

# Less is more: improving proteostasis by translation slow down

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**Protein homeostasis, or proteostasis, refers to a proper balance between synthesis, maturation, and degradation of cellular proteins. A growing body of evidence suggests that the ribosome serves as a hub for co-translational folding, chaperone interaction, degradation, and stress response. Accordingly, in addition to the chaperone network and proteasome system, the ribosome has emerged as a major factor in protein homeostasis. Recent work revealed that high rates of elongation of translation negatively affect both the fidelity of translation and the co-translational folding of nascent polypeptides. Accordingly, by slowing down translation one can significantly improve protein folding. In this review, we discuss how to target translational processes to improve proteostasis and implications in treating protein misfolding diseases.**

## Approaches to alleviate protein misfolding disorders

Protein misfolding disorders [1] constitute a diverse set of diseases that can be grouped according to molecular mechanisms of pathology. The first group results from mutations that reduce folding of important enzymes and other proteins, leading to their loss of function, such as cystic fibrosis caused by mutation in CFTR (cystic fibrosis transmembrane conductance regulator) or Tay–Sachs disease caused by mutation in  $\beta$ -hexosaminidase A. In principle, these defects could be alleviated by improving protein folding. The second group results from folding–reducing mutations that enhance formation of toxic protein aggregates, such as Huntington’s disease caused by expansion of the polyglutamine track in huntingtin or amyotrophic lateral sclerosis (ALS) caused by mutations in superoxide dismutase. In addition to improving protein folding, these diseases could potentially be alleviated by facilitating degradation of misfolded mutant species or toxic protein aggregates.

As various types of protein misfolding pathologies represent a major medical problem, there has been an ongoing

interest in approaches that reduce the accumulation of misfolded protein species. In fact, several protein misfolding diseases can be partially alleviated by induction of autophagy [2,3], and inducers of autophagy have shown efficacy in animal models [4,5]. Another approach has been to facilitate ubiquitin–proteasome-dependent degradation of mutant molecules. For example, recently a group of small molecules has been developed that enhance association of misfolded polypeptides with heat shock protein (Hsp)70 and facilitate their ubiquitination by the E3 ligase CHIP (carboxyl terminus of Hsp70 interacting protein) [6]. These compounds effectively promoted degradation of the mutant androgen receptor, the cause of Kennedy’s disease, and tau, a factor in Alzheimer’s disease pathology [6–9]. Yet another approach has been to enhance the overall proteasomal activity by inhibiting the proteasome-associated ubiquitin hydrolase USP14 with small molecules. These drugs promote degradation of the oxidatively damaged polypeptides and were proposed to facilitate degradation of pathological proteins [10].

A promising strategy for improving protein homeostasis and alleviating protein misfolding diseases has been upregulation of molecular chaperones. Currently, several chaperone-inducing compounds have been reported and tested in cell culture and animal models of a variety of protein misfolding disorders associated with aggregate toxicity (see, e.g., [11–14]). Similarly, this approach has been tested with disorders associated with insufficient function of mutant proteins. For example, the adverse effects of mutant lysosomal glucocerebrosidase in Gaucher disease can be improved by an increase in the chaperone capacity of cells [15]. Because misfolded molecules of the mutant glucocerebrosidase are rapidly degraded via the endoplasmic reticulum (ER)-associated protein degradation pathway, induction of ER chaperones improved folding of glucocerebrosidase and increased its levels [15]. Analogous effects were seen with mutant  $\beta$ -hexosaminidase A [15].

The ribosome has been described as a hub for protein quality control because major events in protein folding and degradation of misfolded polypeptides physically associate with translating ribosomes [16]. Based on these findings, here we suggest that the arsenal of approaches to lessen protein misfolding could be expanded to include regulation of translation. Correcting protein misfolding from the birth of polypeptide chains at the ribosome holds promises to treat protein misfolding diseases.

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## Co-translational quality control contributes to protein homeostasis

### *Co-translational folding*

Our current understanding of protein folding is predominantly based on *in vitro* refolding of denatured full length proteins [17]. However, under native intracellular conditions, protein folding often occurs concurrently with the synthesis of primary polypeptide chains on the ribosomes [18,19]. The journey of a newly synthesized polypeptide starts in the peptidyl transferase center (PTC) of the ribosome, from where it traverses the exit tunnel. The ribosome exit tunnel provides a unique environment for the nascent chain. The length of the exit tunnel spans 80–100 Å, which is able to accommodate a peptide stretch of approximately 30 amino acids [20,21]. The interior of the tunnel is neither straight nor smooth. The narrow interior of the tunnel (10–20 Å) is incompatible with tertiary structure formation of nascent chains. However, some compact secondary structures such as  $\alpha$ -helical conformation can be accommodated by the tunnel [22].

Upon emergence from the ribosome, the amino terminus of the nascent chain faces a drastic environmental change. There is now ample evidence indicating that the ribosome attached nascent chains can acquire specific conformations [19]. Co-translational folding of partially synthesized nascent chains differs from refolding of full length polypeptides due to the vectorial nature and the relatively slow elongation speed of the translation process. A major hurdle to the capture of co-translational folding events is the heterogeneous nature of newly synthesized polypeptides. Recently, an approach was developed to monitor the folding status of nascent chains by coupling the specificity of folding-sensitive reagents and the sensitivity of ribosome profiling [23]. These results revealed efficient co-translational folding as soon as the corresponding sequence became available. Therefore, newly synthesized proteins are the primary subjects of quality control.

### *Co-translational chaperone interaction*

Most newly synthesized polypeptides interact with molecular chaperones [24] that assist co-translational folding of polypeptides attached to the ribosome [16]. In eukaryotes, two ribosome-associated systems interact with newly synthesized polypeptides, the nascent chain-associated complex (NAC) and the Hsp70-based Ssb/Ssz/Zuo triad system [25]. Both systems are physically located in close proximity to the peptide exit tunnel of ribosomes. A series of studies revealed a global view of substrate specificity for NAC and Hsp70 [26,27]. Interestingly, the Hsp70 Ssb preferentially binds to a subset of nascent chains that tend to aggregate and bear disordered regions. These studies highlight the robust network of chaperones acting co-translationally to preventing misfolding and aggregation. However, it is unclear whether chaperone assistance is continuously needed alongside the translation process.

The chaperones mentioned above belong to a class of chaperones linked to protein synthesis (CLIPS), which is distinct from the stress-induced chaperone network [28]. In yeast, in addition to the Ssb/Ssz/Zuo triad system, CLIPS also include the member of the Hsp70 family Ssa1, TRiC/CCT and prefoldin, whereas the stress-induced group of

chaperones is represented by Hsp104, Sti1, or Ssa4. Despite their mechanistic similarity, unlike the latter group, CLIPS-encoding genes are not induced but rather repressed under stress conditions [29]. It remains to be addressed how these two chaperone networks interplay to regulate co-translational and post-translational folding.

### *Co-translational degradation*

In spite of high translation fidelity, effective chaperone machinery, and translation pausing during stress, not all newly synthesized polypeptides fold successfully and assume their functions in a timely manner. It has been estimated that up to 30% of newly synthesized proteins are rapidly degraded, although this notion has been intensely debated for more than a decade [30–32]. Key questions include but are not limited to: are they degraded in a truly co-translational manner? Are there any substrate preferences? How are these substrates distinguished from the normal peptides? What is the enzymatic machinery responsible for degradation of nascent chains emerging from the ribosome? To address some of these questions, ribosome-associated ubiquitination has been measured in budding yeast [33]. Under normal growth conditions, approximately 5% of newly made polypeptides are ubiquitinated. Using an *in vitro* mammalian system, another group reported that 12–15% of nascent polypeptides are ubiquitinated [34]. In both studies, reducing the ribosome-associated chaperone network or inducing misfolding by amino acid analogs dramatically increased levels of co-translational ubiquitination.

Nascent chains are not equally susceptible to co-translational degradation. It is not surprising to find that the length of the polypeptide and the aggregation propensity are positively correlated with co-translational ubiquitination [33]. Interestingly, co-translational ubiquitination is also linked to a higher tRNA adaptation index, suggesting that translation speed also contributes to the fate of newly synthesized polypeptides. In other words, in line with less effective folding, rapidly translated mRNA products are more susceptible to co-translational ubiquitination.

Since the first description of co-translational degradation [35], many studies have aimed to identify E3 ubiquitin ligases catalyzing co-translational degradation. Several candidate ligases responsible for ubiquitination of cytosolic misfolded proteins, including CHIP and Ubr1/Ubr2, do not seem to mediate co-translational ubiquitination [16]. Two ligases, Ltn1 and CCR4/Not, have been implicated in ubiquitination of nascent chains associated with stalled ribosomes. However, these ribosome-associated ligases have been found to have a rather limited role in ubiquitination of polypeptides associated with the actively translating ribosome [33,34]. In fact, it appears that no single ligase is solely responsible for co-translational ubiquitination. The mechanistic aspects of co-translational quality control remain to be investigated.

### *Co-translational stress response*

Recent work reported that newly synthesized polypeptides are more sensitive to proteotoxic stress, than older polypeptides, and they are selectively degraded by the ubiquitin–proteasome machinery [36]. It is not surprising,

therefore, that in addition to induction of chaperones through the heat shock transcription factor Hsf1, cells often rely on translational control for an immediate and rapid response to stress [37].

Indeed, global protein synthesis is reduced in response to many types of adverse conditions, which relieves the burden of the protein quality control system due to reduced protein production [38]. Current models for the mechanism governing this translational attenuation are largely limited to cell signaling pathway-mediated initiation regulation. For instance, eIF4F complex-mediated cap recognition and eIF2-controlled ternary complex formation are two key initiation targets in controlling global mRNA translation [39,40]. However, the repression of global protein synthesis occurs faster than changes in signaling pathways upon stress, suggesting that additional mechanisms might exist to offer rapid shutdown of protein production.

Using a genome-wide ribosome profiling approach, two groups independently reported widespread pausing of ribosomes early in elongation in response to proteotoxic and heat shock stress [41,42]. Interestingly, most of the ribosomes pause within the first 50 codons, a region corresponding to the length of nascent chains occupying the ribosomal exit tunnel. Because ribosome-associated chaperone molecules are located near the exit of the tunnel, it is conceivable that translation elongation is also influenced by chaperone availability. It is still unclear mechanistically how the absence of chaperones brings translation to a halt. This phenomenon nevertheless reveals that translating ribosomes fine tune the elongation rate by sensing the intracellular folding environment. The early elongation pausing may represent a co-translational stress response to maintain the intracellular protein homeostasis.

### Translation elongation rates influence proteostasis

The folding competence of ribosome-attached polypeptides is primarily determined by the amino acid sequence, as well as the fidelity of its translation, and the activity of the CLIPS chaperones. Several lines of evidence indicate that the rate of elongation of the nascent polypeptide strongly affects its ability to fold properly by modulating both the fidelity of translation and the availability of the ribosome-associated folding factors.

The ribosome is a very precise machine that has a relatively low rate of errors. Indeed, in eukaryotes the frequency of incorrect incorporation of amino acids was estimated to be approximately  $10^{-4}$  [43]. Amino acid incorporation is a competitive process between the cognate and near-cognate tRNAs for a given codon. Several studies both with *in vitro* translation systems and cell culture indicated that high rates of translation reduce fidelity and promote misincorporation of amino acids [44–47], which could lead to protein misfolding. Reduced elongation speed, by contrast, allows for a relatively longer dwelling time for the ribosome to search for the correct tRNA pairing. It is thus conceivable that an increased translation speed generates more aberrant translational products. Notably, poor translation fidelity is extremely dangerous exactly because it causes accumulation of misfolded potentially toxic polypeptides. Indeed, mutations that reduce the

translation fidelity cause defects in proteostasis and, eventually, lead to cellular toxicity [48,49]. Importantly, the CLIPS-mediated folding pathway appears to buffer defects in translation fidelity, as deletion of the Ssb Hsp70 chaperones makes cells extremely sensitive to aminoglycoside antibiotics that induce misincorporation of amino acids into nascent polypeptides [50]. Obviously, the combination of all these effects influences the quality of newly synthesized proteins.

The effectiveness of co-translational folding is also a function of the rate of translation elongation. In mammalian cells, the rate of protein synthesis is approximately five residues per second on average [17,51]. However, the ribosome does not proceed at a constant rate but rather in a stop-and-go movement manner [52]. Variations of elongation speed may result from local stable mRNA structures [53], the presence of rare codons [54], or the interactions between the nascent chain and the ribosome and/or exogenous factors [18]. Increasing evidence has supported the notion that the local discontinuous translation (ribosome pausing) temporally separates the translation of segments of the peptide chain and actively coordinates their co-translational folding [55].

The role of the rate of translation in protein folding was also evidenced from the influence of the mRNA sequence (i.e., the codon usage) on the folding efficiency of the corresponding peptide. Codon usage bias has been thought to result from selection for efficient and accurate translation of highly expressed genes. However, the presence of non-optimal codons at specific mRNA positions may facilitate co-translational folding [55,56]. Translational pausing at rare codons provides a time gap to enable independent folding of available portions of the nascent chain and/or delays the appearance of sequences that might interfere with the folding process. Recently, two independent groups reported that non-optimal codon usage is critical for structural and functional properties of proteins involved in circadian rhythm [57,58]. Therefore, the translation machinery interprets two layers of folding information embedded in the mRNA sequences, and the latter evolves to adjust the codon usage to allow optimal folding of encoded polypeptides.

Further indication that the translation rate influences protein folding was recently underscored by the study of translation of eukaryotic proteins in bacterial cells. Translation in bacterial cells is several times faster than in eukaryotic cells and, accordingly, eukaryotic proteins have not evolved to fold properly at these rates. This has serious implications for the production of recombinant proteins in bacterial systems. Importantly, introduction of a mutation that slows bacterial translation speed enhances eukaryotic protein folding efficiency [59].

### Targeting translational processes to improve proteostasis

#### *Improving the quality of translational products by slowing down translation*

It has been proposed that the rate of translation in cells could be suboptimal for protein folding. Accordingly, it may be possible to improve protein folding and reduce the production of misfolded polypeptides by slowing down

translation. Indeed, it was reported that inhibition of translation by only 15–20% strongly reduced accumulation of ubiquitinated species upon proteasome inhibition, and almost completely prevented formation of an agglomerate of protein aggregates, the aggresome [60,61], indicating an overall suppression of production of misfolded species. Furthermore, minor inhibition of translation could significantly improve production of the normally folded functional mutant disease protein CFTRdelta508. Because misfolded species of CFTRdelta508 are rapidly degraded, minor inhibition of translation had a paradoxical effect of increasing the levels of CFTRdelta508 by improving its folding and thus stabilizing it.

To understand the role of the rate of polypeptide growth in improving protein folding, research compared the effects of inhibitors of translation elongation and initiation on folding of individual mutant proteins and the overall production of misfolded species. Inhibitors of elongation improved all these parameters, whereas inhibitors of initiation were ineffective, with the exception of the substrate CFTRdelta508. In the latter case, both the elongation inhibitor emetine and the initiation inhibitor hypuristanol similarly improved folding and increased protein levels.

The unusual effects of the initiation inhibitor on CFTRdelta508 could be because this polypeptide is folded post-translationally in the ER. Accordingly, minor inhibition of translation may improve its folding by reducing the load of newly translated polypeptides on the ER chaperone machinery. It was also reported that mild inhibition of translation initiation via upregulation of eIF2 phosphorylation by the drug guanabenz leads to the overall improvement of proteostasis and of folding of ER proteins, probably by reducing the load on ER chaperones [62]. Similarly, inhibition of translation initiation improved folding of certain mutants of fibulin, which also takes place in the ER [63].

#### *Effects of physiological regulation of translation on protein folding*

Translation in cells is regulated by multiple signaling pathways under physiological conditions. The major regulator of the ribosome dynamics is the mammalian target of rapamycin complex 1 (mTORC1). mTORC1 acts as a master regulator of protein synthesis, promoting cell growth in response to nutrients, growth factors, and cellular energy status [64]. Although constitutive activation of mTORC1 increases protein synthesis, it has a negative effect on the quality of the translational products [65]. Accordingly, inhibition of mTORC1 by rapamycin restores the quality of newly synthesized polypeptides. mTORC1 controls protein synthesis by phosphorylating several translational regulators. Two well-characterized downstream targets are the eukaryotic initiation factor 4E binding proteins (4E-BPs) and the p70 ribosomal S6 kinases (S6Ks) [39,66]. 4E-BPs are the major effectors of mTORC1 in controlling cap-dependent translation initiation, whereas S6Ks act on multiple stages including elongation through eEF2K. Using mouse embryonic fibroblasts (MEFs) lacking these mTORC1 downstream targets, the authors demonstrate that it is S6Ks that contribute to the translation fidelity.

Notably, the phosphorylation of S6Ks is rapamycin-sensitive, whereas the phosphorylation of 4E-BPs is largely rapamycin-resistant [67]. Indeed, rapamycin rescues the quality of translational products mainly by slowing down the rate of ribosomal elongation.

Improvement of protein folding by rapamycin correlated with improved translation fidelity [65]. As noted above, the frequency of mistranslation is about  $10^{-4}$ , and therefore one wrong amino acid is incorporated in approximately ten polypeptide molecules of average size. Considering that only a fraction of the misincorporations would affect folding, the overall contribution of translation infidelity to misfolding appears to be relatively small, and at first glance its improvement cannot account for the overall improvement of folding. On the other hand, evaluations of the rate of misincorporation were done using either *in vitro* systems or reporter systems in cell culture. These evaluations did not measure translation fidelity at conditions of activated mTOR kinase, under which misincorporations could happen more often due to the higher translation rate. Furthermore, stretches of optimal codons in proteins were not considered, which could be translated much faster and have higher rates of misincorporation in critical protein segments. Therefore, the evaluations of mistranslations could significantly underestimate these effects, and along with higher availability of folding chaperones, improvement of fidelity may strongly contribute to improvement of folding upon slowing down translation.

#### *Effects of suppression of translation on aging and a possible role of improved folding*

The insulin-like growth factor (IGF)1 pathway plays a major role in controlling aging. In analyzing mechanisms of effects of this pathway on aging in *Caenorhabditis elegans*, it was reported that regulation of translation contributes to these effects. Accordingly, RNAi-mediated downregulation of multiple components of the translation machinery significantly extended life span. These components included proteins of both large and small ribosome subunits, S6K, and the initiation factors eIF2 $\beta$  and eIF4G [68–70]. It was proposed that suppression of translation in this model extends life span by reducing the load of translated proteins on the chaperone network and therefore improving proteostasis [71]. More recently, using a whole genome RNAi screen, it was demonstrated that depletion of various components of the translation machinery reduces protein aggregation in several models of protein misfolding disorders [72]. Similarly, in the *Drosophila* model it was demonstrated that suppression of the translation initiation factor 4E-BP reduces protein aggregation in muscle and extends the life span [73]. Therefore, overall improvement of protein folding appears to significantly contribute to beneficial effects on life span by slowing down translation.

Interestingly, one potent anti-aging drug is rapamycin, the inhibitor of mTOR kinase, which reduces translation rates. Indeed, this drug in addition to its anticancer and immunosuppressive effects was shown to significantly extend life span in various model organisms from *C. elegans* to mice [74,75]. It is conceivable that the improvement of

proteostasis by rapamycin due to slowing down translation contributes to its anti-aging effects.

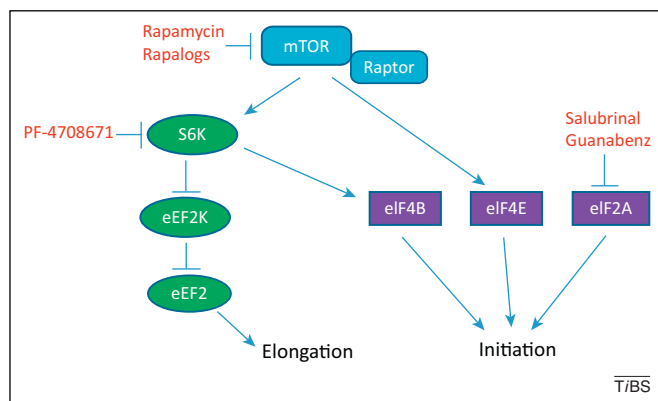
### Approaches to slow down translation for treatment of protein misfolding disorders

The beneficial effects of translation inhibitors on protein folding suggest that this approach could be used for treatment of protein misfolding disorders in parallel to other approaches, such as induction of chaperones or activation of proteasome or autophagy. Currently, a large number of inhibitors of translation in eukaryotes, both natural products and synthetic molecules, have been described. These inhibitors affect various steps in the translation process both in initiation and elongation. Some of these inhibitors are FDA-approved drugs, for example, emetine for treatment of certain parasites [76]. Unfortunately, the major barrier in developing this approach is that the existing inhibitors of translation in eukaryotes demonstrate toxicity, because long-term cell survival requires functional translation. Finding semi-inhibiting doses could be difficult because of the unequal distribution of translation inhibitory drugs (e.g., emetine) between different organs within the organism. This seemingly unsolvable problem could be approached by finding targets whose inhibition leads to only partial suppression of translation.

Current knowledge suggests that translation initiation and elongation can be differentially affected via regulatory pathways (e.g., mTOR). Targeting these pathways has an advantage because modulating these signaling pathways causes only partial inhibition of translation and therefore may not cause significant mechanism-based long-term toxicity. Another advantage is the availability of existing drugs that modulate some of these pathways (e.g., rapamycin). The majority of these drugs have been developed for cancer treatment and therefore there is a possibility of repurposing them for treatment of protein misfolding disorders. A recent report of the mTOR crystal structure may trigger a flurry of studies aimed at developing new drugs targeting mTOR. This would be an important step forward as rapamycin has several drawbacks, and novel mTOR inhibitors (e.g., Everolimus, Temsirolimus, and Ridaforlimus) that have been tested in Phases II–III clinical trials are very well tolerated [77,78].

As compared with inhibition of mTOR, inhibition of S6K provides a different balance between suppression of translation elongation and initiation, because the effects on the mTOR–4EBP1–eIF4E axis are mostly excluded [79] (although there is a feedback loop between S6K and mTOR). Within the translation system, S6K inhibition mostly regulates activity of the elongation factor eEF2 (via eEF2K; Figure 1) [80,81] and, to a lesser extent, translation initiation by phosphorylation of eIF4B [82]. PF-4708671, a well-tolerated inhibitor of S6K, was recently developed [83].

A potent way of affecting the signaling pathway that controls initiation of translation is interference with dephosphorylation of the initiation factor eIF2A. This factor is phosphorylated by several kinases, which leads to inhibition of translation under various stresses [84], including ER stress, viral infection, hypoxia, etc. eIF2A is dephosphorylated by the phosphatase GADD34/PPP1R15A [85].



**Figure 1.** Inhibitors that affect signaling pathways regulating elongation and initiation of translation.

### Box 1. Outstanding questions

- How are translation regulators coordinated with chaperone modulators to promote proteostasis?
- What are the ultimate agents that fine tune the rate of translation?
- How does the powerful ribosome profiling approach help to identify nascent chains that are more susceptible for co-translational misfolding?
- Is there a way to achieve translational adjustment of selective mRNAs without affecting global protein synthesis?

Small molecules that inhibit this phosphatase were discovered, including salubrinal [86] and guanabenz [62], causing hyperphosphorylation of eIF2A and inhibition of translation. Furthermore, guanabenz was shown to protect cells from ER stress [62]. Importantly, guanabenz is a known hypertension drug and is therefore already FDA-approved [87]. Overall, it appears that due to the lack of long-term toxicity, targeting signaling pathways that regulate translation could be an interesting approach towards treatment of protein misfolding disorders.

### Concluding remarks

Recently accumulated experimental evidence demonstrates that translation speed can be controlled by various ribosome-associated factors in addition to the primary sequence of transcripts. It is remarkable that adjusting the rate of elongation may partially correct protein misfolding. This finding opens a new avenue to treatment of disorders associated with protein misfolding. However, we are only beginning to understand the mechanistic details of translational regulation. Many challenges remain in future therapeutic applications (Box 1). It will be exciting to follow these questions and uncover many surprises that will emerge.

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