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SUMMARY

The important role that regulation of protein translation plays in determining longevity in invertebrate organisms became widely appreciated in 2007, with the publication of several papers discussed in last year's review. During 2008, several studies have further strengthened the idea that regulation of translation is one component of a highly evolutionarily conserved pathway that modifies longevity. Importantly, studies published this year also began to provide insights into specific mechanisms by which altered mRNA translation does (and in some cases does not) slow aging in invertebrate model organisms.

Key words: translation, dietary restriction, longevity, target of rapamycin, ribosome, proteotoxicity, degradation

Introduction

Protein translation is a complex, highly-regulated process with a central role in regulating cell function and homeostasis. Therefore, it is not news that health and longevity are dependent on appropriate regulation of translation throughout life. More surprising, however, are the recent observations made by multiple laboratories that perturbing the function of individual components of the key translational machinery can result in substantial increases in life span. Indeed, as described below, modulation of translation in response to nutrient availability appears to be one of the best conserved longevity pathways across invertebrate organisms.

TOR signaling and translation: A conserved longevity pathway.

Over the past several years, a small number of orthologous proteins have become recognized as conserved longevity modifiers (Kaeberlein, 2007; Kennedy, 2008). These include sirtuins, the target of rapamycin (TOR) kinase, insulin-like receptors, and FOXO-family transcription factors. It has remained unclear, however, whether these isolated cases were truly indicative of conserved pathways modulating longevity conserved across eukaryotic kingdoms since quantitative evidence was lacking.

A study published this year in *Genome Research* used the yeast *S. cerevisiae* and the nematode worm *C. elegans*, two organisms separated by approximately 1.5 billion years of evolution, to provide a quantitative evaluation of the degree to which genetic modifiers of aging are conserved (Smith *et al.*, 2008). By analysis of gene sequence, yeast orthologs and homologs were identified that correspond to approximately 300 known worm aging genes (defined as genes whose reduced function leads to increased life span), and yeast replicative life span was then measured for all non-essential single-gene deletion strains. From this comprehensive analysis, 25 long-lived yeast deletions were identified, each representing a conserved ortholog/homolog pair between yeast and worms. This proportion is significantly higher than the fraction of yeast aging genes identified by randomly screening deletions strains (Kaeberlein *et al.*, 2005), providing quantitative evidence for conserved longevity pathways across these two widely-separated species. Of particular relevance for this review is the observation that at least 8 of the 25 conserved longevity genes are

known to modulate protein translation and are likely to function in a single conserved longevity pathway downstream of TOR, a nutrient-responsive kinase (see (Wullschleger *et al.*, 2006) for review). These conserved longevity genes, each with a known effect on translation, include TOR, S6 kinase (S6K), ribosomal proteins of the large subunit, and translation initiation factors (**Table 1**).

Although TOR signaling and regulation of translation initiation are conserved longevity control mechanisms shared between yeast and worms, it is interesting to note that the mechanisms by which reduced ribosome biogenesis influences longevity may be somewhat different in the two species. A functional ribosome is composed of two multi-protein subunits, a 40S "small" subunit and a 60S "large" subunit. In *C. elegans*, RNAi knock-down of ribosomal proteins or rRNA processing factors that mediate production of either ribosomal subunit are associated with increased life span (Chen *et al.*, 2007; Curran and Ruvkun, 2007; Hansen *et al.*, 2007; Pan *et al.*, 2007). In yeast, in contrast, decreased abundance of the large ribosomal subunit seems to be much more important for longevity than the small subunit (Steffen *et al.*, 2008) (see below), with potential exceptions (Chiocchetti *et al.*, 2007). Whether these differences reflect overlapping or entirely different downstream mechanisms of longevity control in yeast and worms is currently unknown.

Is protein translation involved in the response to dietary restriction (DR)?

Three sets of observations, from studies of yeast, worms, and flies, suggest that DR slows aging by altering protein synthesis in response to reduced TOR signaling. First, TOR activity is reduced by dietary restriction (DR) (Juhasz *et al.*, 2007; Morck and Pilon, 2006; Takeshige *et al.*, 1992). Second, inhibition of TOR is sufficient to increase life span (Jia *et al.*, 2004; Kaeberlein *et al.*, 2005; Kapahi *et al.*, 2004; Powers *et al.*, 2006; Vellai *et al.*, 2003). Lastly, several TOR-regulated translation factors play a conserved longevity-modifying role (Fabrizio *et al.*, 2001)..

Epistasis analysis of replicative life span in yeast clearly places *TOR1*, *SCH9* (yeast S6K (Urban *et al.*, 2007)), and ribosomal proteins in the same pathway as DR (Steinkraus *et al.*, 2008), indicating that TOR signaling and TOR-regulated translation factors mediate life span extension by dietary restriction in this organism. In *C.*

elegans, however, the genetic relationships between TOR signaling, protein translation, and DR appear to be more complex. Similar to the case in yeast, inhibition of TOR signaling fails to further increase the life span of worms subjected to DR (Hansen *et al.*, 2007). Epistasis analyses, however, indicate that *rsks-1* (S6K) and translation initiation factors map to a different epistasis group than TOR and DR (Hansen *et al.*, 2008). One explanation that has been proposed is that while DR leads to reduced protein synthesis via reduced TOR activity, the life span extension observed from knock-down of S6K and other protein synthesis factors in well fed animals occurs via a different mechanism (Hansen *et al.*, 2008).

Despite the appeal of a simple linear model with TOR and translation downstream of DR, recent data are emerging to suggest that more complex models are likely to be required to explain the underlying biology. For example, while it is certainly the case that TOR signaling is reduced by DR, there is also evidence that TOR activity and protein synthesis are reduced during normal aging. For example, Linford and colleagues (Linford *et al.*, 2007) have reported that the transcriptional profile of aging mice is indicative of reduced TOR signaling and that DR attenuates this age-associated reduction in TOR activity. A similar trend is observed during replicative aging in yeast, where a reduction in transcription of ribosomal components and translation factors is observed in aged cells (Yiu *et al.*, 2008).

Models of longevity regulation that present protein translation as the primary downstream target of TOR signaling must confront evidence that TOR has multiple additional outputs that have also been implicated in longevity control. For example, autophagy, previously shown to be required for life span extension in response to reduced signaling through the insulin and IGF-I signal pathways, is also regulated by TOR signaling (Melendez *et al.*, 2003). Autophagy is induced by DR (Juhasz *et al.*, 2007; Morck and Pilon, 2006; Takeshige *et al.*, 1992), and three recent studies have indicated that this TOR-mediated induction of autophagy is required for increased life span [22, 26, 27]. Similar to the effect in a long-lived *daf-2* mutant, RNAi knock-down of essential autophagy genes (including the beclin ortholog *bec-1*, the phosphatidylinositol-3-kinase *vps-34*, or yeast *ATG7* ortholog *atg-7*) is sufficient to prevent life span extension in a genetic model of DR, *mutation of eat-2*. It remains to be determined, however, whether this requirement for autophagy is general for other

methods of DR in C. elegans, such as bacterial deprivation during adulthood [28, 29], and whether autophagy is essential for life span extension in a variety of different organisms. Interestingly, Hansen et al. (Hansen *et al.*, 2007) provide additional evidence that induction of autophagy is not sufficient to promote longevity in *C. elegans*, and that mutation of S6K does not induce autophagy. Taken together, these observations may suggest that the life span extension associated with DR is likely to require multiple TOR-regulated outputs, including both enhanced autophagy and altered protein synthesis.

In yeast, nutrient signaling kinases including TOR and PKA, when active, inhibit the activity of transcription factors such as Msn2 and Msn4 by maintaining them in the cytoplasm. Reduced TOR or PKA signaling leads to nuclear accumulation of Msn2/4 and activation of target genes (Beck and Hall, 1999; Smith *et al.*, 1998). A recent report finds that yeast replicative lifespan extension in the *tor1Δ* requires *MSN2/4* (Medvedik *et al.*, 2007), suggesting a translation-independent mode of lifespan modulation by TOR. The authors go on to report that activation of Msn2/4 leads eventually to increased Sir2 activity, although this has not been found by another group (Kaeberlein *et al.*, 2005). Similarly yeast chronological lifespan extension by *sch9Δ* or *tor1Δ* has recently been reported to require the Rim15 kinase (Fabrizio *et al.*, 2003; Wei *et al.*, 2008), which activates Msn2/4 and another stress responsive transcription factor, Gis1 (Cameroni *et al.*, 2004; Pedruzzi *et al.*, 2000).

Potential mechanisms of longevity control via regulation of protein translation

1. More efficient allocation of resources to promote longevity.

When considering how altered translation might lead to increased life span, one obvious hypothesis is that decreased translation simply reflects a more efficient usage of cellular energy and resources. Protein synthesis requires a large amount of ATP, and has been estimated in amphibians to account for at least 10% of total metabolic rate and greater than 10% of total energy consumption (Fuery *et al.*, 1998). In yeast, a ribosome biogenesis alone may account for 50% of the transcription in the cell (Warner *et al.*, 2001). It is reasonable to speculate that reduced protein synthesis

might allow a higher percentage of cellular resources to be allocated to pro-longevity maintenance pathways, perhaps accompanied by a reduction in allocation of resources to reproduction. Such as trade-off may account for a portion of the longevity effects associated with reduced IIS or DR (Partridge *et al.*, 2005), and this idea is consistent with the observation that *C. elegans* with reduced S6K or *ifg-1* activity also show a reduction in fecundity (Pan *et al.*, 2007). However, at least in certain cases, the longevity benefit of DR or reduced insulin signaling may be separable from the reproductive costs (Piper *et al.*, 2008). The extent to which these phenotypes may be separable in long-lived translation mutants is unknown.

2. Differential translation of a subset of longevity-modulating mRNAs

A second potential mechanism by which altered protein translation could modulate longevity is by differentially influencing the production of a small number of key longevity control proteins. While a majority of mRNAs are expected to be translated less efficiently in translation-deficient long-lived animals, one or more mRNAs coding for longevity-promoting proteins may be translated at their normal levels (or possibly even more efficiently) under conditions where translation initiation is impaired.

One specific example of such a mechanism was described recently in the yeast replicative aging system (Steffen *et al.*, 2008). Gcn4 is a starvation responsive transcription factor that is post-transcriptionally regulated via upstream open reading frames present in the Gcn4 5'-UTR (Hinnebusch, 2005). Under nutrient-replete conditions, when translation is highly active, translation of the *GCN4* open reading frame is negligible; however, under conditions where translation initiation is reduced (leading to less efficient translation of most mRNAs), translation of *GCN4* translation is enhanced. *GCN4* translation is also enhanced when 60S, but not 40S, ribosomal subunit abundance is reduced (Steffen *et al.*, 2008). The life span extensions associated with deletion of several different ribosomal large subunit genes, *SCH9* (yeast S6K) or *TOR1* are all attenuated in cells lacking Gcn4.

The role of Gcn4 as a transcription factor, combined with the requirement of increased *GCN4* expression for life span extension, suggests that one or more Gcn4

target genes are likely to contribute to the enhanced longevity observed in translation-deficient yeast cells. In addition to regulating amino acid biosynthetic genes, Gcn4 targets include genes involved in purine biosynthesis, autophagy, ER stress response, and mitochondrial function (Jia *et al.*, 2000; Natarajan *et al.*, 2001; Patil *et al.*, 2004). *GCN4* orthologs are present in invertebrates and mammals, contain 5'-UTR upstream open reading frames, and appear to have similar functions (Hinnebusch, 2005). An important goal for future studies will be to determine whether these orthologs also modulate longevity in multicellular eukaryotes.

Post-transcriptional regulation of *GCN4* is one example of how differential translation of a specific mRNA can contribute to longevity control. It is also possible that differential translation of entire classes of gene products could be altered in response to reduced TOR signaling, inhibition of translation initiation, or altered abundance of ribosomal subunits. For example, certain types of mRNAs may be more or less efficiently translated under specific translation states based on their structural features or subcellular localization (e.g. endoplasmic reticulum-associated translation).

Perhaps most intriguing is the possibility that distinct varieties of ribosomes, each with its own constituent protein composition, might be active within the cell at the same time. In support, recent evidence in yeast suggests specific roles for individual ribosomal subunits multiple aspects of translational regulation (Komili *et al.*, 2007). Such a model suggests that variant subunit compositions (e.g. lacking one ribosomal protein or containing a ribosomal protein or RNA with differential post-translational modifications) confer differential affinity for translation of subsets of target mRNAs. Mutations that alter the abundance or activity of individual ribosomal proteins or ribosomal processing factors may alter relative abundance of ribosomal subtypes and influence longevity in this manner. Clearly, much work needs to be done to clarify the importance of differential mRNA translation in aging.

3. Improved protein homeostasis

One of the questions posed in last year's Hot Topics review of protein translation in aging (Kaeberlein and Kennedy, 2007) was whether improved protein homeostasis

might account for some or all of the longevity benefits associated with reduced translation. During 2008, evidence has continued to accumulate suggesting that longevity and resistance to proteotoxicity are correlated, and the hypothesis that proteotoxicity may underlie many features of aging continues to gain momentum. This correlation is particularly apparent in *C. elegans*, where several different longevity-associated genes have been shown to influence aggregation of transgenically expressed toxic peptides (e.g see refs. [9, 45, 46]).

Particularly noteworthy, in this regard, was the observation that DR is sufficient to substantially delay age-associated paralysis caused by proteotoxicity in transgenic nematode models of polyglutamine disease and amyloid beta disease (Steinkraus *et al.*, 2008). This effect was shown both for environmental dietary restriction (bacterial food deprivation (Kaeberlein *et al.*, 2006; Lee *et al.*, 2006)) and for several genetic models of dietary restriction. In this study (Steinkraus *et al.*, 2008), DR was also shown to suppress similar phenotypes using a generic proteotoxicity model in which an aggregation-prone form of GFP (GFP-degron) is expressed in body wall muscle cells (Link *et al.*, 2006). These observations can be interpreted to suggest that DR acts by increasing resistance to a variety of different proteotoxic insults.

Like DR, RNAi knock-down of the insulin/IGF-1-like ("IIS") receptor *daf-2*, which had been previously shown to reduce amyloid beta-induced proteotoxicity (Cohen *et al.*, 2006), is also sufficient to reduce polyglutamine- and GFP-degron-induced proteotoxicity (Steinkraus *et al.*, 2008). The genetic relationship between IIS and DR with respect to proteotoxicity parallels their relationship with respect to longevity: (1) effects of reduced *daf-2* activity require the FOXO family transcription factor *daf-16*, whereas effects of DR do not, and (2) combining *daf-2(RNAi)* with DR leads to an apparent additive phenotypic response. Interestingly, however, both *daf-2(RNAi)* and DR require the heat shock transcription factor, *hsf-1*, to increase life span and suppress proteotoxicity (Hsu *et al.*, 2003; Steinkraus *et al.*, 2008). This observation could be interpreted to suggest that both interventions act on aging and resistance to proteotoxicity via a common downstream mechanism involving enhanced chaperone activity and improved protein folding. Alternatively, the phenotypic suppression associated with *hsf-1(RNAi)* may reflect a secondary effect

that is mechanistically distinct from the pro-longevity and anti-proteotoxicity effects of these interventions.

Is the suppression of proteotoxity observed in response to DR or *daf-2(RNAi)* related to altered protein translation? This question is still unanswered; however, as pointed out in last year's review (Kaeberlein and Kennedy, 2007), reduced protein translation might be expected to improve protein homeostasis by reducing the flux of proteins through endogenous repair and degradation pathways. It is also possible that protein repair or degradation machinery may be differentially translated under conditions of reduced ribosome biogenesis or translation initiation. In this regard, it is worth noting that in many eukaryotes (including yeast, worms, and humans), at least one of the genes coding for ubiquitin is expressed as a fusion protein with a ribosomal protein (Catic and Ploegh, 2005). Ribosomal protein expression is known to be regulated by TOR and S6K, and it is likely that ribosomal protein levels are tightly coupled to overall translation state. Thus, the highly conserved co-expression of ubiquitin and ribosomal proteins may serve as one mechanism to link proteasomal activity with global protein synthesis.

Translation, TOR signaling, and Aging in Mammals

It is clear that TOR signaling and translation control are central determinants of longevity in invertebrates; indeed, other than DR, a reduction in TOR signaling is the only intervention shown to increase life span in both of the yeast aging paradigms (replicative and chronological), as well as in nematodes and fruit flies. The importance of this pathway in mammalian aging has yet to be determined. In the absence of mammalian longevity data, however, there is reason to be optimistic that reduced TOR signaling may be beneficial for a variety of age-associated diseases in mammals. For example, mice treated with rapamycin show resistance to cancer, neurodegeneration, and cardiac disease (Gao *et al.*, 2006; Wullschleger *et al.*, 2006). Additionally, S6 kinase knockout mice show phenotypes consistent with a genetic mimic of dietary restriction, including improved insulin sensitivity, reduced adiposity and resistance to age-associated obesity (Um *et al.*, 2004). There is also emerging data that inhibition of TOR is likely to have beneficial health effects in humans. Rapamycin (Sirolimus) is used clinically as an immunosuppressant and to prevent

coronary stent restenosis (Cheng-Lai and Frishman, 2004), and is also in clinical trials as an anti-cancer therapeutic (Weil, 2008). It is noteworthy that reduced age-associated cancer incidence is a primary feature of DR in rodents, suggesting that rapamycin mimics at least some DR phenotypes in humans. It will be of great interest to determine whether rapamycin exposure can prevent or treat other age-associated human diseases.

The mechanistic explanations underlying these beneficial effects are likely to be complicated, and the consequences of reduced TOR or SK6 activity may well be different from tissue to tissue. Moreover, crosstalk with the insulin/IGF-1 pathway (and other pathways) occurs at multiple levels. For instance, insulin stimulation results in Akt-dependent TOR activation and phosphorylation of S6 kinase (see (Um *et al.*, 2006) for review). In addition, S6 kinase phosphorylates insulin receptor substrate 1 (IRS1) and dampens insulin signaling (Um *et al.*, 2004). With respect to longevity or age-related disease, it will be necessary to determine whether beneficial interventions in either pathway (IIS and TOR) are attributable to canonical downstream targets (e.g. translation initiation with S6 kinase), or alternately to effects on other pathways through cross-regulation. One recent report, for example, finds that rates of protein synthesis are not appreciably decreased in myoblast cultures isolated from mice lacking S6 kinase activity, even though the myoblasts were smaller and myofiber size is reduced in vivo (Mieulet *et al.*, 2007; Ohanna *et al.*, 2005). Whether this *in vitro* model accurately reflects the situation *in vivo* or in other tissues remains to be determined.

It is of vital importance to know whether reduced TOR activity enhances longevity in mammals. Fortunately, initial longevity experiments with mice fed a diet supplemented with rapamycin are well underway as part of the National Institute on Aging Interventions Testing Program (Miller *et al.*, 2007) and results should be available soon. In the last few years, the relationship between protein translation and aging has begun to come into sharper focus. The next few years should see increasingly successful efforts to elucidate the mechanisms underlying enhanced longevity resulting from reduced TOR signaling and/or translation initiation, as well as a better understanding of whether these pathways are therapeutic targets for age-related disease.

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Table 1. Conserved longevity modifying genes that influence protein translation. Several genes that regulate protein translation have been reported to modulate longevity in two or more organisms. Putative orthologs for these genes are shown below. Shading indicates published data demonstrating a role for that gene in aging of the noted organism. *Our unpublished data.

Yeast	Worm	Fly	Mouse
TOR1	*let-363*	dTOR	mTOR
KOG1*	*daf-15*		Raptor
SCH9	*rsks-1*	dS6K	RPS6KB1
RPL19A	*rpl-19*	RpL19	RPL19
RPL22A	*rpl-22**	RpL22	RPL22
RPL6B	*rpl-6*	RpL6	RPL6
RPL9A	*rpl-9*	RpL9	RPL9
TIF1	*inf-1*	eIF-4A	EIF4A2, EIF4A1
TIF2	*inf-1*	eIF-4A	EIF4A2, EIF4A1
TIF4631	*ifg-1*	eIF4G	EIF4G1, EIF4G3

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