REVIEW

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Co-translational mechanisms of quality control of newly synthesized polypeptides

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During protein synthesis, nascent polypeptides emerge from ribosomes to fold into functional proteins. Misfolding of newly synthesized polypeptides (NSPs) at this stage leads to their aggregation. These misfolded NSPs must be expediently cleared to circumvent the deleterious effects of protein aggregation on cell physiology. To this end, a sizable portion of NSPs are ubiquitinated and rapidly degraded by the proteasome. This suggests the existence of co-translational mechanisms that play a pivotal role in the quality control of NSPs. It is generally thought that ribosomes play a central role in this process. During mRNA translation, ribosomes sense errors that lead to the accumulation of aberrant polypeptides, and serve as a hub for protein complexes that are required for optimal folding and/or proteasome-dependent degradation of misfolded polypeptides. In this review, we discuss recent findings that shed light on the molecular underpinnings of the co-translational quality control of NSPs.

Introduction

Protein synthesis is one of the most energy-consuming processes in the cell[1] and is a major step in the regulation of gene expression.[2] Notwithstanding the relatively high fidelity of mRNA translation (approximately 2–3 errors for every 10,000 amino acids added to a NSP in mammalian cells),[3] rates of incorrect amino acid incorporation arise in an estimated 20% of proteins due to an accumulation of errors in protein synthesis and/or upstream gene expression regulatory mechanisms (e.g., transcription).[4,5] In addition, aberrant proteins can be generated by anomalous post-translational modifications and dysfunction of *trans*-acting factors that influence the folding of NSPs.[5] Eukaryotes have evolved a variety of mechanisms to ensure high-quality surveillance of their proteomes. Accordingly, a substantial proportion of NSPs are thought to be rapidly degraded during or shortly after their synthesis by the ubiquitin-proteasome system.[6,7] The exact percentage of NSPs that are degraded remains the subject of debate. Pioneering studies contend that approximately 30–50% of NSPs are co-translationally degraded.[8,9] However, cells in these studies were deprived of amino acids prior to metabolic labeling in order to minimize competition between the radiolabeled and endogenous unlabeled amino acids. Subsequently, it was demonstrated that amino acid deprivation induces a feedback loop mechanism, whereby proteasomes are activated to degrade mature, non-aberrant proteins to replenish the endogenous amino acid pool.[10] This observation strongly suggests that the proportion of degraded NSPs was overestimated due to methodological shortcomings.[10] More recently, the extent of NSP degradation was assessed using an alternative method that centers on the level of ubiquitination in lieu of metabolic labeling. Here, nascent polypeptide chains were labeled with a biotin-conjugated fluorescent version of puromycin.[11] Using this approach, the authors have found that approximately 15% and 8% of NSPs are co-translationally ubiquitinated in mammalian cells and in yeast, respectively.[11] Taken together, these findings indicate that a sizable portion of NSPs are co-translationally ubiquitinated and degraded by the proteasome.

In addition to their well-established role in mRNA translation, ribosomes play a central role in the regulation of folding and degradation of NSPs. Herein, we will review recent findings that illuminate the molecular mechanisms underlying the role of the ribosome and other components of the translational machinery in the quality control of NSPs and discuss the functional consequences of these processes.

The role of ribosomes in the folding of NSPs

As they emerge from the ribosomes, nascent polypeptide chains fold into complex three-dimensional structures that characterize the functional protein.[12] The dimensions of the ribosome exit tunnel are limiting (80–100Å long and 10–20Å wide in yeast),[13,14] only allowing folding of the elongating polypeptide chain up to the secondary structural elements.[15] Consequently, the final stages of NSP folding must be accomplished in the crowded cytoplasmic environment. Nascent polypeptides that emerge from the ribosome contain surface-exposed hydrophobic domains that can engage in nonspecific interactions with an array of cytosolic proteins, as well as with the other nascent polypeptides.[16,17] Ribosome-associated chaperones protect these

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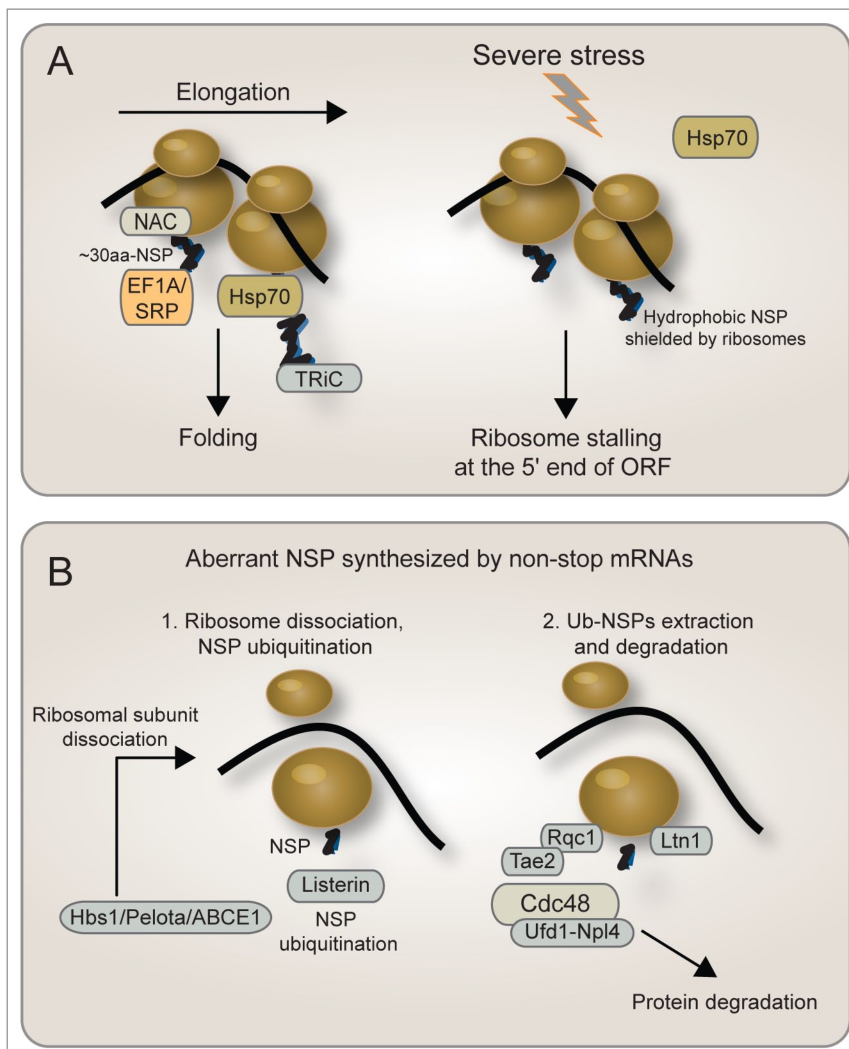

Figure 1. Mechanisms of ribosome stalling, ribosomal subunit dissociation and NSP ubiquitination. **A)** During elongation, NAC, eEF1A, TRiC and Hsp70 chaperones assist the folding of the polypeptide chains fostering their passage through the exit tunnel. Severe stress decreases the association of Hsp70 with ribosomes and nascent chains. Consequently, ribosomes stall on the first ~100 nucleotides of the open reading frame (ORF) to shield the nascent chains, thereby blocking elongation. **B)** Ribosomes are stalled on the poly(A) tail of mRNAs with no stop codon due to strong electrostatic interactions between positively charged poly-lysine and the negatively charged peptide exit tunnel. Hbs1/Pelota/ABCE1 complex dissociates stalled ribosomes while Listerin ubiquitin E3 ligase ubiquitylates abnormal nascent polypeptide chains. Cdc48 AAA-ATPase forms a complex with Ufd1-Npl4 that binds the ribosome through Rqc1 and Tae2. This complex is thought to "pull out" the ubiquitinated stalled nascent polypeptide chains from the ribosomes and delivers them to the proteasome for degradation.

partially folded polypeptides from these nonspecific interactions, thereby increasing the likelihood of accurate folding.[18]

The growing nascent chain first encounters the nascent polypeptide-associated complex (NAC).[19] The NAC is a heterodimeric complex that associates with ribosomes in close proximity to the ribosome exit tunnel (**Fig. 1A**).[19] It shields approximately 30 N-terminal amino acids of the nascent polypeptide.[19] As the nascent polypeptide chain lengthens, the NAC is replaced by other ribosome-associated factors, such as the signal recognition particle (SRP) and the elongation factor eEF1A, both of which have a higher affinity for the hydrophobic regions of the nascent polypeptide chain than the NAC (**Fig. 1A**).[20] Subsequently, additional chaperones are recruited to the exposed and partially folded hydrophobic domains of NSPs. Heat shock proteins Hsp70, Hsp90 and the eukaryotic TCP-1 ring complex (TRiC) were identified as the predominant classes of these chaperones (**Fig. 1A**).[21-25] In eukaryotic cells, Hsp70 chaperones associate with ribosomes, bind elongating polypeptide chains and facilitate their folding, which promotes the association of NSPs with downstream chaperones such as Hsp90[26] or TRiC.[21,27] Hsp70 chaperones preferentially promote the folding of long nascent chains that are characterized by abundant hydrophobic regions.[23] Although some NSPs that had been partially folded by Hsp70 are transferred to Hsp90,[26] the role of Hsp90 chaperones in co-translational folding remains largely elusive. Hsp90 chaperones appear to regulate the folding of a restricted subset of proteins such as steroid receptors and Src-related kinases.[28] TRiC is a large multimeric complex that bears a resemblance to the bacterial GroEL double ring.[29] It was originally identified as a chaperone complex that binds to polar regions of newly synthesized cytoskeletal proteins and facilitates their optimal folding.[30,31] More recently, the TRiC interactome has been further explored.[32] Like its prokaryotic homolog GroEL, the TRiC complex appears to associate with a plethora of structurally and functionally distinct NSPs, which indicates that it plays a general role in the folding of a broad spectrum of NSPs.[32,33]

In yeast, NAC cooperates with Hsp70 chaperone Stress 70B (SSB)–Ribosome Associated Complex (RAC) and the Hsp110 chaperone Sse1 in the folding of NSPs.[34] RAC is a heterodimeric complex formed by members of the conserved Hsp70 (Ssz) and Hsp40 chaperone families (Zuotin).[35] Zuotin interacts with rpL31,[25] which surrounds the peptide exit tunnel.[36] In yeast, deletion of Sse1 and SSB results in protein aggregation, which is further exacerbated by the loss of NAC.[34] Intriguingly, depletion of Sse1 and SSB also causes reduction of small and large ribosomal subunits with concomitant enrichment of ribosomal proteins and rRNA in the pool of aggregated NSPs. This suggests the presence of a regulatory feedback loop whereby an increase in NSP aggregation in cells with abrogated chaperone activity downregulates protein synthesis, thereby preventing further accumulation of misfolded NSPs.[34] These findings show that RAC and associated chaperones play an important role in the optimal folding of NSPs and moreover, that they serve as a

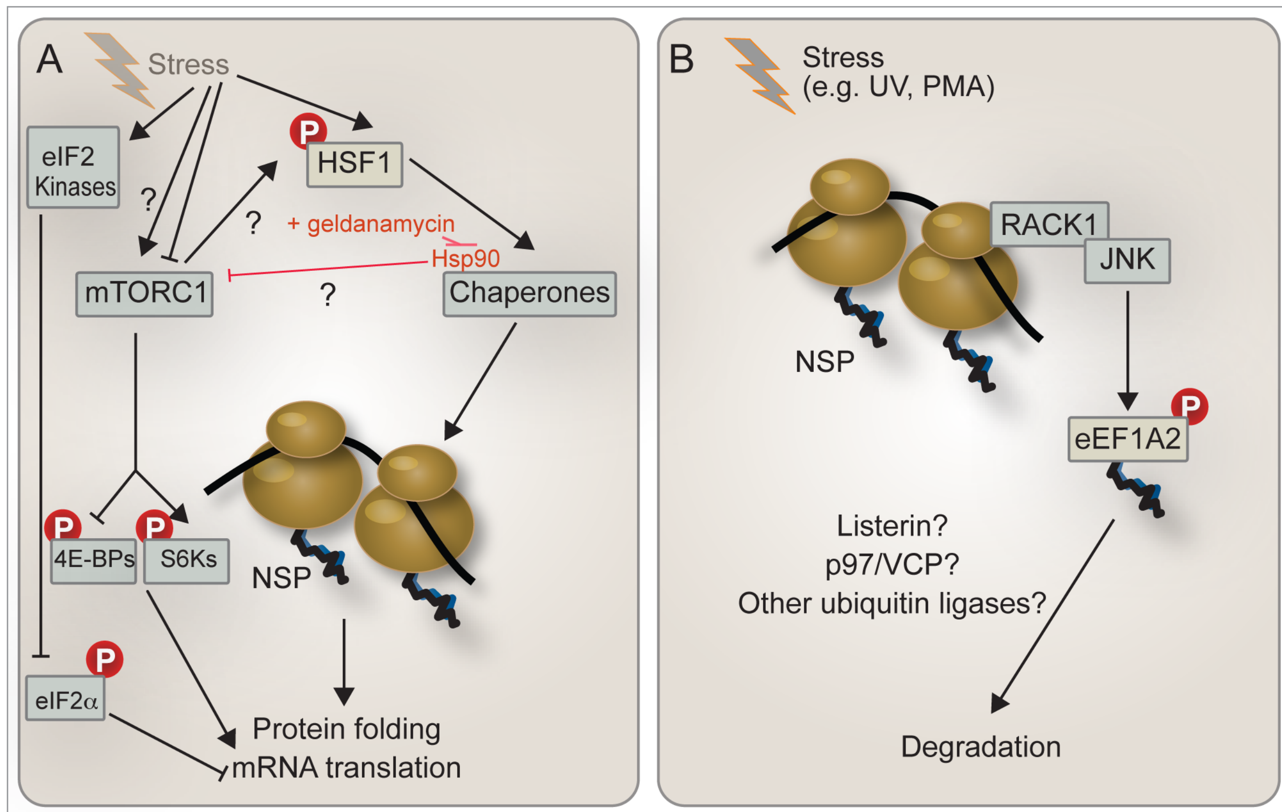

Figure 2. Potential cross-talk between chaperone system, mTORC1 and eIF2 kinases in coordination of NSP folding and translation rates and the role of JNK in mediating degradation of NSPs during stress. **A)** Chaperones stimulate folding of NSPs and their depletion and/or inhibition leads to translational arrest. At an early phase of stress response, however mTORC1 appears to phosphorylate the heat shock transcription factor 1 (HSF1) to induce the expression of chaperone-encoding genes. Chaperone activity prevents accumulation of misfolded NSPs whereas mTORC1 stimulates mRNA translation by phosphorylating 4E-BPs (4E-BP1, 2 and 3 in mammals) and ribosomal protein S6 kinases (S6 kinase 1 and 2 in mammals). In stark contrast, various types of prolonged stress including nutrient deprivation have been shown to inhibit mTORC1 and downregulate protein synthesis rates. In response to various types of stress eIF2 kinases (PERK, PKR, GCN2 and HRI in mammals) phosphorylate eIF2α resulting in the inhibition of global protein synthesis and concomitant stimulation of translation of mRNAs bearing inhibitory upstream open reading frames that encode stress response proteins (e.g., ATF4 and CHOP). Inhibition of the chaperone system (e.g., geldanamycin) appears to suppress mTORC1 activity, but the role of mTORC1 in co-translational protein degradation is still largely elusive. **B)** Stress such as UV-irradiation and altered levels of intracellular Ca^{2+} (PMA) activate JNK. Activated JNK is recruited to ribosomes via interaction with the structural ribosomal protein RACK1. On the ribosomes, JNK phosphorylates eEF1A2 and stimulates its binding to NSPs thereby bolstering their degradation by the proteasome. In mammals, the link between eEF1A2 and proteasome remains unknown.

central node of protein quality control, coordinating both protein folding and rates of mRNA translation (see below).

Ribosomes Link Translational Control and NSP Folding

Folding of nascent polypeptides may be coordinated with translation rates, which in turn are determined by both protein length and mRNA sequence.[37,38] Mild inhibition of protein synthesis has been shown to facilitate folding of NSPs.[39] Interestingly, in yeast, proteins that are synthesized rapidly are more prone to co-translational ubiquitination, an effect that appears to be mediated by a complex network of E3 ubiquitin ligases, including ribosome-bound Rkr1 and Hel2.[40] In general, the translation rate and folding efficiency is determined, at least in part, by features of the mRNA that are being translated. For example, yeast TRP3 mRNA contains a cluster of consecutive rare codons that correspond to a highly-structured region of PYK protein that separates its two enzymatic domains. [41]In light of these and similar findings, it has been proposed that mRNA regions encompassing elongation pausing sites define the domain boundaries and facilitate the structural organization of NSPs.[42-45] Potential effects of codon bias on protein folding also highlight hitherto unappreciated effects that synonymous single-nucleotide polymorphisms (SNPs) may exert on protein function. Namely, SNPs are commonly not expected to affect synthesis, folding and function of polypeptides as the genes harboring them encode the same amino acids as their wild-type counterparts. However, it has been demonstrated that SNPs in the multidrug resistance (MDR1) gene that encodes transmembrane ABC-transporter MDR1/P-glycoprotein affect its folding and transporter activity.[46,47] In addition, an elongation pause site in signal recognition particle receptor (SRα) mRNA is required for co-translational ER membrane anchoring of SRα receptor, inasmuch as synonymous mutations that alleviate elongation pausing impede ER localization of this receptor.[48] Therefore, optimal protein

function requires well-orchestrated regulation of translation and protein folding.

Ribosome-Dependent Regulation of Ubiquitination and Proteasomal Degradation of NSPs

Misfolded NSPs must be rapidly targeted for degradation to avoid the accumulation of protein aggregates that have deleterious repercussions for cells. Accordingly, several key components of the ribosome-associated NSP quality surveillance system have been recently described including Listerin (Ltn1 in yeast), translation elongation factor 1A (EF1A), and 97-kDa valosin-containing protein (p97/VCP; Cdc48 in yeast) (**Fig. 1A and B**).

Listerin mediates degradation of NSPs encoded by non-stop mRNAs

The role of the ribosome in orchestrating co-translational degradation of aberrant NSPs appears to be an essential part of the mRNA surveillance mechanism referred to as non-stop decay. Non-stop decay eliminates mRNAs that are missing appropriate stop codons (non-stop mRNAs) and give rise to anomalous NSPs.[49,50] During the translation of non-stop mRNAs, the poly(A) tail is translated into a polylysine stretch at the C-terminus of the NSP.[51,52] The positively charged polylysine stretch engages in strong electrostatic interactions with the negatively charged surface of the ribosome exit channel, thereby inducing ribosome stalling.[53] To free stalled ribosomes, these aberrant non-stop polypeptides must be removed and degraded.[51] A ribosome-dependent quality control pathway that removes NSPs encoded by non-stop mRNAs has been recently described. This pathway appears to be preserved from yeast to mammals and is centered on Listerin (Ltn1 in yeast), a highly conserved E3 ubiquitin ligase.[54] Listerin contains a lysine-rich domain at the N-terminus and a RING finger at the C-terminus.[55] The RING domain has intrinsic E3 ubiquitin ligase activity.[55] Listerin associates with the large, 60S ribosomal subunit[54,56] and promotes ubiquitination of aberrant non-stop polypeptides in yeast[54] and humans.[57] Recruitment of Listerin to the 60S ribosome is facilitated by the Hbs1/Pelota/ABCE1 recycling complex that dissociates stalled ribosomes from non-stop mRNAs, leading to their degradation by the exosome (**Fig. 1B**).[58] Hbs1 is a paralogue of the translation terminator factor, eukaryotic release factor 3 (eRF3)[59]. It binds to the sarcin-ricin loop of the large ribosomal subunit, which represents a highly conserved region of 28S rRNA that interacts with translation elongation factors.[60] The binding of Hbs1 to this region displaces elongation factor-tRNA complexes from stalled ribosomes. Pelota (Dom34 in yeast) is a paralogue of the eukaryotic release factor 1 (eRF1)[61] that associates with the A-site of stalled ribosomes and promotes their dissociation in conjunction with ABCE1, which is a conserved member of the ATP-binding cassette (ABC) family of proteins.[62,63] It is worthwhile to note that, in yeast, the Hbs1/Dom34 complex plays a central role in the mRNA surveillance mechanism known as no-go decay (NGD) that is induced by prolonged ribosome pausing.[64] Taken together, these findings highlight the mechanism whereby the surveillance complex composed of Hbs1/Pelota/ABCE1 dissociates ribosomes stalled on non-stop mRNAs, while Listerin targets non-stop polypeptides for proteasomal degradation (**Fig. 1B**).

The role of the eukaryotic translation elongation factor 1A (eEF1A) in degradation of NSPs

eEF1A is an abundant elongation factor which delivers aminoacyl-tRNAs to the A-site of the ribosome in a ternary complex with GTP.[65] Upon anticodon/codon recognition, ribosomes activate the GTPase activity of eEF1A, leading to GTP hydrolysis and release of GDP-bound eEF1A from the ribosome.[66,67] Interestingly, in addition to its essential role in translation elongation, archaebacterial translation elongation factor 1A (aEF1a) appears to sense stalled ribosomes during no-go and non-stop mRNA decay. aEF1a recruits ribosome release factor1 (aRF1) and aPelota to the ribosome.[68,69] aRF1 recognizes the stop codon and terminates mRNA translation by stimulating peptidyl-tRNA bond hydrolysis, whereas aPelota dissociates ribosomes that are stalled on aberrant mRNAs. Thus, aEF1a orchestrates the translation elongation, termination and quality control of NSPs.[2,68,69]

The potential role of eEF1A in NSP quality control has also been reported in a variety of eukaryotic organisms ranging from yeast to mammals. To this end, eEF1A has been demonstrated to bind ubiquitinated NSPs and associate with the proteasomes in yeast and *C. elegans*.[20,70,71] In addition, eEF1A appears to be necessary for the degradation of a subset of N-acetylated polypeptides in vitro[72] and has also been recently identified as a component of the "translasome complex" which is composed of translation factors, ribosomes and proteasomal components.[73] Taken together, these findings suggest that eEF1A is a central factor in the regulation of NSP surveillance and implies that its role may be to deliver aberrant NSPs to the proteasome.

p97/VCP: transferring ubiquitinated NSPs from ribosome to proteasome

In addition to Listerin and eEF1A, p97/VCP (Cdc48 in yeast) has recently emerged as a pivotal regulator of ribosome-mediated NSP surveillance. p97/VCP is a multifunctional AAA-ATPase that has been implicated in a variety of cellular processes including autophagy, endosomal sorting and ubiquitin/proteasome-dependent proteolysis.[74] This protein acts as a central node in the endoplasmic reticulum-associated degradation (ERAD) pathway, where it facilitates the retrotranslocation of ubiquitinated proteins from the endoplasmic reticulum and delivers them to the proteasome.[75,76] Cdc48 recognizes ubiquitin chains on target proteins in conjunction with the heterodimeric Ufd1-Npl4 complex, which is followed by the activation of its ATPase activity and subsequent extraction of ubiquitinated proteins from the ER.[77] Cdc48/Ufd1-Npl4 complexes have been recently shown to associate with the 60S ribosomal subunit,[78] which also hosts Ltn1 (**Fig. 1B**). Moreover, ubiquitinated NSPs are accumulated on the ribosomes of Cdc48-deficient yeast.[78] Importantly, ribosomal localization of Cdc48 requires ribosome-associated factors Tae2 and Rqc1, factors that have also been shown to interact with Ltn1 (**Fig. 1B**).[56,79] Therefore, a model has been proposed whereby the Cdc48/Ufd1-Npl4 complex recognizes erroneous NSPs that are co-translationally ubiquitinated by Ltn1 and delivers them to proteasomes (**Fig. 1B**).

The Cross-Talk between Ribosomes and Chaperones Determines Translation Rates under Stress

Cells respond to various types of stress, such as UV-irradiation, nutrient deprivation and ER-stress, by reprogramming gene expression and shutting down global mRNA translation.[80] A stress-induced decrease in the global protein synthesis rate is mediated by the members of the eukaryotic translation initiation factor 2 (eIF2) kinase family (PERK, PKR, GCN2 and HRI).[81] eIF2 forms a ternary complex with the initiator tRNA (Met-tRNAi) and GTP that delivers Met-tRNAi to the 40S ribosomal subunit to form the 43S pre-initiation complex.[82] At the end of initiation, eIF2-bound GTP is hydrolyzed and the binary eIF2/GDP complex is released from the ribosome.[83] eIF2B is a guanine nucleotide exchange factor (GEF) that exchanges GDP bound to eIF2 for GTP allowing the formation of the ternary complex and another round of initiation.[83] eIF2 kinases inactivate eIF2 via phosphorylation of its regulatory eIF2α subunit, which stabilizes eIF2/GDP/eIF2B complexes and inhibits GEF activity of eIF2B.[84-86] Whereas phosphorylation of eIF2α inhibits global translation, it promotes the translation of a specific subset of mRNAs harboring short, inhibitory upstream ORFs (uORFs) that encode master transcriptional regulators of stress response such as CHOP and ATF4 (**Fig. 2A**).[87] In non-stressed cells, uORFs impede the progression of scanning ribosomes toward the downstream initiation codon.[88,89] In contrast, stress-induced eIF2α phosphorylation attenuates re-initiation on uORFs, thereby favoring the translation of downstream ORFs.[90]

In addition to the well-established role of eIF2α phosphorylation in stress-induced downregulation of mRNA translation, several recent studies implicate chaperones in this process. Proteotoxic stress is a condition in which protein homeostasis (protein synthesis, folding, and clearance) is altered by various stressors including heat-shock or oxidation.[91] Cells respond to proteotoxic stress by decreasing mRNA translation rates and increasing the chaperone capacity.[92] Recent findings show that in addition to its role in the folding of NSPs, Hsp70 bolsters translation elongation rates by facilitating extrusion of nascent chains from the ribosome exit tunnel.[21,93] In cells exposed to heat stress or toxic amino acid analogs, Hsp70 chaperones dissociate from ribosomes, presumably due to excess misfolded proteins out-competing nascent polypeptides for Hsp70. The overall reduction in ribosome-associated Hsp70 is paralleled by a genome-wide elongation pausing ~100 nucleotides downstream of the initiation codon.[93,94] Under these conditions, stalled ribosomes shield the first ~30–40 amino acids of nascent polypeptide chains (**Fig. 1A**). Consistently, depletion or pharmacological inhibition of Hsp70 recapitulates the elongation pausing phenotype even in the absence of proteotoxic stress.[93] Therefore, elongation pausing of ribosomes is considered to be a strategy to prevent potentially harmful unspecific interactions of nascent polypeptides, and to allow time for more Hsp70 chaperones to become available, as well as to block other ribosomes from engaging the mRNA under conditions where chaperone capacity is diminished.[93,94]

Although the mechanism underlying the pausing of ribosomes during elongation remains poorly understood, emerging data indicate that chaperones and the ribosome exit tunnel collaboratively orchestrate protein folding and ribosome scanning (**Fig. 1B**).[95] Ribosomal proteins of the large subunit rpL4 and rpL22 appear to form what resembles a gated opening in the ribosome exit tunnel[36] and have a role in ribosome stalling.[15] Hsp70 chaperones associate with rpL22 and rpL4 and this interaction may cooperate to resize the ribosome exit tunnel.[36,93] These studies provide the first look into the mechanisms that chaperones may employ to coordinate protein folding and translation elongation rates.

NSP Quality Control during Stress

Although ribosome-mediated quality control of NSPs represents an important mechanism of proteome surveillance, mechanisms that regulate this process in response to stress remain largely obscure. Below, we discuss recent findings that provide the first evidence implicating the mammalian target of rapamycin complex 1 (mTORC1) and c-jun N-terminal kinase (JNK) in the regulation of NSP folding and degradation under stress.

Chaperones and mTORC1

In addition to affecting translation rates by stimulating elongation of polypeptides, recent findings suggest that chaperones may influence protein synthesis at the rate-limiting initiation step via modulation of mTORC1 activity. mTORC1 stimulates mRNA translation by phosphorylating a multitude of downstream targets including eukaryotic translation initiation factor 4E (eIF4E)-binding proteins (4E-BPs), ribosomal protein S6 (rpS6) kinases (S6Ks) and their downstream targets (e.g., rpS6, eIF4B, PDCD4 and eukaryotic elongation factor 2 kinase), as well as by stimulating ribosome biogenesis and tRNA synthesis (**Fig. 2A**).[96]

The mTORC1 pathway is the major anabolic pathway whose activity is downregulated when cells are exposed to adverse conditions such as nutrient or oxygen deprivation.[96] Paradoxically, during the initial phase of cellular response to a variety of stressors, including misfolding agents (heat shock, hydrogen peroxide) or unrelated stressors (such as UV-irradiation), mTORC1 appears to be activated (**Fig. 2A**).[97,98] Recent observations suggest that partial reduction in availability of the chaperone Hsp90 during the early stress response stimulates mTORC1 signaling, whereas prolonged exposure to stress that exceeds buffering capacity of the chaperones appears to inhibit mTORC1 activity.[99] Depletion of chaperone function by genetic (loss of heat shock factor protein 1 [HSF1]) or pharmacological means (using the Hsp90 inhibitor geldanamycin) resulted in a more rapid inhibition of mTORC1 signaling during the course of stress as compared with control cells (**Fig. 2A**).[99] This suggests that a reduction in chaperone availability during moderate stress may modulate mTORC1 activity. Dietary restriction and optimal chaperone capacity have been linked to an increase in longevity in numerous species.[100-103] Notwithstanding that further studies are required to establish the underlying mechanism of chaperone-dependent modulation of mTORC1 signaling, moderately reduced chaperone capacity

and nutrient excess may have synergistic effects on the activation of mTORC1. Consistently, hyperactivation of mTOR is linked to various pathological states including cancer and diabetes, whereas chronic suppression of mTOR signaling has been linked to lifespan extension in a variety of organisms.[96] Furthermore, a recent report implied that mTORC1 may modulate the chaperone capacity of the cell by phosphorylating HSF1,[104] which suggests the existence of regulatory feedback loops that coordinate mTORC1 activity, translation rates and chaperone availability (**Fig. 2A**).

The RACK1/JNK/eEF1A2 complex

Stress-activated c-Jun N-terminal kinase (JNK) is a major regulator of cellular stress responses that has been implicated in several protein surveillance mechanisms.[105] Nonetheless, the role of JNK in NSP quality control remains poorly understood. We demonstrated that JNK co-translationally regulates stability and multimerization of the tumor suppressor protein p53.[106] E2-ubiquitin conjugating enzyme Ubc13 associates with p53 on polysomes. This results in K63-ubiquitination of p53 and inhibition of its tetramerization and transcriptional activity.[107] UV-irradiation or phorbol ester-induced activation of JNK leads to phosphorylation of polysome-associated p53 and dissociation of p53/Ubc13 complexes. This is accompanied by tetramerization of p53 and its subsequent transcriptional activation.[106]

Lately, a more general role for JNK in NSP decay has been suggested.[108,109] Upon activation, JNK is recruited to the 40S ribosomal subunit via the receptor for activated C kinase 1 (RACK1) and it co-sediments with polysomes (**Fig. 2B**).[109] RACK1 is a scaffold protein that interacts with a wide repertoire of signaling molecules including JNK.[110] It is also a structural component of the 40S ribosomal subunit that localizes proximally to the mRNA exit channel.[111] The yeast homolog of RACK1 (Asc1p) promotes the degradation of erroneous nascent polypeptides encoded by the non-stop mRNAs.[112] Moreover, association of RACK1 with the ribosome appears to be necessary for ubiquitination of NSPs.[108] In mammalian cells, activated JNK binds and phosphorylates the eEF1A2 isoform of eEF1A on evolutionarily conserved serine/proline motifs (Ser205 and Ser358) that are notably absent in the eEF1A1 isoform.[109] As mentioned previously, eEF1A is considered to play an evolutionarily conserved role in folding and co-translational degradation of NSPs. Accordingly, JNK-mediated phosphorylation of eEF1A2 stimulates its recruitment on polysomes and its binding to NSPs.[109] This is paralleled by increased proteasomal degradation of NSPs (**Fig. 2B**).[109] Remarkably, while critical for stress-induced NSP decay, JNK-mediated phosphorylation of eEF1A2 does not appear to have a major effect on translation elongation rates.[109] Although it remains to be assessed whether eEF1A2 activates E3 ubiquitin ligases, directly delivers NSPs to the proteasome, or whether this is mediated by as-yet unknown factors, these results indicate that ribosome-associated RACK1/JNK/eEF1A2 complexes represent a central node of the stress-induced NSP quality control network in mammalian cells (**Fig. 2B**). A mass spectrometry-based approach revealed that, in addition to eEF1A2 and a number of proteasomal components, ribosome-associated JNK complexes contain a variety of chaperones including Hsp70 chaperones and TRiC subunits.[109] This suggests that JNK may be a major regulator of NSP folding and degradation under stress.

Future Challenges and Concluding Remarks

Emerging data indicate that, in addition to their well-established role in translation, ribosomes and other components of the translational machinery play a major role in NSP surveillance. However, cellular networks that link protein synthesis, chaperones, and proteasome machineries are still largely unknown. Alterations in chaperone capacity modulate protein synthesis rates,[94,93] whereas inhibition of translation appears to downregulate chaperone production by disrupting the transcriptional activity of heat shock factor protein 1 (HSF1).[113] In turn, HSF1 has been shown to regulate mTORC1 activity during stress and to stimulate rDNA transcription in cancer cells, thus suggesting that it may bolster translation.[99,114] In addition to HSF1, SKN-1 (*C. elegans* ortholog of mammalian nuclear respiratory factors Nrf1, 2 and 3), a master transcriptional regulator of oxidative stress, has been shown to coordinate rates of protein synthesis and degradation by modulating the activity of the ubiquitin-proteasome system.[115] Inhibition of the proteasome leads to the induction of SKN-1, whereas suppression of translation leads to its inactivation.[115] Importantly, SKN-1 promotes resistance to oxidative stress and extends the lifespan of worms.[116] These findings suggest that HSF1 and SKN-1 may serve as central nodes of cellular stress network that sense alterations in translation rates and proteotoxic stress to coordinate protein synthesis, folding and decay to maintain organismal and cellular homeostasis.

In addition to knowledge gaps related to the molecular underpinnings of cellular networks that coordinate mRNA translation, NSP quality control and degradation, it is still not clear whether NSP surveillance equally affects all cellular proteins, or whether it targets specific subsets of NSPs. The potential importance of selective mechanisms of NSP surveillance is substantiated by defective ribosomal products (DRiPs) that are generated by rapid degradation of a specific subset of NSPs shortly after completion of their synthesis and serve as peptide ligands for MHC class I molecules.[117,118] These findings suggest that targeted degradation of NSPs may play a major role in essential cellular processes such as immune response and immunosurveillance of tumors.[119] The plausibility of the selective degradation of NSPs also puts forward a model, whereby decay of a subset of NSPs would produce changes in the proteome that are required for the optimal stress response (e.g., by degrading nascent polypeptides that are not required during stress). In this way, degradation of NSPs may serve not only as a quality control mechanism, but also as the crucial step in the regulation of gene expression.

In this review, we summarized emerging mechanisms of co-translational surveillance of NSPs and the networks that coordinate translation rates, folding and degradation of NSPs. The results of these studies strongly suggest that the co-translational surveillance of NSPs is achieved via the orchestrated action of the translational machinery, chaperones and the ubiquitin-proteasome system.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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