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Hot Topics in Aging Research: Protein Translation, 2009

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Summary

In the last few years, links between regulation of mRNA translation and aging have been firmly established in invertebrate model organisms. This year, a possible relationship between mRNA translation and aging in mammals has been established with the report that rapamycin increases life span in mice. Other significant findings have connected translation control with other known longevity pathways and provided fodder for mechanistic hypotheses. Here we summarize advances in this emerging field and raise questions for future studies.

Keywords

proteotoxicity; longevity; dietary restriction; rapamycin; TOR; ribosome; translation; degradation

Introduction

It is clear from a number of prior studies that reduced or altered protein translation can promote longevity in invertebrate model organisms (Kaeberlein & Kennedy 2007; Syntichaki *et al.* 2007b). Life span extending mutations have been identified in a growing number of genes that code for translation-related proteins, including kinases that signal to promote mRNA translation, translation initiation factors, structural components of the ribosome, and ribosomal RNA processing factors (Table 1). The two foremost questions arising from these studies address (1) what are the mechanistic principles linking translational control pathways to aging in invertebrates? and (2) do similar mechanisms of life span control exist in mammals? Reports in the last year have shed light on both of these questions. It is now clear that inhibition of the mammalian target of rapamycin (mTOR), a key regulator of mRNA translation in response to nutrient and growth cues, can result in life span extension in mice (Harrison *et al.* 2009). Moreover, findings from several groups have provided mechanistic insight into how factors that regulate translation could also modulate aging.

Targeting mTOR extends mammalian life span

Extending human healthspan, which can be defined as the period of life in which relatively good health is maintained, is a fundamental goal of aging research. A primary strategy for achieving this goal is through the identification of compounds that extend life span in mammals and/or prevent the onset of age-related diseases. The National Institute on Aging Intervention Testing Program (ITP) was initiated to rigorously test putative anti-aging

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compounds for effects on mouse life span (Nadon *et al.* 2008). Compounds are nominated by members of the scientific community, and those selected for inclusion in the ITP are tested in parallel at three independent sites. In the last year, investigators with the ITP reported that dietary supplementation with rapamycin, a specific inhibitor of the TOR complex I (TORC1), is the first pharmacological intervention found to reproducibly confer robust life span extension in mice (Harrison *et al.* 2009). Several features of this study are of interest, in particular its support for the hypothesis that reduced or altered protein synthesis resulting from mTOR inhibition promotes enhanced longevity in mammals (Kaeberlein & Kennedy 2009). A second important point from the study (Harrison *et al.* 2009) is that animals were not treated with rapamycin until 600 days of age, late middle age for a mouse and roughly comparable to 60 years of age for a person. This demonstrates that significant increases in longevity are attainable even when treatment is initiated late in life (Harrison *et al.* 2009).

Rapamycin and derivatives thereof, termed rapalogs (Tsang *et al.* 2007), have been tested and, in some cases approved, for a variety of clinical applications (Guertin & Sabatini 2009; Stanfel *et al.* 2009). For instance, rapalogs are used in treatment of renal cell carcinoma (Le Tourneau *et al.* 2008) and coronary artery disease (Serruys *et al.* 2006). Furthermore, rapamycin, either alone or as a combination therapy with other agents, may prove effective in a wide range of other metastatic, neurodegenerative and metabolic diseases (Stanfel *et al.* 2009). Rapamycin and derivatives are also used as immune suppressants after organ transplants (Morath *et al.* 2007), raising the possibility that while rapamycin is effective at increasing longevity in a pathogen free mouse facility, deleterious effects on the immune system may preclude long-term use in humans. At issue is whether a level of rapamycin can be achieved that provides beneficial effects on aging without unwanted side-effects. An important follow-up study will be to test the immune performance of mice under age-extending doses of the drug. Other unwanted side effects are occasionally reported in individuals taking rapamycin (and derivatives), and impaired skeletal muscle performance may be another concern in response to long-term exposure. Clearly, more detailed studies of these issues will be needed before rapamycin derivatives are considered for use in the human population as anti-aging drugs.

The report by Harrison et al. (Harrison *et al.* 2009) provides strong evidence that TOR signaling influences mammalian aging. Whether this effect is mediated in part by reduced mRNA translation will be an important question for future studies. Although there is no direct evidence yet that mRNA translation was reduced in the long-lived mice fed rapamycin, several prior studies from invertebrate models have demonstrated that inhibition of TOR is sufficient to reduce mRNA translation while simultaneously increasing life span, and that altered mRNA translation underlies at least a portion of the longevity effect (Kaeberlein & Kennedy 2008; Stanfel *et al.* 2009). Along these lines, it will be of particular interest to learn whether other components of the mTOR pathway that are involved in mRNA translation, such as ribosomal S6 kinase, also modulate life span in mice. Mutation of S6 kinase homologs leads to increased life span in yeast, nematodes, and flies, and promotes resistance to a high-fat diet in mice (Stanfel *et al.* 2009). Although the longevity of S6 kinase knock-out mice has not yet been reported, if altered mRNA translation is causally involved in the life span extension from rapamycin, then reduced S6 kinase activity should increase life span in mice as well.

TORC1 and TORC1 in translation control and aging

The mTOR kinase exists in two complexes, TORC1 and TORC2, which contain both shared and unique components (Ma & Blenis 2009; Polak & Hall 2009). Both TOR complexes are nutrient-responsive. Control of mRNA translation by mTOR is regulated primarily by

TORC1, which coordinates protein synthesis and degradation in response to growth status; TORC2 controls cell stress responses and actin cytoskeletal function and, more recently (see below), has been linked to body growth rate (Cybulski *et al.* 2009). Studies in the last year have shed light on the contribution of these two complexes to aging and metabolism.

Rapamycin directly inhibits TORC1 function (Loewith *et al.* 2002), although long-term exposure of cells in culture to rapamycin can reduce TORC2 function as well (Zeng *et al.* 2007). Nevertheless, the assertion that reduced TORC1 function can result in life span extension has ample supporting evidence, in addition to the studies reporting life span extension from rapamycin in yeast and mice (Powers *et al.* 2006; Medvedik *et al.* 2007; Harrison *et al.* 2009). First, mutation of TORC1-specific components is sufficient to increase life span in both *S. cerevisiae* and *C. elegans*. In yeast, Tor1 functions solely in TORC1, while Tor2 acts in both TORC1 and TORC2; deletion of *TOR1* increases life span (Kaeberlein *et al.* 2005; Powers *et al.* 2006; Bonawitz *et al.* 2007), while deletion of *TOR2* is lethal. In nematodes, mutation of *daf- 15*, which codes for the homolog of the TORC1 component *Raptor*, results in life span extension (Jia *et al.* 2004), as does RNAi knock-down of the *let-353* gene, which codes for the *C. elegans* TOR homolog (Vellai *et al.* 2003). While life span studies have not been reported for mice with reduced *Raptor* levels, two studies in the last year have reported the effects of tissue-specific *Raptor* knockouts (Polak & Hall 2009). Adipose-specific *Raptor* knockout mice have properties characteristic of long-lived mice, including increased leanness and resistance to diet-induced obesity (Polak *et al.* 2008). This is accompanied by improved glucose tolerance and insulin sensitivity. Possible underlying molecular changes include increased mitochondrial uncoupling, a reduction in a feedback loop involving ribosomal S6 kinase-mediated inhibition of IRS1, and/or improved adipose-mediated endocrine signaling. These findings give a strong rationale for longevity analysis of adipose-specific *Raptor* knockout mice.

Knockout of *Raptor* in skeletal muscle results in muscular dystrophy attributable to reduced mitochondrial biogenesis, which leads in turn to lower muscle oxidative capacity and enhanced glycogen storage (Bentzinger *et al.* 2008). These findings indicate that the consequences of reduced TORC1 activity may be advantageous in some tissues while deleterious in others, and underscore the importance of exploring Do rapamycin-treated mice have signs of muscular dystrophy? One possibility is that the effects of rapamycin were differential in peripheral tissues, perhaps because of differential uptake, variable responsiveness or other factors. Alternatively, a uniform reduction in TORC1 activity across many tissues may have less deleterious effects than an unbalanced reduction is skeletal muscle, as brought about by the tissue-specific *Raptor* knockout (Bentzinger *et al.* 2008). Clearly, findings from these three studies (Bentzinger *et al.* 2008; Polak *et al.* 2008; Harrison *et al.* 2009), while establishing TORC1 function as important for mammalian longevity, also raise fundamental questions to be resolved in the future.

In mammals, two key TORC1 targets that are important for increased protein synthesis are 4E-BP1 (eIF4E-binding protein 1) and ribosomal S6 kinase (Ma & Blenis 2009). Active 4E-BP1 inhibits cap-dependent translation by binding to and inhibiting the activity of eIF4E; phosphorylation by mTOR inhibits 4E-BP1, leading to enhanced translation initiation. In contrast, mTOR-dependent phosphorylation of S6 kinase activates this enzyme, and leads to enhanced protein synthesis as well as increased ribosomal RNA synthesis (Hannan *et al.* 2003; Wullschleger *et al.* 2006). 4E-BPs and S6 kinase have both been linked to aging in invertebrate models. Genetic approaches to reduce the activity of putative S6 kinase orthologs lead to life span extension in yeast (Fabrizio *et al.* 2001; Kaeberlein *et al.* 2005), nematodes (Hansen *et al.* 2007; Pan *et al.* 2007) and flies (Kapahi *et al.* 2004). Similarly, reduced expression of components of eIF-4F (which contains eIF4E and is targeted by 4E-BPs) leads to life span extension in *C. elegans* (Henderson *et al.* 2006; Hansen *et al.* 2007;

Pan *et al.* 2007; Syntichaki *et al.* 2007a). Related mouse longevity studies have not yet been reported.

An interesting report from the last year indicates that rapamycin can have differential effects on TORC1-dependent phosphorylation of S6 kinase and 4E-BPs (Choo *et al.* 2008). In cell culture studies, rapamycin was shown to inhibit both S6 kinase and 4E-BP1 phosphorylation; however, in some cells 4E-BP1 phosphorylation is restored in a TORC1-dependent but rapamycin-resistant manner. These findings, if they are relevant *in vivo*, call for a careful analysis of the consequences of long-term rapamycin treatment in mice at concentrations that extend life span (Harrison *et al.* 2009).

Two studies in the last year have directly characterized the role of Drosophila 4E-BP with regard to aging and age-related disease. Zid et al. (Zid *et al.* 2009) report that 4E-BP is (1) up-regulated by dietary restriction, (2) required for full life span extension by dietary restriction, and (3) sufficient when overexpressed to extend life span under normal dietary conditions. These findings further cement the connection between the TOR, mRNA translation and dietary restriction in invertebrate models. The results of Zid et al. (Zid *et al.* 2009) also resemble the adipose-specific *Raptor* knockout mice, in that enhanced mitochondrial function was proposed to underlie the beneficial effects on longevity (Polak *et al.* 2008). Interestingly in the case of 4E-BP, enhanced translation of mitochondrial genes (and others) with short 5′ UTRs was observed during dietary restriction, leading the authors to propose a mechanism whereby dietary restriction stimulates 4E-BP production, leading to reduced CAP-dependent translation and a relative increase in translation of messages with simple, less CAP-dependent 5′ UTRs.

The differential translation of a subset of mRNAs described by Zid *et al.* (2009) in response to dietary restriction in flies is reminiscent of a similar mode of regulation observed in long-lived yeast cells with reduced abundance of 60S ribosomal subunits (Steffen *et al.* 2008). In the yeast experiment, translation of the Gcn4 mRNA was enhanced at the same time that global mRNA translation was reduced, due to the presence of translation-inhibiting upstream open reading frames (uORFs) present in the *GCN4* 5′ UTR that are less efficiently translated in these long-lived mutants. This differential translation of Gcn4 was shown to underlie a portion of the life span extension associated with depletion of 60S ribosomal subunits as well as dietary restriction in yeast. Although the differentially translated mRNAs described by Steffen *et al.* in yeast and by Zid *et al.* in flies are different, these studies suggest that specific changes in translation of mRNAs based on structural determinants of their 5′ UTRs may represent a conserved strategy for modulating longevity in response to nutrient availability.

A second recent study in *Drosophila* focused on the role of 4E-BP in a measure of functional aging, age-related cardiac decline (Wessells *et al.* 2009). Previous studies had shown that reduced TOR signaling was protective in this assay (Luong *et al.* 2006). Here the authors examine downstream targets of TOR, finding that either overexpression of 4E-BP or reduced-function mutants of S6 kinase protected against cardiac stress (Wessells *et al.* 2009). Interestingly, in the case of 4E-BP, protection was cell autonomous to cardiac tissue, whereas the benefits of reduced S6 kinase were non-cell autonomous and attributed to altered endocrine effects on insulin-like signaling. Clearly the connection between insulin-like signaling and TOR signaling is complex, and teasing out the mechanistic underpinnings of longevity benefits of mutations in either pathway will be a key goal of future studies.

In addition to affecting translation initiation, TORC1 controls other cellular processes that have been linked to longevity. For instance, reduced TOR signaling leads to increased autophagy in a range of organisms from yeast to mammals (Noda & Ohsumi 1998; Chang *et*

al. 2009). In nematodes, RNAi-mediated inhibition of autophagy has been reported to block life span extension by dietary restriction and mutation of *daf-15* (Jia & Levine 2007; Hansen *et al.* 2008), as well as *daf-2* mutants with reduced insulin-like signaling (Melendez *et al.* 2003). This important role of autophagy in the response to dietary restriction may be conserved in yeast chronological aging, as two reports in the last year from Aris and colleagues have indicated that autophagy-deficient yeast are short-lived (Alvers *et al.* 2009a) and that chronological life span extension by rapamycin requires a functional autophagy pathway (Alvers *et al.* 2009b).

Metabolic control downstream of TOR signaling has also been linked to aging in yeast. Due to their predilection for fermentation, rapidly dividing yeast grown on rich media have low respiratory levels. *TOR1* deletion strains exhibit enhanced respiration and this altered metabolic response is required for chronological life span extension (Bonawitz *et al.* 2007; Shadel & Pan 2009). One consequence of this shift in metabolism is that less organic acids are produced as a byproduct and secreted into the extracellular milieu. A report published earlier this year points to toxicity by these extracellular organic acids as a molecular mechanisms that causes chronological aging in yeast (Burtner *et al.* 2009). Notably, dietary restriction has effects on yeast metabolism similar to *TOR1* deletion, promoting enhanced respiration during the growth phase prior to arrest in a non-proliferative state. These latter findings indicate that reduced organic acid production contributes to chronological life span extension from dietary restriction. Collectively, these findings indicate that multiple downstream effectors of TOR signaling can be linked to longevity in different organisms, and that altered mRNA translation is unlikely to be the whole story.

During the last year, evidence has emerged that the TORC2 complex may also modulate aging. Souskas et al. report the isolation and characterization of a mutant in *C. elegans* Rictor (*rict-1*), a TORC2 specific component, finding that *rict-1* hypomorphic mutants exhibit robust life span extension depending on the bacterial food source (Soukas *et al.* 2009). Their results indicate that on a nutrient-rich bacterial food source, *rict-1* mutants reduce food consumption and that the life span extension may be an indirect effect of this reduction, in other words a phenocopy of dietary restriction. *rict-1* mutants also have an array of other phenotypes including increased body fat storage, reduced body size, and increased energy expenditure. These findings are largely in line with a second report examining *rict-1* mutants (Jones *et al.* 2009), although life span was not directly examined in this latter study. Both studies link TORC2 signaling to the serum and glucocorticoid-induced kinase *sgk-1*, noting that *sgk-1* mutants have remarkably similar phenotypes to *rict-1* mutants and implicating this kinase as a primary effector of TORC2 signaling (Jones *et al.* 2009; Soukas *et al.* 2009). Whether altered TORC2 activity has effects on translation in nematodes and whether this might be linked to longevity or metabolic phenotypes remains to be assessed.

As with TORC1 (Guertin *et al.* 2006), mice require TORC2 for development (Shiota *et al.* 2006). Unlike adipose-specific *Raptor* knock-out mice, which are resistant to diet-induced obesity (Polak *et al.* 2008), adipose-specific knock-out of *Rictor* results in increased body and non-adipose organ size, independent of dietary fat content (Cybulski *et al.* 2009). These mice have elevated insulin-like growth factor 1 and IGF-1 binding protein 3, and are hyperinsulinemic, but glucose-tolerant. These findings suggest TORC2 function in adipose tissue regulates body growth through an as yet undefined mechanism. Whether this is linked to phenotypes apparent in *rict-1* mutant nematodes is also not known. As with *Raptor*, phenotypes associated with *Rictor* mutation are tissue-specific; skeletal muscle ablation of *Rictor* has no apparent phenotypic consequence (Bentzinger *et al.* 2008).

Emerging links between mRNA translation and aging-related processes

Much of the evidence for the important role that mRNA translation plays in modulating aging comes from studies showing that interventions resulting in decreased mRNA translation can increase life span, and these studies have been a major focus of preceding reviews in this Hot Topics series (Kaeberlein & Kennedy 2007; Kaeberlein & Kennedy 2008). Evidence is accumulating, however, that mRNA translation also influences a variety of aging-related processes and impinges on multiple longevity pathways. Some of these links have been mentioned above, and three additional reports published this year illustrate this point in different fashions.

Reduced expression of genes encoding protein components of the ribosome has been shown to result in life span extension in both yeast and nematodes (Kaeberlein & Kennedy 2007; Kaeberlein & Kennedy 2008), and a recent study by Wang et al. (Wang *et al.* 2008) suggests that these mutations can also affect mitochondrial function in a surprising way. Wang et al. generated yeast expressing a mutant allele of mitochondrial adenine nucleotide translocase Aac2, which is analogous to the human ANT1 allele associated with autosomal dominant progressive external opthalmoplegia (Kaukonen *et al.* 2000; Wang *et al.* 2008). Yeast expressing this mutant Aac2 experience age-dependent mitochondrial degeneration resulting in short replicative life span (Wang *et al.* 2008). Interestingly, both the mitochondrial degeneration and the short life span were suppressed by deletion of ribosomal proteins previously associated with enhanced longevity in wild type cells. Age-related mitochondrial decline has been reported in organisms ranging from yeast to humans (Finley & Haigis 2009) and it is interesting to speculate that protection against mitochondrial dysfunction by mutations that reduce mRNA translation could extend beyond the yeast system, as well.

Another recent study links protein translation to the hypoxic response in *C. elegans* (Anderson *et al.* 2009). Regulation of the hypoxic response by the HIF-1 transcription factor has recently been shown to be an important determinant of longevity in nematodes (Chen *et al.* 2009; Mehta *et al.* 2009; Zhang *et al.* 2009). In a screen for hypoxia resistance in worms, Anderson et al. recovered mutants in *rrt-1*, encoding an arginyl-transfer RNA (tRNA) synthetase, and additional experiments indicated that reduced translation rate was associated with increased resistance to hypoxia. This resistance required the unfolded protein response, suggesting that possibility that reduced translation could improve the ER stress response during hypoxia. Interestingly, Chen *et al.* (Chen *et al.* 2009) reported that a functional ER stress response is also important for life span extension associated with deletion of *HIF-1*. Thus, as noted previously (Kaeberlein & Kennedy 2008), improved protein quality control in the ER and other cellular compartments may also be an important feature associated with mutations that reduce mRNA translation and increase life span. It would be of interest to know whether the alleles of *rrt-1* associated with resistance to hypoxia also slow aging.

A third interesting report this year links mRNA mistranslation to enhanced expression of the nicotinamidase Pnc1 in yeast (Silva *et al.* 2009). The authors elevated the rate of mistranslation by expressing a $tRNA_{CAG}$Ser synthetase from a pathogenic yeast, *C.albicans*, which decodes the leucine CUG codon as serine. Surprisingly, a quantitative proteomics approach revealed that cells expressing the $tRNA_{CAG}$Ser synthetase have higher Pnc1 protein levels. Pnc1 converts nicotinamide into nicotinic acid, which can be further converted into NAD(+) (Ghislain *et al.* 2002). Since nicotinamide inhibits the histone deacetylase Sir2 and NAD(+) activates it (Lin *et al.* 2000; Anderson *et al.* 2003; Gallo *et al.* 2004), this suggests that mistranslation could promote higher Sir2 activity. Both enhanced Sir2 and Pnc1 function are known to result in life span extension (Kaeberlein *et al.* 1999; Anderson *et al.* 2003). These findings provide a potential novel mechanistic connection

between translation efficiency and Sir2 function, a link that will doubtless be explored further in future studies.

Lastly, it is important to note that several ribosomal proteins have extra-ribosomal functions. Interested readers should consult the following review (Warner & McIntosh 2009). While none of these functions have been directly linked to aging, this is a formal possibility at least in the case of a subset of ribosomal protein gene mutations that result in life span extension.

Conclusions

The first clear demonstration of a drug that robustly increases life span in mammals has brought to center stage the strategy of pharmacologically targeting a conserved aging pathway to slow aging and extend healthspan. The fact that this drug inhibits mTOR signaling and probably affects aging at least in part through altered levels of mRNA translation only increases the importance of understanding the myriad and complicated links between translational control and aging. Further work is likely to focus on the development of drugs that target nodes other than mTOR in translational control pathways, such as S6 kinase and translation initiation factors. The possible complications associated with mTOR inhibition may (or may not) preclude the long-term chronic use of rapalogs as anti-aging drugs, and intervening at other levels may ultimately help separate positive effects from unwanted side effects. Manipulating mTOR activity and mRNA translation could also provide therapeutic leverage against diseases that are not necessarily a reflection of the normal aging process, such as mitochondrial degenerative diseases. Ongoing studies are poised to address some of these key questions, and we look forward to more advances in this area of aging-related research in 2010.

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Table 1
Translation-related proteins implicated in longevity control

Table 1 represents a compilation of studies performed by several labs (Lee *et al.* 2003; Hamilton *et al.* 2005; Hansen *et al.* 2005; Chen *et al.* 2007; Curran & Ruvkun 2007; Hansen *et al.* 2007; Kim & Sun 2007; Pan *et al.* 2007; Syntichaki *et al.* 2007a; Hansen *et al.* 2008; Managbanag *et al.* 2008; Smith *et al.* 2008; Steffen *et al.* 2008).

Function	Yeast	Nematode	Fly
Signaling	TOR1, SCH9	LET-363, RSKS-1	Tor, S6k, Tsc1, Tsc2
Translation initiation factor	TIF1, TIF2, TIF4631	D2085.3, EGL-45, EIF-3.B, EIF-3.F, IFE-2, IFF-1, IFG-1, INF-1, IFTB-1, T27F7.3, Y39G10AR.8	
Ribosomal proteins	RPL6B, RPL7A, RPL9A, RPL13A, RPL10, RPL19A, RPL20B, RPL21B, RPL22A, RPL23A, RPL29, RPL31A, RPL33B, RPL34B, RPL37B, RPL43B, RPP2B, RPS6B, RPS18A, RPS18B,	RPL-4, RPL-6, RPL-9, RPL-19, RPL-30, RPS-3, RPS-5, RPS-6, RPS-8, RPS-10, RPS-11, RPS-15, RPS-22, RPS-23, RPS-26	
Others (rRNA processing, nucleolar proteins, mitochondrial ribosome)	LOC1, NOP12, TMA19, REI1, SSF1	B0261.4, DRR-2, EXOS-3, F09G8.3, F33D4.5, F53F4.11, F59A3.3, NOL-5, TAG-264, W09D10.3, Y37D8A.18, Y48G1A.4, Y55F3B_743.b, Y71H2_378.a	