies in the nematode *C. elegans* revealed that impeding mRNA translation significantly affects longevity, indicating that the levels of protein synthesis may affect ageing (Hansen et al., 2007; Kaeberlein and Kennedy, 2007; Pan et al., 2007; Syntichaki et al., 2007).

Down-regulation of protein synthesis extends lifespan

Protein synthesis or the translation of mRNA is a conserved process involving three main steps: initiation, elongation and termination. Initiation of translation requires the concerted action of a large number of proteins known as translation initiation factors (eIFs). These factors recognize the cap structure at the 5' end of mRNA and allow the binding of the 40S ribosome subunit that scans downstream for the initiation codon. The next step is the elongation, during which the fully assembled ribosome reads the transcript and uses amino acid-charged tRNAs to synthesize the peptide chain, with the participation of elongation factors. Finally, the process is terminated when a release factor binds to the final (STOP) codon and releases the complete polypeptide from the ribosome that breaks apart into its two subunits (Gebauer and Hentze, 2004; Proud, 2007).

All steps of protein synthesis are tightly regulated and are carried out with superb precision, ensuring the fidelity of the proteins. Many studies have demonstrated that mRNA translation fidelity does not change

during ageing (Filion and Laughrea, 1985). However, it has been shown that the rate of protein synthesis declines with age in a variety of organisms (Makrides, 1983; Partridge and Gems, 2002; Rattan, 1996).

Initiation, the first step of mRNA translation, is a rate-limiting process and the most common target of protein synthesis control. The initiation factor eIF4E plays a key role in the process, by recognizing the 5'-end cap structure of most eukaryotic mRNAs and facilitating their recruitment to the ribosomes (Gingras et al., 1999). In the nematode *C. elegans*, five eIF4E isoforms, termed IFE-1 - IFE-5, are encoded in the genome (Keiper et al., 2000). IFE-1, IFE-3 and IFE-5 are expressed in the germline, whereas IFE-2 and IF-4 are expressed specifically in somatic cells (Keiper et al., 2000). Depletion of the isoforms that are expressed in the germline, or of the somatic isoform IFE-4, does not affect lifespan (Syntichaki et al., 2007). However, loss of IFE-2, which is the most abundant in the soma causes significant extension of lifespan (Syntichaki et al., 2007). This result indicates that down-regulation of protein synthesis specifically in the soma extends lifespan. Interestingly, the observed lifespan extension does not require a functional germline, since lack of germline does not suppress the effect of IFE-2 deficiency (Syntichaki et al., 2007). Notably, depletion of the IFE-1 during adulthood leads to moderate adult lifespan extension, indicating that IFE-1 also modulates longevity (Pan et al., 2007).

Other studies in the nematode have further revealed that elimination of other translation initiation factors or their regulators, after completion of the development of the organism, results in similar effects on adult lifespan. For example, knocking down of eIF4G (*ifg-1*) by RNAi or the eIF2 beta subunit (*iftb-1*) during adulthood results in 30% increase of lifespan (Chen et al., 2007; Hansen et al., 2007; Pan et al., 2007). In addition, RNAi with several ribosomal proteins or the ribosomal-protein S6 kinase (S6K) –after completion of development- leads to increased lifespan. In all cases, the rate of protein synthesis in "long-lived" animals is reduced, compared to wild type control animals (Hansen et al., 2007; Pan et al., 2007). Furthermore, an RNAi screen for essential genes that extend lifespan when inactivated post-developmentally has revealed many genes encoding for several components of the eIF complex and the ribosome. These include *C. elegans* homologs of eIF2G, eIF3F and eIF4A (Chen et al., 2007; Curran and Ruvkun, 2007).

Signaling pathways that regulate protein synthesis and ageing

Translation of mRNA is a highly regulated process that enables the cell to fine-tune gene expression by stimulating or repressing translation of specific mRNAs, usually through the reversible phosphorylation of mRNA translation factors. Various signaling pathways, activated by hormones, growth factors and nutrients regulate protein synthesis (Figure 1). For example, the insulin-like pathway, the TOR pathway and the MAPK pathway are key signal transduction pathways implicated in ageing that also modulate protein synthesis (Gingras et al., 2004; Proud, 2007).

The insulin-IGF-1 pathway plays a vital role in the regulation of somatic growth and cellular proliferation and in parallel is a key modulator of ageing in various organisms (Guarente and Kenyon, 2000). The pathway is engaged by the insulin receptor that binds the insulin ligand and phosphorylates the phosphatidylinositol 3 kinase (PI3K) that generates phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) or phosphatidylinositol-3,4-biphosphate (PtdIns(3,4)P_2), which in turn activate the 3-phosphoinositide-dependent protein kinase 1 (PDK1). Subsequently, PDK1 activates the serine-threonine protein kinase AKT/protein kinase B (PKB). These kinases target the FOXO transcription factor DAF-16 and block its transcriptional activity. Mutations that down-regulate this signaling cascade- for example in the insulin/IGF-like receptor DAF-2 or the phosphatidylonisol-3-OH kinase (PI3K) AGE-1 have been found to extend nematode life span. The extension is dependent on the activity of DAF-16, a forkhead (FOXO) transcription factor that controls the expression of a variety of genes involved in stress resistance (superoxide dismutase, catalase, glutathione, heat-shock protein 16 and others), metabolism (apolipoproteins, glyoxylate cycle and cytochrome P450s) fat accumulation and fertility (Gems and Partridge, 2001; Kenyon, 2005). Similarly, mutations in the insulin-IGF-1 pathway in *Drosophila* (in the insulin-like receptor), also increase the lifespan of flies (Clancy et al., 2001; Tatar et al., 2001). For example, a mild reduction of the insulin-like receptor (Inr) increases mean female lifespan by up to 85% (Partridge and Gems, 2002). In mammals, different receptors for insulin and IGF-1 participate in divergent pathways in different tissues (Yang et al., 2005). Mutations that down-regulate either the insulin pathway or the IGF pathway, result in prolonged lifespan (Bluher et al., 2003; Flurkey et al., 2001; Holzenberger et al., 2003). In addition, such long lived mice have decreased rate of protein synthesis compared with the control animals (Hsieh and Papaconstantinou, 2004).

TOR (target of rapamycin) signaling is stimulated by serum, insulin and growth factors, and promotes protein synthesis through multiple outputs (Gingras et al., 2004; Proud, 2007). The most characterized effector is the ribosomal S6 kinase (S6K), which induces mRNA translation through phosphorylation of ribosomal protein S6 and through regulation of translation initiation factors, such as

eukaryotic initiation factor 4B (eIF4B) (Proud, 2004). In addition, TOR signaling promotes translation by regulating the activity of the initiation factor eIF4E. More specifically, TOR phosphorylates the eIF4E–binding protein (4E-BP) and liberates the eIF4E, enabling it to interact with eIF4G and to form a complex competent to mediate cap-dependent translation. Moreover, it has been found that the TOR pathway promotes transcription of genes encoding for ribosomal proteins. In many cell types, the TOR pathway is impaired under amino acid starvation. In this case many of the above proteins undergo dephosphorylation. Studies in *C. elegans* revealed that TOR deficiency, which dampens the rate of translation, extends lifespan (Vellai et al., 2003).

MAPK signaling pathway is also clearly linked with the control of the protein synthesis, by affecting a number of mRNA translational machinery components to promote the assembly of initiation factor complexes and the activation of the elongation machinery (Hsieh and Papaconstantinou, 2004; Proud, 2007). It contains several modules of which the best understood are the classical MAPK (ERK), p38 MAPK α/β and JNK (c-Jun N-terminal kinase) pathways. Each involves kinases that phosphorylate components of the translational machinery and/or other proteins that regulate mRNA translation. ERK activates the protein kinase RSKs (or $p90^{RSK}$s) that phosphorylates other kinases leading to activation of the TOR pathway. Moreover, RSKs phosphorylate at least two other proteins involved in translational control: the eEF2 kinase and eIF4B, promoting its association with eIF3. p38 MAPK α/β activates MK-2, which regulates the stability of mRNAs, probably through the phosphorylation of ARE-binding proteins. In addition, the MAPK signaling pathway leads to the phosphorylation and activation of the kinases Mnk1 and 2. Mnks bind to eIF4G and mediate eIF4E phosphorylation (Waskiewicz et al., 1999). The physiological significance of this phosphorylation is still unclear. However, it is notable that such phosphorylation is generally mediated by mitogen- or stress- and cytokine-activated signaling. In addition to eIF4E, Mnks also phosphorylate eIF4G.

Protein damage during ageing

One of the most common symptoms of ageing at the molecular level is the accumulation of altered proteins both within the cells and extracellularly (Hipkiss, 2006; Rothstein, 1975, 1979, 1989). Protein damage may result in the loss of protein function or it can also lead to protein aggregation. In the latter case, the interaction of damaged proteins with normal cellular proteins may cause sequestration and inhibition of key molecules, like transcription factors, cytoskeletal proteins, molecular chaperones and hydrolytic enzymes

(Hipkiss, 2006). Although protein modifications are continuously generated, through a variety of processes, cellular homeostatic mechanisms either suppress the formation of altered proteins, or enhance their destruction, thereby preventing their accumulation. Such protective mechanisms are the lysosomal and proteasomal pathways. Many studies, involving both biochemical and micro array expression assays, have shown that proteolytic activity decreases with age in many cell types (Gems and McElwee, 2003; Makrides, 1983; Martinez-Vicente et al., 2005; Sarkis et al., 1988; Szweda et al., 2002). Certain pathways of lysosomal protein degradation, such as macroautophagy and chaperone-mediated autophagy, exhibit age-dependent decline in function (Arslan et al., 2006). In addition, alterations in the activity of certain lysosomal enzymes, including cathepsins, have been reported to occur during ageing (Sarkis et al., 1988). Moreover, decline in the function of the proteasome during ageing has been observed in cultured cells and in tissues from various organisms, resulting in an increased half-life of oxidized proteins (Sitte et al., 2000a; Sitte et al., 2000b). This can be attributed to down regulation of genes that encode proteasome subunits and the accumulation of proteasome inhibitory proteins as a function of ageing. Interestingly, many age-related pathologies such as Alzheimer's disease, Parkinson's disease and atherosclerosis are characterized by increased levels of altered proteins, which are thought to be the cause of aged-related pathology (Hipkiss, 2006).

Why is lifespan extended when protein synthesis is reduced?

It is well known that mRNA translation is one of the most energy-consuming cellular processes (Proud, 2002). The addition of a single amino acid in the polypeptide chain during mRNA translation requires the energy released by the hydrolysis of four ATP molecules. The amount of total cellular energy that is devoted to translation varies between different cell types and growth states. For example, dividing cells spend more energy on mRNA translation than post-mitotic cells, given their higher requirement for protein synthesis. In all cases, a high proportion of cellular metabolic energy is used in translation and almost all is consumed during the elongation phase. Therefore, reduction of protein synthesis would lead to significant conservation of cellular energy. Extra energy could be channeled towards mechanisms of maintenance and repair, contributing to cell survival under stress conditions, such as oxidative stress (Figure 2). Interestingly, the basic concept of the "disposable soma" theory of ageing is that soma is mortal and frail because fails to divert energy towards repairing stochastic damage that accumulates during life (Kirkwood, 1977; Kirkwood and Austad, 2000). By contrast, the germ line may achieve immortality by investing most of the energy to mechanisms of repair.

In support of this hypothesis, it has been found that *ife-2 C. elegans* mutants, which are defective for a somatic isoform of the translation initiation factor eIF4E and have reduced rate of protein synthesis, are more resistant than wild type animals to oxidative stress induced by the herbicide paraquat and sodium azide, an inhibitor of the respiratory chain cytochrome c oxidase, (Syntichaki et al., 2007). Moreover, IFE-2 deficiency increases oxidative stress resistance and extends lifespan of *mev-1* mutants that are continuously under oxidative stress due to their lack of the cytochrome b large subunit in complex II of the mitochondrial electron transport chain (Ishii et al., 1998; Syntichaki et al., 2007). Resistance to oxidative stress corresponds to increased capability for detoxification and repair, indicating a higher capacity for damage repair in these mutants. Thus, down regulation of protein synthesis in the soma, due to elimination of a specific initiation factor of translation (eIF4E) leads to increased oxidative stress resistance and increased lifespan.

Concluding remarks and future prospects

Protein synthesis and protein degradation are the two essential processes that determine the rate of cellular protein turnover. The recent finding that down-regulation of mRNA translation leads to increase of lifespan establishes a direct link between protein synthesis and ageing (Hansen et al., 2007; Pan et al., 2007; Syntichaki et al., 2007). However, the exact mechanism through which protein biosynthesis affects ageing still remains unknown. Given that mRNA translation is one of the most energy consuming processes; its reduction would result in notable energy savings. This energy could be diverted to cellular repair and maintenance processes, thus contributing to longevity. Moreover, reduction of mRNA translation may prevent the synthesis of unwanted proteins that could interfere with the cellular stress response. Interestingly, under stress, global mRNA translation is attenuated, while there is a switch to selective translation of proteins that are required for cell survival under stress (Clemens, 2001; Holcik and Sonenberg, 2005). The mechanisms that regulate this switch are poorly understood. Mild stress is known to stimulate maintenance and repair mechanisms, a phenomenon known as “hormesis” (Mattson, 2008) Hormesis is associated with reduced accumulation of damaged proteins, stimulation of proteasomal activity and increased cellular resistance to toxic agents (Cypser and Johnson, 2002; Rattan, 2004). Hormesis has also been found to prolong lifespan. It is possible that hormesis depends on lowering protein synthesis to levels that increase energy availability but enable production of essential and vital proteins. In this context, it remains to be seen whether reducing protein synthesis is part of hormetic effects on ageing.

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Figure Legends

Figure 1. Signaling pathways that modulate protein synthesis by regulating the activity of specific translation factors.

Insulin-insulin growth factor 1 (INS-IGF-1) signaling is activated by the binding of insulin or IGF-1 to the insulin receptor and results in the activation of the phosphatidylinositol 3 kinase (PI3K). PI3K converts phosphatidylinositol (4,5) –bisphosphate (PIP2) to phosphatidylinositol (1,4,5)-trisphosphate (PIP3). PIP3 in turn activates the serine threonine protein kinase Akt (also known as protein kinase B; Akt/PKB) which phosphorylates and activates the S6 kinase (S6K), while it suppresses the serine threonine protein kinase GSK3 (glycogen synthase 3). The GSK3 kinase regulates the activity of the eukaryotic translation initiation factor 2B (eIF2B). S6K phosphorylates the small ribosomal subunit S6 and the eukaryotic initiation factor 4B (eIF4B). S6K can also be activated by the target of rapamycin (TOR) signaling pathway. In addition to S6K, the TOR pathway leads to the phosphorylation of the eukaryotic initiation factor 4E-binding protein (4E-BP), which inhibits protein synthesis by blocking the eukaryotic translation initiation factor 4E (eIF4E). In addition, TOR signaling can be activated by the mitogen activated protein kinase (MAPK) signaling cascade. The MAPK pathway stimulates the RSKs or $p90^{RSK}$s kinases that phosphorylate the eukaryotic translation initiation factor 4B (eIF4B) and the kinase of the eukaryotic elongation factor 2 (eEF2K). Moreover, MAPK signaling activates the mitogen-activated protein kinase-interacting kinases (Mnks) that in turn phosphorylate the eukaryotic translation initiation factor 4E (eIF4E) and the eukaryotic translation initiation factor 4G (eIF4G). Arrows indicate positive regulation events whereas the bar lines indicative negative regulation events.

Figure 2. A working model linking down regulation of protein synthesis to prolonged lifespan.

A) Normal lifespan: Cellular energy is distributed between both protein synthesis and repair. However, since protein synthesis is a highly energy-consuming process, the energy remaining for mechanisms of repair and maintenance is limited, resulting in progressive structural and functional deterioration and ageing.

B) Increased lifespan: Down-regulation of protein synthesis may lead to prolonged lifespan by allowing for more cellular energy to be channeled towards repair mechanisms, enabling the cell to better withstand stress.

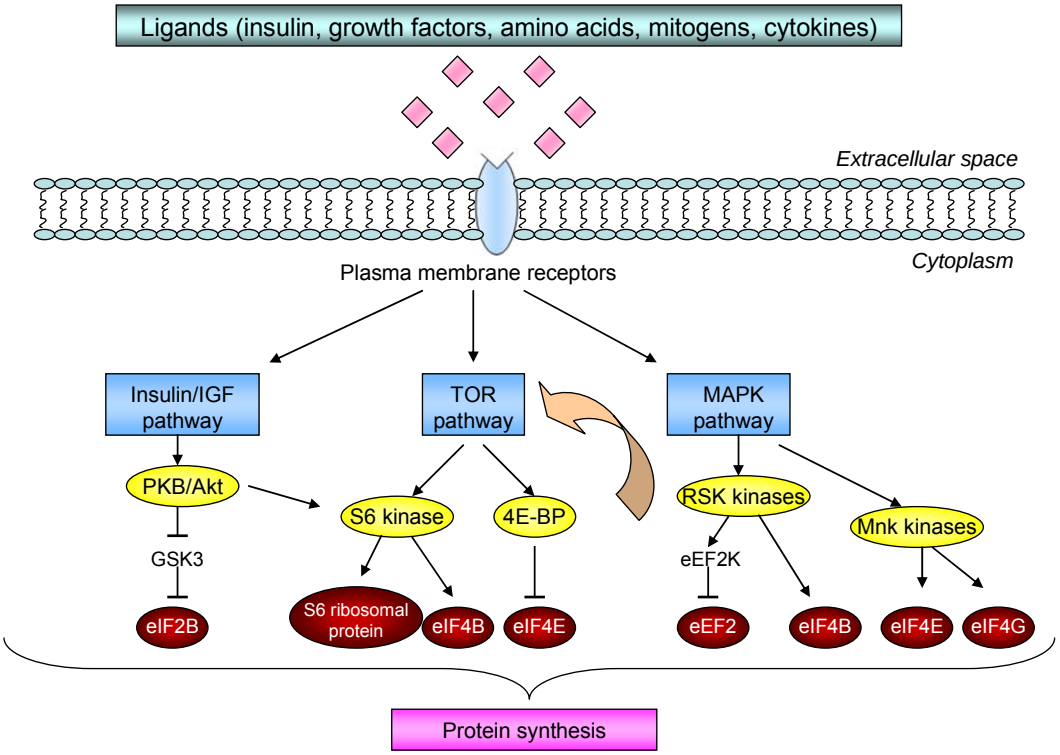

Figure 1
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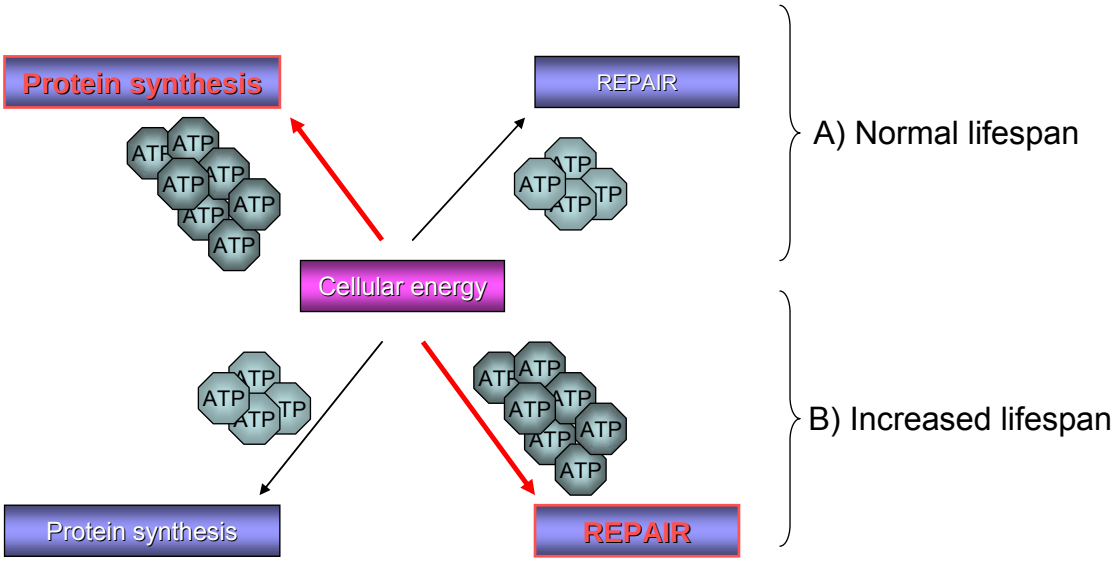

Figure 2
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