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HOT TOPICS

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Summary

Translation of RNA to protein is essential for life. It should perhaps not be surprising, therefore, that appropriate regulation of translation plays a key role in determining longevity. This Hot Topic Review discusses papers published in the last year related to the importance of translation, and its regulation by signaling through the target of rapamycin (TOR) kinase, in modulating aging and age-associated disease.

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

Introduction

The link between aging and environmental nutrients is well established. In organisms ranging from yeast to mammals, dietary restriction (DR) increases lifespan and delays onset of a variety of age-associated pathologies. Although the mechanisms accounting for these effects are not completely understood, recent progress has mapped some of the important players. Foremost among these is the nutrient-responsive target of rapamycin (TOR) kinase, an evolutionarily conserved gatekeeper that integrates nutrient and hormonal cues to modulate growth and longevity. When nutrients are abundant, TOR activity is high, which favors faster growth and cell division; when nutrients become limiting, TOR activity is decreased, leading to reduced growth, enhanced resistance to stress, and increased lifespan.

Evidence supporting a role for TOR signaling in DR comes primarily from work in invertebrate organisms. In worms and flies, genetic mutations resulting in decreased TOR activity have been shown to increase lifespan. In yeast, genetic or pharmacological inhibition of TOR increases both replicative and chronological lifespan, and genetic epistasis indicates that TOR acts downstream of DR. It remains to be determined whether decreased TOR activity increases lifespan in mammals; however, mammalian TOR (mTOR) responds to nutrient deprivation in a manner similar to invertebrate TOR, and inhibition of mTOR mimics at least some of the health benefits associated with DR, such as decreased cancer risk and resistance to neurodegenerative disease .

TOR signaling is known to regulate several different processes that could contribute to its role in aging. These downstream functions of TOR include repressing autophagy and stress response pathways, regulating mitochondrial metabolism, and promoting ribosome function and mRNA translation (**Figure 1**). Among these, regulation of translation has emerged as a potent determinant of longevity. Here we summarize some of the most important recent reports on the role of mRNA translation in longevity and discuss potential mechanisms for how reduced mRNA translation could slow aging in different organisms.

Translation regulatory factors determine longevity

TOR signaling promotes translation through multiple outputs (**Figure 1**). The best characterized of these is ribosomal S6 kinase, which functions to promote translation initiation via phosphorylation of ribosomal protein S6 and through regulation of translation initiation factors,

such as eIF4B. TOR signaling also promotes translation by repressing the activity of 4E-BP's, which inhibit eIF4E, and in yeast (but perhaps not higher eukaryotes) TOR activity also directly promotes transcription of ribosomal protein genes.

The relevance of translation regulation for longevity has been best characterized in *C. elegans*, with translation initiation appearing to be of particular import. In two studies published in Aging Cell late last year, Pan et al. and Hansen et al. reported that knock-down or deletion of the *C. elegans* S6K homolog, *rsks-1*, increases lifespan. Lifespan extension was also observed in worms with reduced expression of translation initiation factors, including *ifg-1* (eIF4G), *iftb-1* (eIF2B), and *ife-2* (eIF4E). Syntichaki et al. reported similar lifespan extension associated with knock-down of *ife-2*. More recently, additional translation initiation factors have been identified by the Kapahi and Ruvkun laboratories from screens for development genes that result in increased lifespan upon RNAi inhibition specifically during adulthood. These include *C. elegans* homologs of eIF2G, eIF3F, and eIF4A.

Decreased function of ribosomal proteins increases lifespan in yeast and worms

In addition to defining the role of translation initiation in longevity determination, recent studies have documented the importance of ribosome components themselves in the aging process. Lifespan extension associated with mutation to structural components of the ribosome was first noted from a partial genome-wide screen for single-gene deletion mutations that increase replicative lifespan in yeast. In this study, deletion of either *RPL31A or RPL6B*, each of which codes for a ribosomal large subunit protein, was found to significantly increase lifespan.

Chiocetti et al. have recently expanded on this observation by showing that additional ribosomal proteins modulate replicative aging in yeast. Diploid cells heterozygous for *RPL10* and haploid cells deleted for either *RPL6A*, *RPS18A* or *RPS18B* were reported to be significantly long-lived.

A similar importance of ribosomal proteins in aging has been observed in *C. elegans*. In the studies mentioned above from the Kapahi, Kenyon, and Ruvkun labs, RNAi knockdown of several ribosomal proteins was found to increase adult lifespan. Both large and small subunit proteins were identified from these screens, including *rpl- 4*, *6*, *9*, *19*, *30*, *rps- 3*, *6*, *8*, *10*, *11*, *15*, *22*, and *26*, as well as processing factors involved in maturation of ribosomal RNA. Taken together, these studies strongly suggest that decreased ribosome abundance can increase lifespan in both yeast and *C. elegans*.

Identification of the yeast S6 kinase. S6 kinase (S6K) plays a central role in multicellular eukaryotes linking TOR signaling to aging. S6K is activated by TOR and functions to promote translation via multiple outputs, including translation initiation, translation elongation, and post-translational regulation of ribosomal protein activity. Mutations that decrease S6K activity increase lifespan in both worms and flies, and S6K1 knockout mice have intriguing phenotypes, including resistance to age- and diet-induced obesity, though the longevity of these mice has not been reported.

Although TOR activity is known to modulate longevity in yeast, determining whether S6K plays a similar role in this organism has been difficult until recently, due to the lack of an obvious S6K ortholog by sequence homology. Urban et al. provide compelling evidence that the functional S6

kinase ortholog in yeast is, in fact, the nutrient responsive Sch9 kinase. Sch9 has long been known to play an important role in regulating ribosome biogenesis in yeast in response to nutrient availability; however, Sch9 had been previously suggested as the ortholog of mammalian Akt proteins. Urban et al. show that Sch9 is specifically phosphorylated by the rapamycin-sensitive TOR complex 1. Further they demonstrate that Sch9 specifically phosphorylates ribosomal S6 kinase. While these findings do not preclude an Akt-related function for Sch9, they do provide compelling evidence that Sch9 acts in a manner similar to S6 kinase.

This demonstration that Sch9 functions analogously to S6 kinase has important implications for aging research. Deletion of Sch9 is known to increase both replicative and chronological lifespan and is thought to be a genetic model of DR in yeast. In addition to TOR, Sch9/S6K is the only other protein known to modulate longevity in both yeast aging paradigms, as well as in worms and flies. Thus, it appears that a linear pathway has been conserved (at least in yeast, worms, and flies) through which longevity is determined in response to nutrient availability via reduced TOR signaling, reduced S6K activity, and altered mRNA translation.

Is protein homeostasis the key? How might reduced translation increase lifespan in evolutionarily divergent organisms? It has been speculated that an age-associated accumulation of misfolded, aggregated, and damaged proteins may underlie many of the pathologies of aging (**Figure 2**). One intriguing hypothesis is that decreased translation leads to improved protein homeostasis by reducing the levels of damaged proteins. A reduction in translation rate, which would be predicted to reduce both normal protein production and the flux of damaged proteins

through the various repair pathways, may allow the endogenous protein repair and degradation machinery to maintain toxic proteins at a lower steady-state level. A key experimental validation of this hypothesis will be to determine whether long-lived animals with reduced translation indeed have lower levels of damaged proteins. If correct, this hypothesis also suggests a potential therapeutic avenue toward treating human diseases of proteotoxicity by targeting translation regulatory pathways.

The idea that protein homeostasis is important for aging has received further support by work from the Dillin lab implicating both the FOXO family transcription factor *daf-16* and the heat shock transcription factor *hsf-1* in the response to transgenic expression of a toxic amyloid beta peptide in *C. elegans* . Both *daf-16* and *hsf-1* are known to act downstream of IIS to promote longevity, and have been previously implicated in polyglutamine toxicity. What this new work added to the picture is the idea that *daf-16* and *hsf-1* maintain protein homeostasis by different mechanisms. Cohen et al. proposed that soluble amyloid beta oligomers are the toxic species, and that *daf-16* promotes the formation of non-toxic insoluble aggregates while *hsf-1* promotes disaggregation of the soluble oligomers. Perhaps *daf-16* and *hsf-1* promote longevity in wild-type animals at least in part by detoxifying endogenously generated protein damage.

In addition to (1) making fewer proteins or (2) repairing damaged proteins, cells have a third option for dealing with proteotoxicity – degradation. Both ubiquitin-mediated degradation via the proteosome and autophagic degradation of proteins have been suggested as an important defense against age-associated accumulation of damaged proteins**.** Autophagy, in particular, has received much attention as a potential longevity promoting process based, among other things,

on the observation that reduced IIS fails to increase the lifespan of *C. elegans* defective for autophagy.

In this regard, it is noteworthy that, in addition to regulating translation, another primary function of TOR signaling is to repress autophagy. Under conditions of reduced TOR signaling, autophagy is induced and, consequently, degradation of damaged proteins is likely to be increased. DR is known to cause increased autophagy in rodents, consistent with the idea that DR promotes enhanced clearance of damaged proteins via the TOR pathway. Important experimental validation of this hypothesis remains to be established, however, including determining whether induction of autophagy is required for the lifespan extension associated with either TOR inhibition or DR, and determining whether induction of autophagy (or proteosomal degradation) is sufficient to increase lifespan and/or delay age-associated diseases.

Conclusions

Recent progress has demonstrated that TOR, S6K, and translation regulatory factors are key modulators of longevity in each of the three primary invertebrate aging models. Many of the mechanistic details remain unknown, but given the rapid progress in this area of aging research, we anticipate a continued stream of reports from invertebrate organisms addressing these questions. We have proposed that improved protein homeostasis may underlie many of the beneficial effects associated with DR, reduced TOR signaling, and reduced translation. Alternative possibilities should not be excluded, however. For example, reduced translation may lead to improved efficiency of energy utilization accompanied by reduced production of

mitochondrial reactive oxygen species. Alternatively, it may be the case that a general reduction in translation is accompanied by a relative increase in translation of a specific subset of post-transcriptionally regulated mRNAs (e.g. GCN4 in yeast) that could be important for longevity.

A critical question that has not been sufficiently pursued is whether TOR signaling and translation play a similar role in modulating longevity and disease susceptibility in mammals. TOR signaling is known to regulate the same processes in mammals as in invertebrates in response to nutrient and growth cues, making this pathway a leading candidate for mediating at least some of the beneficial effects of DR. Indeed, TOR inhibitors are known to have potent anti-cancer activity, one of the hallmarks of DR in rodents. It will be of great interest to discover whether TOR inhibition mimics other phenotypes associated with DR and whether pharmacological intervention in TOR signaling can increase lifespan in mammals.

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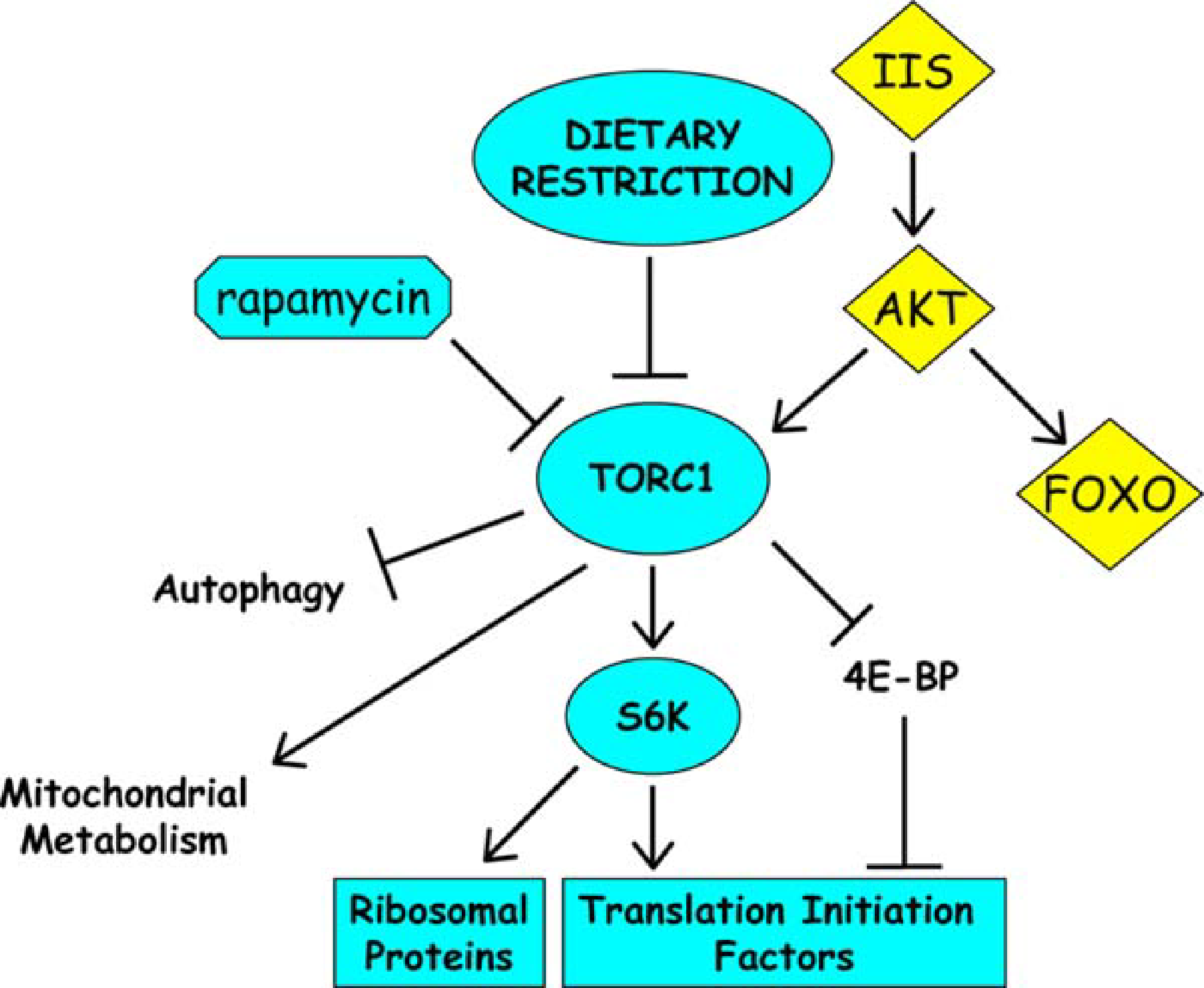
Figure Legend.

Figure 1. A conserved longevity pathway involving TOR signaling and protein translation. Components of the TOR signaling pathway have been shown to modulate longevity in yeast, worms, and flies, and may represent a mechanism for lifespan extension in response to dietary restriction. All shaded factors are known to affect aging in invertebrate organisms. IIS = insulin/IGF-1-like signaling.

Figure 2. Model for how protein homeostasis can influence aging. The age-associated accumulation of damaged or misfolded toxic proteins may contribute to pathologies of aging. Decreased translation rate could reduce the amount of toxic proteins and increase lifespan in two ways: by reducing the production of proteins with translation errors and by reducing the overall protein burden, allowing the endogenous repair and degradation machinery to maintain a lower steady-state level of protein damage. Enhanced repair, increased degradation, and the formation of insoluble aggregates are alternative strategies for reducing the pool of toxic soluble proteins. Each of these alternative strategies has also been linked to longevity in invertebrate organisms.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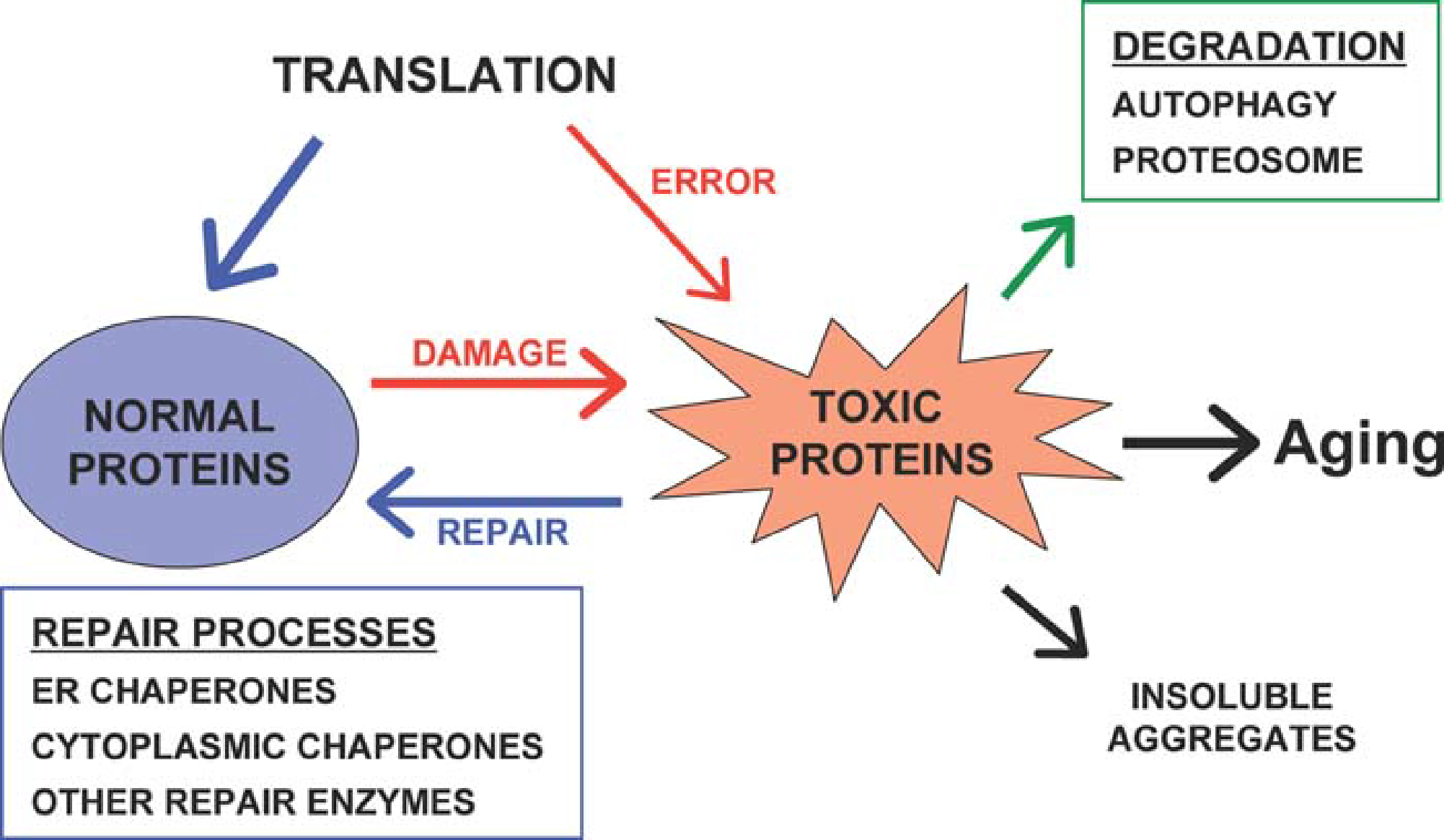
FOXO
Autophagy
S6K
4E-BP
Mitochondrial Metabolism
Ribosomal Proteins
Translation Initiation Factors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