Title

Ubiquitin-dependent translation control mechanisms: Degradation and beyond.

Journal

Cell Reports, 43(12)

Authors

Ford, Pierce

Narasimhan, Mythreyi

Bennett, Eric

Publication Date

2024-12-24

DOI

10.1016/j.celrep.2024.115050

Peer reviewed

Published in final edited form as:

Cell Rep. 2024 December 24; 43(12): 115050. doi:10.1016/j.celrep.2024.115050.

Ubiquitin-dependent translation control mechanisms: degradation and beyond

Pierce W. Ford[1,*], **Mythreyi Narasimhan**[1,*], **Eric J. Bennett**[1,2]

[1]School of Biological Sciences, Department of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA 92093, USA

Abstract

Translation control mechanisms connect the largely static genome to the highly dynamic proteome. At each step in the translation cycle, multiple layers of regulation enable efficient protein biogenesis under optimal conditions and mediate responses to acute environmental challenges. Recent research has demonstrated that individual ribosomal protein ubiquitylation events act as molecular signals to specify quality control pathway outcomes. Here, we synthesize current knowledge of ubiquitin-mediated translation control mechanisms and highlight key outstanding questions. We compare and contrast ubiquitin-dependent mechanisms that regulate ribosome associated quality control pathways at several steps in the translation cycle. We also explore how distinct ribosome ubiquitylation events on specific ribosomal proteins impact translation activity and how defects in specific ubiquitin-mediated regulatory steps impact physiology and health.

Introduction

Protein biogenesis is central to all cellular activities. Rapid and faithful decoding of the genetic material into proteins is required to construct dynamic proteomes that respond to developmental and proliferative cues[1,2]. Protein biogenesis and degradation bookend the protein lifecycle, and both processes are regulated to coordinate stress responses. In this context, the ubiquitin proteasome system regulates the degradation of most cytosolic and nuclear proteins[3]. Protein ubiquitylation involves appending ubiquitin to target lysine residues through the ordered activities of E1 activating enzymes, E2 conjugating enzymes, and E3 ubiquitin ligases[4]. The canonical fate of ubiquitylated proteins is proteasome-mediated degradation, but some ubiquitylation events instead regulate substrate activity[3]. Post-translational modification of many translation-associated factors has been demonstrated to regulate translation activity[5]. Perhaps unsurprisingly, proteomic approaches have described ubiquitylation of many translation-associated factors, including most ribosomal proteins, providing a possible major mechanism for ubiquitin-mediated translation regulation[6–9]. Many of these identified ribosomal ubiquitylation events likely

[2]Corresponding author - e1bennett@ucsd.edu.
[*]These authors contributed equally to this work.

Declaration of Interests
The authors declare no competing interests.

target orphaned (non-complexed) ribosomal proteins for decay[10–12]. However, a subset of ribosomal protein ubiquitylation events are conserved across eukaryotes at the level of the individual modification site, and these ubiquitylation events have been shown to directly regulate assembled and active ribosomes[13,14]. The site-specificity of these regulatory ribosomal ubiquitylation events contrasts with typical ubiquitylation reactions, where plasticity of ubiquitin-site utilization is often observed when proteasomal degradation is the ultimate outcome. Here, we focus on describing how conserved ribosomal ubiquitylation regulates translation activity and capacity.

Overview of ribosome-associated quality control pathways

Though translation is a stunningly complex process, most translation reactions properly decode individual mRNAs into cognate proteins. However, despite this robustness, ribosome progression can be impeded by a variety of mechanisms that require the activity of ribosome-associated quality control (RQC) pathways[15]. Since the early genetic characterization of the RQC pathway in yeast[16,17], recent research progress has uncovered new RQC pathways that respond to specific insults during the translation cycle[15,16,18,19]. Ribosomal protein ubiquitylation events are consistently identified as key steps in these increasingly diverse RQC pathways.

There are numerous conditions in which ribosome progress can be delayed during the initiation, elongation, and termination phases of translation. For ease of discussion, we consider three general categories of delayed ribosome progression: paused ribosomes, stalled ribosomes where the stall is resolvable, and trapped ribosomes that are permanently stuck with progression impossible (Fig. 1A). Ribosome pauses can occur upon depletion of individual tRNAs, consecutive decoding of non-optimal codons, ribosome assembly defects that impair decoding, or reduced abundance or activity of translation elongation, initiation, or termination factors. Ribosome stalling occurs when physical blockades are encountered. Examples include rigid mRNA structure, mRNA modifications, crosslinked mRNA with ribosomes or other RNA binding proteins (RBPs), defective ribosomes that prevent initiation or elongation, polypeptide interactions with the 60S exit channel, and translation elongation or initiation inhibitors. In principle, each of these stalls can be resolved to allow ribosomes to continue translating past the impediment. However, truncated or cleaved mRNAs that do not contain a termination codon present a blockade where the ribosome cannot proceed past the insult and result in what we refer to here as a trapped ribosome (Fig. 1A). Paused versus stalled ribosomes can be differentiated using kinetic parameters (e.g. paused ribosomes are more transient than stalled ribosomes), and a central challenge for RQC pathways is the faithful discrimination between normal pausing events and more problematic stalls.

Each of the scenarios outlined above requires RQC activity for successful resolution, and the precise mechanisms and outcomes often vary. Regulatory ribosomal ubiquitylation events are often required for subsequent steps of RQC pathways. Surprisingly, these ubiquitylation events are highly site-specific, with multiple distinct ligases being employed to ubiquitylate individual ribosomal proteins on specific lysine residues (Fig. 1B). The ubiquitylation site-specificity suggests that individual ribosomal ubiquitylation events impart distinct functional outcomes. In this review, we predominately focus on the distinct ribosomal ubiquitylation

events which serve to resolve stalled ribosomes, with some discussion of ubiquitin-mediated resolution of paused ribosomes.

Elongation ribosome-associated quality control

The elongation phase of the translation cycle normally proceeds through a set ordered reactions that decode the A-site codon, catalyze peptidyl transferase to extend the growing nascent polypeptide chain, and translocate the ribosome to position a new codon within the A-site (Fig. 2). The normal elongation cycle can be slowed by a variety of impediments. One initial conundrum was how stalled ribosomes that require RQC activity are differentiated from normally translating ribosomes. Initial elegant kinetic modeling and subsequent experiments in *E. coli* from the Subramaniam research group indicated collision events between a stalled leading ribosome and a trailing ribosome lead to abortive termination[20]. Subsequent clever and rigorous biochemical, structural, and *in vivo* studies from the Inada, Hegde, and Zaher research groups provided evidence that ribosome collisions act as a common trigger to activate RQC pathways[21–23]. The collision model simplifies the discrimination between paused and stalled ribosomes and provides unique biochemical entities (e.g. ribosome collision interfaces) to engage RQC factors. While 80S ribosome collisions clearly stimulate RQC pathway activation, it remains unclear if collisions are strictly required for all RQC pathway outcomes.

RQC activation generally results in truncated polypeptide destruction, mRNA decay, and ribosome recycling. However, there are several exceptions to this general description as well as context, organism, and tissue type specificities in RQC responses[15,18,19]. Failure to degrade truncated nascent chains due to loss or mutation of RQC factors leads to neurodegeneration, underscoring the physiological importance of RQC-mediated resolution of stalled ribosomes[24,25]. In this section we discuss ubiquitin-mediated steps and mechanisms that occur during RQC pathway activation and subsequent resolution of stalled and collided ribosomes.

Ubiquitin-mediated sensing and ribosome splitting

A stalled 80S elongating ribosome that persists long enough to allow a trailing 80S ribosome to collide with it triggers the classical elongation-centric RQC pathway (Fig. 2). Collisions are initially sensed by the E3 ligase ZNF598 in mammals, which is recruited to the collision interface and mediates distinct regulatory ribosomal ubiquitylation events[21,22]. ZNF598 ubiquitylates ribosomal proteins eS10 (RPS10) at K138 and K139 and uS10 (RPS20) at K4 and K8 (Fig. 2). These ubiquitylation events are hierarchical: eS10 ubiquitylation precedes and is required for uS10 ubiquitylation[26–29]. These site-specific ubiquitylation events are reversed by the deubiquitylating enzymes USP21 and OTUD3[26]. While OTUD3 specifically demodifies ZNF598-mediated eS10 and uS10 ubiquitylation, USP21 exhibits promiscuous ribosomal deubiquitylation activity[26]. Initial studies reported that ZNF598 specifically monoubiquitylates eS10 and uS10[26,28,29]. However, recent studies demonstrate that ZNF598 also mediates K63-linked polyubiquitylation on eS10 and uS10[30,31]. Loss of ZNF598 or mutation of the ubiquitylation sites on either eS10 or uS10 results in RQC failure and readthrough of stall inducing sequences in multiple reading frames[28,29]. This observation

indicates that concurrent ubiquitylation of two different ribosomal proteins are needed for subsequent RQC steps in mammals. Hel2, the yeast ortholog of ZNF598, similarly ubiquitylates uS10 but interestingly also ubiquitylates uS3 and eS7 (RPS7)[32]. However, mutation of the uS10 ubiquitylation sites alone completely phenocopy the stall readthrough effects of *Hel2* deletion, suggesting uS10 ubiquitylation is the most consequential RQC ubiquitylation event in yeast[32]. This conserved regulatory ribosomal ubiquitylation is a pivotal step in initiating downstream RQC events.

The mechanistic role of these regulatory ribosomal ubiquitylation events has been a key area of active investigation. Ribosomal ubiquitylation is directly recognized by the ribosome quality control trigger complex (RQT) in yeast (ASC-1 complex in mammals) to promote collided ribosome dissociation (Fig. 2)[30–37]. In mammals, the ASC-1 complex consists of a stable complex of the RNA helicase ASCC3, CUE domain containing protein ASCC2, TRIP4, and an additional ASCC1 component which may be dispensable for ribosome clearance[34,35,38]. The yeast RQT complex is comprised of Slh1 (ASCC3 homolog), Cue3 (ASCC2 homolog), and Rqt4 (TRIP4 homolog)[32,37]. The ATPase-dependent helicase activity of the core component ASCC3/Slh1 is important for dissociation of collided ribosomes and its loss results in stall readthrough, phenocopying ZNF598/Hel2 loss[33–35,38]. The presence of a ubiquitin-binding CUE domain within ASCC2/Cue3 led to the hypothesis that it serves as the elusive reader of ribosome ubiquitylation. However, there are contradictory reports regarding the significance of the CUE domain of ASCC2 during RQC-stimulated ribosomal clearance[34,35]. Loss of ASCC2 results in ribosome stall readthrough that is fully rescued by re-expressing wildtype ASCC2. However, rescue experiments using ASCC2 with a mutant CUE domain abates the loss of function phenotype either partially[34] or completely[35], indicating that the ubiquitin-binding CUE domain is at least partially dispensable *in vivo* in mammals. In contrast, recent evidence shows that a ubiquitin-binding deficient ASCC2 fails to promote subunit dissociation in an *in vitro* ribosome splitting assay, emphasizing the biochemical importance of ribosomal ubiquitin recognition and binding for ASC-1 complex recruitment[30]. Experiments in yeast show that the ubiquitin binding CUE domain of Cue3 and N-terminal residues of Rqt4 independently associate with ubiquitylated ribosomes[36]. While either *Cue3* or *Rqt4* single mutants fail to abolish ribosome disassembly, their combined deletion phenocopies *Hel2* loss[36]. Together, these observations illustrate a mechanism where, during elongating ribosome collisions, the ubiquitin moieties on uS10 and eS10 recruit the ASC-1/RQT complex via ASCC2/Cue3 or TRIP4/Rqt4 ubiquitin binding to stimulate clearance of stalled ribosomes.

The cryo-EM structure of the yeast RQT complex revealed its exclusive association with the 40S subunit of the leading stalled ribosome, close to the mRNA entry channel and ribosomal protein uS10. During a ribosomal collision, the RQT-associating region of the trailing ribosome is occluded by the lead ribosome, making it inaccessible to the RQT complex[33]. This observation is consistent with *in vitro* data demonstrating that the RQT complex specifically disassociates the leading stalled ribosome in a collision, resulting in either elongation resumption by the trailing ribosome past the blockade or subsequent RQT-mediated clearance of the next leading ribosome in the collision queue[33,35]. The interaction of the RQT complex with the lead ribosome also relies on the accessibility of the 3’ mRNA overhang emerging from the stalled ribosome. A minimum of 30 nucleotides downstream

of the stalled ribosome P-site is essential for efficient subunit splitting by ASCC3/Slh1[31,33]. As an interesting contrast, the mammalian mRNA surveillance Pelota-Hbs1 complex can promote subunit dissociation of stalled ribosomes with either an empty A site or an occupied A site with a very short 3' mRNA overhang[35]. The Pelota-Hbs1 complex fails to act on ribosomes when the mRNA overhang extends beyond 12 nucleotides[30,35,38]. This ubiquitin-independent mechanism therefore serves to split and rescue terminally trapped ribosomes. Thus, distinct mechanisms exist to clear the different classes of impeded ribosomes.

The RQT complex cryo-EM structure further suggests Slh1 promotes subunit splitting by applying a pulling force on mRNA with the trailing ribosome acting as a wedge between the subunits of the two collided ribosomes. According to this model, the presence of at least one neighboring ribosome would be required to drive subunit dissociation, implying that the last 80S in the collision queue would not be dissociated[33]. While these findings are in agreement with the biochemical evidence suggesting ASCC3/Slh1 dissociates monosomes far less efficiently than disomes or trisomes[30,35], a recent study indicates ASCC3 can efficiently dissociate all queued ribosomes regardless of their position as long as they are ubiquitylated by ZNF598[31]. This study also showed that even a single stalled monosome can be ubiquitylated by ZNF598 and dissociated by the ASC-1 complex, implying that ribosome collisions are not a prerequisite for recruitment of either ZNF598 or the ASC-1 complex *in vitro*[31]. However, it is unclear if this observation holds true *in vivo* where ribosome abundance is far in excess of ZNF598, ASCC2, and ASCC3. In such a scenario, ribosome collisions may be required to ensure selective recruitment of RQC factors to problematic translation events.

Ubiquitin-mediated turnover of nascent polypeptide chains in stalled ribosomes

Ribosomes that require RQC-dependent stall resolution generally have incompletely synthesized polypeptides emerging from the ribosomal exit tunnel. Destruction of these partially synthesized proteins is an important RQC outcome that is needed to avoid accumulation of toxic unfolded protein intermediates. ASC-1/RQT complex-mediated subunit dissociation results in a free 40S subunit, and an uncommon 60S-peptidyl-tRNA conjugate. To facilitate recycling of the 60S subunit, degradation of the associated trapped nascent chain is necessary (Fig. 2). This degradation is mediated through ubiquitylation by the E3 ligase Listerin in mammals (Ltn1 in yeast)[17,39]. The obstructed 60S subunit, but not empty 60S or 80S, is specifically recognized by NEMF in mammals (Rqc2 in yeast). This recognition is facilitated by NEMF interaction with the P-site peptidyl-tRNA as well as the intersubunit surface of the 60S, which is occluded by the associated 40S subunit during active translation. Interactions between NEMF and the peptidyl-tRNA confer the ability to discriminate a 60S-peptidyl-tRNA conjugate from empty an 60S. NEMF in turn recruits Listerin to the peptidyl-tRNA-obstructed 60S and positions the Listerin RING domain next to the exit tunnel to promote K48-linked polyubiquitylation of the nascent chain[40,41]. Genetic screens[16,42] and *in vitro* studies[43] identify TCF25 in mammals (Rqc1 in yeast), as essential for efficient nascent chain polyubiquitylation by Listerin. The ubiquitylated, but still trapped, nascent chain can not immediately be processed for degradation by the proteasome. The targeted polypeptide must first be detached from the covalently bound tRNA and extracted out of the 60S subunit. ANKZF1 in mammals

(Vms1 in yeast) facilitates this function by binding to the 60S subunit and mediating endonucleolytic cleavage of the P-site tRNA acceptor arm, thereby releasing the nascent chain bound to the 3'-CCA end of the tRNA[44–47]. Once cleaved, p97 (Cdc48 in yeast) and its cofactors UFD1 and NPLOC4 (Ufd1 and Npl4 in yeast) are recruited to the polyubiquitylated nascent chain where they extract the nascent polypeptide for eventual proteasome-mediated degradation (Fig. 2)[16,42,48]. These elaborate tiered mechanisms for RQC-dependent nascent chain ubiquitylation are required to prevent spurious targeting of nascent chains elongating from normally translating ribosomes. Ribosome ubiquitylation thus serves a part of a failsafe mechanism that confines Listerin activity.

While *in vitro* reconstitution experiments have been instrumental in defining RQC mechanisms, what fraction of the total cellular ribosome pool participates in RQC events at steady state or upon stress remains an open question. A recent study using *in situ* cryo-electron tomography in mammalian cells sought to interrogate cellular RQC capacity. This study revealed that ribosomal collisions triggered by treatment with low-dose elongation inhibitors leads to a significant accumulation of peptidyl-tRNA bound 60S[49]. Interestingly, there was no increase in the fraction of 60S-peptidyl-tRNA complexes bound to RQC factors NEMF and Listerin compared to that of untreated cells[49]. This suggests that while the cellular levels of NEMF and Listerin are adequate to resolve the obstructed 60S subunits resulting from steady-state collisions, an increase in collision stress saturates these RQC factors[49]. As a result, under conditions that elevate the occurrence of ribosomal stalls, the availability of free 60S subunits for subsequent rounds of translation may become limiting. This paucity could, in turn, serve as a trigger for activation of cellular stress response pathways associated with RQC events[38,50–55].

Initiation ribosome-associated quality control

The importance of uS10 and eS10 ubiquitylation within the elongation RQC pathway indicates other observed ribosome ubiquitylation events may regulate distinct steps in the translation cycle. Eukaryotic translation begins when the 40S subunit of the ribosome engages a suite of translation initiation factors and the ternary complex, which is comprised of eIF2, GTP, and methionine-initiator-tRNA (Fig. 3A). This 43S pre-initiation complex associates with the eIF4 mRNA cap-binding complex and scanning of the 5'UTR commences. Upon encountering a sufficiently strong initiation codon, typically AUG, the complex remodels, GTP hydrolysis occurs, the 60S subunit joins, and the ribosome transitions from the initiation phase to the elongation phase[56–58]. One prominent mechanism of translation regulation is the phosphorylation of the alpha subunit of the eIF2 translation initiation factor that occurs upon activation of the integrated stress response (ISR) and limits ternary complex formation[59]. Prior to the characterization of ZNF598/Hel2-mediated RQC ubiquitylation events, site-specific monoubiquitylation of uS3 (RPS3) at K214 and uS5 (RPS2) at K54 and K58 was demonstrated to be induced upon ISR activation[60]. These ubiquitylation events do not require ZNF598 but are instead catalyzed by the E3 ligase RNF10 and require the ribosomal protein RACK1[26,29,61,62]. Site-specific ubiquitylation of uS3 on K214 is required for subsequent ubiquitylation of uS5 on K54 and K58, and these events are antagonized by the deubiquitylating enzyme USP10 (Fig. 3B)[29,61,62]. Because distinct pharmacological agents that act on initiating ribosomes potently stimulate RNF10-

dependent uS3 and uS5 ubiquitylation (Fig. 3A), this new RQC branch was named the initiation ribosome-associated quality control (iRQC) pathway[61].

While ZNF598-mediated ubiquitylation is required to rescue stalled and/or collided 80S ribosomes, the surprising outcome of RNF10-mediated ribosome ubiquitylation is degradation of the entire 40S subunit (Fig. 3B)[61,62]. Prolonged ubiquitylation of uS3 and uS5 by either RNF10 overexpression or USP10 depletion leads to an approximately 17% decrease in cellular 40S ribosomal subunits[61,62]. Surprisingly, 40S degradation in this context does not require canonical autophagy, suggesting a potential 40S disassembly event followed by proteasomal degradation[61]. Though previous results had linked USP10 loss to programmed lysosomal degradation of the ribosome[63], recent work has implicated an additional uS5 ubiquitylation event on K275 as the modification responsible for lysosomal targeting in the absence of USP10 as part of a protective senescence response to oncogenic transformation[64]. These distinct but overlapping roles of USP10 highlight the specificity within the regulatory ribosomal ubiquitylation code.

The physiologically relevant context for iRQC-mediated 40S decay is not fully understood. The observation that approximately 15% of total uS3 is ubiquitylated upon USP10 depletion in the absence of any cellular stressor suggests that RNF10-mediated ribosome ubiquitylation occurs regularly during normal cellular proliferation[61]. One possible iRQC function during normal steady state conditions may be to target defective or improperly assembled 40S subunits for degradation.

Ubiquitin-mediated targeting of non-functional ribosomes

Ribosome biogenesis is a highly regulated process with many quality control checkpoints that ensure production of functionally competent ribosomes[65–67]. Detection and removal of defective 60S and 40S biogenesis intermediates can take place prior to cytoplasmic export of late biogenesis intermediates, which prevents the possible utilization of poorly assembled ribosomes within the translation cycle. Distinct from defects in early nucleolar or nuclear ribosome biogenesis steps, imperfect quality control detection of the later cytoplasmic 40S maturation steps results in the accumulation of premature 40S subunits within presumably translationally active 80S monosome and polysome fractions in yeast[65,68–70]. Although disrupting late cytoplasmic 40S biogenesis steps in human cells did not result in increased 40S biogenesis intermediates migrating with translating 80S fractions[71], premature 60S or 40S subunits that engage with mRNA and translation initiation or elongation factors would reduce overall translation capacity. Degradation pathways would be needed to remove these potentially detrimental defective ribosomal subunits.

Research on prokaryotic ribosomes revealed that mutating highly conserved residues within the decoding center of 16S rRNA (G530U, U534G and A1492C: *E. coli* numbering)[72] or the peptidyl transferase center of 23S rRNA (A2451G, C2452U and U2585A: *E. coli* numbering)[73] does not alter overall ribosomal structure and these defective rRNAs escape early quality control checkpoints and populate mature subunits. Ribosomes that contain these mutant rRNAs cannot function during translation and cause dominant lethality in *E. coli*[72–74]. Building upon these early studies, pioneering research from the Moore group described non-functional ribosomal decay (NRD) pathways in *S. cerevisiae*. These

mechanistically distinct 18S and 25S NRD pathways act to destroy mutant defective rRNAs that become incorporated into mature ribosomal subunits[75,76]. While these studies focused exclusively on rRNA decay and did not directly observe ribosomal protein decay, rRNA decay would likely be impossible without simultaneous destruction of the closely associated ribosomal proteins.

Elegant studies from the Inada research group extended these early NRD characterizations and directly linked the 18S NRD pathway to ribosome ubiquitylation. Work from the Inada research group demonstrated that site-specific ubiquitylation of uS3 is a required step within the 18S NRD pathway (Fig. 3C)[77]. The ubiquitin ligase Mag2 in yeast (RNF10 ortholog) catalyzes uS3 ubiquitylation on the conserved uS3 lysine residue K212 that is also targeted by RNF10 (K214 in mammals)[77]. Subsequent genetic screens identified a second ubiquitin ligase, Fap1, whose deletion impairs 18S NRD. Fap1 extends a polyubiquitin chain on uS3 that was previously ubiquitylated by Mag2 (Fig. 3C)[78]. Incorporation of mutant 18S rRNA molecules that impair the decoding steps of translation is thought to prevent the progression of 80S ribosomes beyond the start codon. Consistent with this idea, sequencing of mRNAs from Mag2-associated ribosomes revealed predominantly non-optimal codons positioned within the A-site of Mag2-associated ribosomes, indicating Mag2 generally associates with slow ribosomes[78]. These results are also consistent with structural studies where Fap1 was shown to interact with the 40S at both the mRNA entry and exit sites. These studies suggest that mRNA movement within an elongating ribosome is incompatible with Fap1 binding, providing a possible mechanism for how Mag2 and Fap1 specifically target stationary ribosomes.

It is currently unknown if the 18S NRD pathway is conserved in mammals. The molecular constituents and mechanisms shared between the characterized mammalian iRQC pathway and the yeast 18S NRD pathway provide evidence that RNF10 could target defective 40S subunits for degradation in mammals. However, the previously highlighted observation that a significant fraction (15%) of uS3 is ubiquitylated in USP10 depleted cells without any perturbation of 40S function challenges the idea that the sole role of the iRQC pathway lies in targeting defective 40S subunits. Cells producing that quantity of defective 40S at steady state is unlikely as it would be highly inefficient and a waste of cellular anabolic resources. There are also key differences between the yeast 18S NRD pathway and the mammalian iRQC pathway that indicate the mammalian pathway has evolved new regulatory functions. For example, the RNF10-mediated uS5 ubiquitylation events are essential in mammals but do not occur in yeast. ISR pathway activation robustly induces mammalian uS3 and uS5 ubiquitylation whereas similar ISR induction does not trigger uS3 ubiquitylation in yeast. Additionally, polyubiquitylation of uS3 is required for 18S NRD in yeast but has thus far never been detected following iRQC activation in mammalian studies.

Though some of the key molecular constituents of the iRQC pathway have been characterized, much remains unknown about the pathway. The mechanism by which 40S subunits targeted for RNF10-mediated ubiquitylation are destroyed is a key area of active study in the field. Identification of the uS3 and uS5 ubiquitylation reader as well as the factors that facilitate the final ribosome destruction will enable the use of new molecular levers to pry apart the cellular consequences associated with failed iRQC events. Further, the

precise ribosomal species targeted for ubiquitylation by RNF10/Mag2 is unknown. Current hypotheses for the relevant iRQC-inducing species include stalled initiation complexes, initiation complex collisions, scanning 40S-80S collisions, stalled initiating 80S ribosomes, and free non-functional pre-40S subunits (Fig. 3B). As we discussed previously, kinetic modeling studies informed predictions for the canonical elongation RQC pathway that elongating 80S ribosome collisions could serve as a stimulating event for translation regulation[20]. Similarly, recent modeling studies focused on translation dynamics during decoding of upstream open reading frames (uORFs) suggest that scanning 40S subunits dissociate when they collide with stalled elongating 80S ribosomes within uORFs[79]. Whether this 40S-80S collision event is the critical ribosomal species required for iRQC activation *in vivo* remains to be determined.

Ribosome stalling due to trapped termination and elongation complexes

Pharmacological inhibitors of distinct steps within the translation cycle have been essential torches used to illuminate ribosome states that trigger RQC pathway activation. For example, low concentrations of elongation inhibitors were shown to stimulate ZNF598-dependent ribosome ubiquitylation in a manner that was not observed with high concentrations of the same inhibitors. These observations were among the first to inform the hypothesis that collisions between elongating ribosomes activate RQC pathways. Recent studies using a translation inhibitor known to prevent eEF1A release from the ribosome during translation elongation, Ternatin-4, unveiled a new and distinct ribosome quality control pathway that targets non-ribosomal proteins for degradation (Fig. 4A). Ternatin-4 treatment results in the surprising degradation of eEF1A. CRISPR-based screens identified the E3 ligases RNF25 and RNF14 as factors required for Ternatin-4-induced eEF1A degradation[80]. The two ligases target distinct proteins to facilitate eEF1A degradation. RNF14 is the ligase that directly ubiquitylates the A-site trapped eEF1A leading to eEF1A degradation. Interestingly, this RNF14-mediated eEF1A ubiquitylation first requires RNF25-mediated site-specific ubiquitylation of the ribosomal protein eS31 (RPS27A) at K107 and K113 (Fig. 4A)[49,80,81]. This study also identified the ISR coactivator, GCN1, to be required for Ternatin-4-stimulated eEF1A degradation. Because GCN1 binds to collided 80S ribosomes[81], this result suggests that ribosome collisions are a necessary precondition for targeting eEF1A trapped within elongating ribosomes. All regulatory ribosomal ubiquitylation discussed thus far has taken place on the 40S subunit proteins. However, recruitment of RNF14 to the ribosome also leads to ubiquitylation of several 60S proteins within the vicinity of eEF1A[80]. Whether these 60S ubiquitylation events represent functional regulation or are incidental from RNF14 proximity is currently unknown, but these are interestingly the only known RQC-related 60S ribosomal subunit ubiquitylation events.

Prior studies had identified a small molecule that promoted readthrough of premature stop codons by stimulating degradation of the translation termination factor eRF1[82]. Follow-up studies demonstrated that the mechanism of eRF1 degradation in this context is identical to the RNF14 and RNF25-dependent mechanism described to facilitate degradation of trapped eEF1A (Fig. 4A)[80,83,84]. Combined, these studies identified a new ribosomal ubiquitylation pathway that is required to destroy elongation or termination factors that become glued

to the ribosomal A-site using small molecules not normally present in mammalian cells. While these pharmacological agents illuminated a hidden translation control pathway, the physiological relevance of this trapped A-site RQC pathway was imminently a focus for follow-up work.

Recent work identifies a possible physiological role for this pathway in resolving formaldehyde-induced mRNA-protein crosslinking events (mRPCs). Treatment of cells with 4-thiouridine followed by UV light treatment (4-SU + UV) was used to model RNA-specific formaldehyde-induced crosslinking of RBPs to RNA (Fig. 4A)[85,86]. This treatment paradigm leads to RNA:protein crosslinks which can physically block ribosome progression resulting in collisions of elongating ribosomes. RNF14 and RNF25 are again identified as necessary for clearance of these crosslinked mRPCs, and the RNF25 hallmark eS31 K107 and K113 ubiquitylation events are enriched by 4SU + UV treatment[85,86]. Degradation of mRPCs is also blocked by pre-treatment with elongation inhibitors, indicating ribosome collisions are likely essential for activation of this RQC pathway as well[85]. Collectively, these findings indicate that the RNF25 and RNF14-mediated RQC pathway exhibits a remarkable flexibility toward eliminating translational roadblocks (Fig. 4A).

Ubiquitin-mediated detection of paused ribosomes

Due to differences in tRNA abundance and availability, the rate of decoding for individual codons varies[87]. Thus, ribosome progression can be paused during decoding of nonoptimal codons. Transcripts containing nonoptimal codons are destroyed by mechanisms that rely on ongoing translation to target slowly decoding mRNAs for decay[88–91]. The CCR4-NOT complex is the major mRNA deadenylase that, with decapping enzymes and exonucleases, facilitates mRNA decay[87,91]. The CCR4-NOT complex comprises both deadenylase and ubiquitin ligase activity and the Not4 (CNOT4 in mammals) component ubiquitylates the ribosomal protein eS7[92–95]. Not4-mediated eS7 ubiquitylation plays a key role in promoting mRNA decay during slow decoding of non-optimal codon-enriched reporter mRNAs (Fig. 4B)[95]. Both deletion of *Not4* and mutation of the four eS7 ubiquitylated lysine residues stabilize the decay of nonoptimal reporter mRNA. Structural studies determined that the amino-terminal domain (NTD) of Not5 (CNOT3 in mammals) occupies the empty E-site of targeted ribosomes, providing a structural rationale for how slow decoding ribosomes with empty E and A sites are selectively targeted by the CCR4-NOT complex (Fig. 4B)[95]. Interestingly, deletion of the Not5 NTD, while impairing the ribosome association of the larger CCR4-NOT complex and the decay of nonoptimal reporter mRNAs, did not impact eS7 ubiquitylation. This result suggests that eS7 ubiquitylation itself is not sufficient to trigger mRNA decay[95]. How Not4-mediated eS7 ubiquitylation is initially stimulated and the role ubiquitylated eS7 plays in recruiting the larger CCR4-NOT complex remain open questions. Further, recent studies investigating the human CCR4-NOT complex revealed notable differences from the previous studies in yeast. Surprisingly, CNOT4-mediated eS7 ubiquitylation is not required for ribosome association of the larger CCR4-NOT complex[96]. These studies suggest that eS7 ubiquitylation is functioning to recruit downstream factors distinct from the CCR4-NOT complex or as a ribosome quality control check to prevent new rounds of translation by blocking 40S association with initiation factors. This hypothesis is supported by structural evidence that eS7 ubiquitylation would sterically clash with eIF4A

and eIF4G in the human translation initiation complex[97]. Elucidation of key factors that act as eS7 ubiquitylation readers will help fill a mechanistic void while also serving as potential tools to enrich for CCR4-NOT targeted ribosomes.

Conclusions and perspectives for future insights

Ribosomal ubiquitylation events serve as discriminating signals in the growing network of translation quality control pathways. Despite the specificity of the distinct E3 ligases involved in carrying out regulatory ribosomal ubiquitylation, RQC pathways often engage in crosstalk by nature of the fact that they all collectively act on ribosomes engaged in translation. As an example of the complexity of crosstalk between RQC branches, constitutive activation of the classic elongation RQC pathway (eRQC) by treatment with elongation inhibitors also directly activates the iRQC pathway, presumably by generating stalled ribosomes close to the start codon that subsequently inhibits scanning 40S complexes. However, unresolved ribosome collisions also lead to eIF2α phosphorylation, which in turn suppresses iRQC activation by limiting the availability of ternary complex for scanning[50]. Thus, eRQC can either stimulate or suppress iRQC activation, and the factors that play a role in determining the ultimate outcome depend on the severity of the activating signals. The fact that multiple distinct pathways act on the same molecular machine also highlights the likely scenario that a singular ribosome may harbor multiple distinct ubiquitylated sites at any given time. There is a growing need to develop reagents and approaches that can examine if multiple hallmark RQC ubiquitylation events occur on individual ribosomes simultaneously. These reagents will prove valuable towards identifying the mechanisms cells utilize to decipher the grammar of the ribosome ubiquitin code to promote the appropriate physiological response.

Similarly, cellular availability of various RQC components varies by cell type in steady state or under stress conditions and may modulate the activation of different RQC branches. Considering that quality control factors are generally available in sub-stoichiometric levels relative to ribosomes, it is likely that RQC pathway activity is limited in its capacity to handle aberrant translational events. Expanded use and development of cryo-electron tomography (cryo-ET) approaches will rapidly enhance our ability to visualize and quantify ribosome quality control activity in cells and tissues with minimal perturbation. Recent results using cryo-ET approaches suggest that RQC pathways may be easily saturated by increasing the presence of collided elongating ribosomes. Interestingly, overwhelming the RQC pathway and the subsequent increase in peptidyl-tRNA trapped 60S subunits also results in an increase in the proportion of aberrant pre-initiation complexes bound to scanning factors[49]. This result suggests that saturating RQC activity may subsequently trigger iRQC pathway activation.

Translation elongation and initiation inhibitors have been invaluable tools used to identify and characterize RQC mechanisms. However, physiologically relevant contexts that do not require the use of inhibitors to activate RQC pathways remain largely uncharacterized. RNA damage that induces protein:RNA crosslinks represents one such scenario, but how these pathways operate within the key detoxifying tissues remains unknown. While ribosome collisions have emerged as a centralizing mechanism that allows for RQC-dependent

discrimination between problematic and functional ribosomes, recent work indicates that collision events are not strictly necessary for ZNF598-mediated ubiquitylation[31]. These findings highlight the need to identify and utilize specific physiological or genetic triggers for RQC induction that can be used to determine how these pathways operate *in vivo*.

Studies in yeast, mammalian cells, and *in vitro* systems have not only resulted in the identification of required RQC factors, but also greatly enhanced our mechanistic understanding of the greater RQC network. However, our understanding of the *in vivo* relevance of the various RQC pathways remains incomplete. Knocking out the collision sensor ZNF598/Hel2 in mammalian cells, fruit flies, zebrafish, or yeast does not result in overt phenotypes or impact cell survival under normal growth conditions[98–100]. Similarly, loss of RNF10/Mag2 does not result in cell proliferation defects. It is therefore important to identify the physiological stress contexts in which prompt recognition and resolution of RQC-inducing events are necessary for survival. Once those contexts are identified, the already accumulated wealth of foundational knowledge regarding RQC mechanisms can be leveraged toward interventions that enhance or impair RQC activity as needed to promote successful recovery from translational stress.

Acknowledgements

This review was supported by funding from the National Institutes for Health (R35GM148339 (E.J.B.) and T32GM133351 (P.W.F.)).

References

1. Hershey JWB, Sonenberg N, and Mathews MB (2019). Principles of Translational Control. Cold Spring Harb Perspect Biol 11. 10.1101/cshperspect.a032607.
2. Saba JA, Liakath-Ali K, Green R, and Watt FM (2021). Translational control of stem cell function. Nat Rev Mol Cell Biol 22, 671–690. 10.1038/s41580-021-00386-2. [PubMed: 34272502]
3. Oh E, Akopian D, and Rape M (2018). Principles of Ubiquitin-Dependent Signaling. Annu Rev Cell Dev Biol 34, 137–162. 10.1146/annurev-cellbio-100617-062802. [PubMed: 30110556]
4. Varshavsky A (2017). The Ubiquitin System, Autophagy, and Regulated Protein Degradation. Annu Rev Biochem 86, 123–128. 10.1146/annurev-biochem-061516-044859. [PubMed: 28654326]
5. Simsek D, and Barna M (2017). An emerging role for the ribosome as a nexus for post-translational modifications. Curr Opin Cell Biol 45, 92–101. 10.1016/j.ceb.2017.02.010. [PubMed: 28445788]
6. Udeshi ND, Mertins P, Svinkina T, and Carr SA (2013). Large-scale identification of ubiquitination sites by mass spectrometry. Nat Protoc 8, 1950–1960. 10.1038/nprot.2013.120. [PubMed: 24051958]
7. Wagner SA, Beli P, Weinert BT, Nielsen ML, Cox J, Mann M, and Choudhary C (2011). A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. Mol Cell Proteomics 10, M111 013284. 10.1074/mcp.M111.013284.
8. Xu G, Paige JS, and Jaffrey SR (2010). Global analysis of lysine ubiquitination by ubiquitin remnant immunoaffinity profiling. Nat Biotechnol 28, 868–873. 10.1038/nbt.1654. [PubMed: 20639865]
9. Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, Sowa ME, Rad R, Rush J, Comb MJ, et al. (2011). Systematic and quantitative assessment of the ubiquitin-modified proteome. Mol Cell 44, 325–340. 10.1016/j.molcel.2011.08.025. [PubMed: 21906983]
10. Nguyen AT, Prado MA, Schmidt PJ, Sendamarai AK, Wilson-Grady JT, Min M, Campagna DR, Tian G, Shi Y, Dederer V, et al. (2017). UBE2O remodels the proteome during terminal erythroid differentiation. Science 357. 10.1126/science.aan0218.

11. Sung MK, Porras-Yakushi TR, Reitsma JM, Huber FM, Sweredoski MJ, Hoelz A, Hess S, and Deshaies RJ (2016). A conserved quality-control pathway that mediates degradation of unassembled ribosomal proteins. Elife 5. 10.7554/eLife.19105.
12. Juszkiewicz S, and Hegde RS (2018). Quality Control of Orphaned Proteins. Mol Cell 71, 443–457. 10.1016/j.molcel.2018.07.001. [PubMed: 30075143]
13. Dougherty SE, Maduka AO, Inada T, and Silva GM (2020). Expanding Role of Ubiquitin in Translational Control. Int J Mol Sci 21. 10.3390/ijms21031151.
14. Monem PC, and Arribere JA (2024). A ubiquitin language communicates ribosomal distress. Semin Cell Dev Biol 154, 131–137. 10.1016/j.semcdb.2023.03.009. [PubMed: 36963992]
15. Filbeck S, Cerullo F, Pfeffer S, and Joazeiro CAP (2022). Ribosome-associated quality-control mechanisms from bacteria to humans. Mol Cell 82, 1451–1466. 10.1016/j.molcel.2022.03.038. [PubMed: 35452614]
16. Brandman O, Stewart-Ornstein J, Wong D, Larson A, Williams CC, Li GW, Zhou S, King D, Shen PS, Weibezahn J, et al. (2012). A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. Cell 151, 1042–1054. 10.1016/j.cell.2012.10.044. [PubMed: 23178123]
17. Bengtson MH, and Joazeiro CA (2010). Role of a ribosome-associated E3 ubiquitin ligase in protein quality control. Nature 467, 470–473. 10.1038/nature09371. [PubMed: 20835226]
18. D'Orazio KN, and Green R (2021). Ribosome states signal RNA quality control. Mol Cell 81, 1372–1383. 10.1016/j.molcel.2021.02.022. [PubMed: 33713598]
19. Yip MCJ, and Shao S (2021). Detecting and Rescuing Stalled Ribosomes. Trends Biochem Sci 46, 731–743. 10.1016/j.tibs.2021.03.008. [PubMed: 33966939]
20. Ferrin MA, and Subramaniam AR (2017). Kinetic modeling predicts a stimulatory role for ribosome collisions at elongation stall sites in bacteria. Elife 6. 10.7554/eLife.23629.
21. Ikeuchi K, Tesina P, Matsuo Y, Sugiyama T, Cheng J, Saeki Y, Tanaka K, Becker T, Beckmann R, and Inada T (2019). Collided ribosomes form a unique structural interface to induce Hel2-driven quality control pathways. Embo j 38. 10.15252/embj.2018100276.
22. Juszkiewicz S, Chandrasekaran V, Lin Z, Kraatz S, Ramakrishnan V, and Hegde RS (2018). ZNF598 Is a Quality Control Sensor of Collided Ribosomes. Mol Cell 72, 469–481 e467. 10.1016/j.molcel.2018.08.037. [PubMed: 30293783]
23. Simms CL, Yan LL, and Zaher HS (2017). Ribosome Collision Is Critical for Quality Control during No-Go Decay. Mol Cell 68, 361–373 e365. 10.1016/j.molcel.2017.08.019. [PubMed: 28943311]
24. Martin PB, Kigoshi-Tansho Y, Sher RB, Ravenscroft G, Stauffer JE, Kumar R, Yonashiro R, Müller T, Griffith C, Allen W, et al. (2020). NEMF mutations that impair ribosome-associated quality control are associated with neuromuscular disease. Nat Commun 11, 4625. 10.1038/s41467-020-18327-6. [PubMed: 32934225]
25. Endo R, Chen YK, Burke J, Takashima N, Suryawanshi N, Hui KK, Miyazaki T, and Tanaka M (2023). Dysregulation of ribosome-associated quality control elicits cognitive disorders via overaccumulation of TTC3. Proc Natl Acad Sci U S A 120, e2211522120. 10.1073/pnas.2211522120.
26. Garshott DM, Sundaramoorthy E, Leonard M, and Bennett EJ (2020). Distinct regulatory ribosomal ubiquitylation events are reversible and hierarchically organized. Elife 9. 10.7554/eLife.54023.
27. Garzia A, Jafarnejad SM, Meyer C, Chapat C, Gogakos T, Morozov P, Amiri M, Shapiro M, Molina H, Tuschl T, and Sonenberg N (2017). The E3 ubiquitin ligase and RNA-binding protein ZNF598 orchestrates ribosome quality control of premature polyadenylated mRNAs. Nat Commun 8, 16056. 10.1038/ncomms16056. [PubMed: 28685749]
28. Juszkiewicz S, and Hegde RS (2017). Initiation of Quality Control during Poly(A) Translation Requires Site-Specific Ribosome Ubiquitination. Mol Cell 65, 743–750 e744. 10.1016/j.molcel.2016.11.039. [PubMed: 28065601]
29. Sundaramoorthy E, Leonard M, Mak R, Liao J, Fulzele A, and Bennett EJ (2017). ZNF598 and RACK1 Regulate Mammalian Ribosome-Associated Quality Control Function by

Mediating Regulatory 40S Ribosomal Ubiquitylation. Mol Cell 65, 751–760 e754. 10.1016/j.molcel.2016.12.026. [PubMed: 28132843]

30. Narita M, Denk T, Matsuo Y, Sugiyama T, Kikuguchi C, Ito S, Sato N, Suzuki T, Hashimoto S, Machova I, et al. (2022). A distinct mammalian disome collision interface harbors K63-linked polyubiquitination of uS10 to trigger hRQT-mediated subunit dissociation. Nat Commun 13, 6411. 10.1038/s41467-022-34097-9. [PubMed: 36302773]
31. Miścicka A, Bulakhov AG, Kuroha K, Zinoviev A, Hellen Christopher U.T., and Pestova Tatyana V. (2024). Ribosomal collision is not a prerequisite for ZNF598-mediated ribosome ubiquitination and disassembly of ribosomal complexes by ASCC. Nucleic Acids Research. 10.1093/nar/gkae087.
32. Matsuo Y, Ikeuchi K, Saeki Y, Iwasaki S, Schmidt C, Udagawa T, Sato F, Tsuchiya H, Becker T, Tanaka K, et al. (2017). Ubiquitination of stalled ribosome triggers ribosome-associated quality control. Nat Commun 8, 159. 10.1038/s41467-017-00188-1. [PubMed: 28757607]
33. Best K, Ikeuchi K, Kater L, Best D, Musial J, Matsuo Y, Berninghausen O, Becker T, Inada T, and Beckmann R (2023). Structural basis for clearing of ribosome collisions by the RQT complex. Nat Commun 14, 921. 10.1038/s41467-023-36230-8. [PubMed: 36801861]
34. Hashimoto S, Sugiyama T, Yamazaki R, Nobuta R, and Inada T (2020). Identification of a novel trigger complex that facilitates ribosome-associated quality control in mammalian cells. Sci Rep 10, 3422. 10.1038/s41598-020-60241-w. [PubMed: 32099016]
35. Juszkiewicz S, Speldewinde SH, Wan L, Svejstrup JQ, and Hegde RS (2020). The ASC-1 Complex Disassembles Collided Ribosomes. Mol Cell 79, 603–614 e608. 10.1016/j.molcel.2020.06.006. [PubMed: 32579943]
36. Matsuo Y, Uchihashi T, and Inada T (2023). Decoding of the ubiquitin code for clearance of colliding ribosomes by the RQT complex. Nat Commun 14, 79. 10.1038/s41467-022-35608-4. [PubMed: 36627279]
37. Sitron CS, Park JH, and Brandman O (2017). Asc1, Hel2, and Slh1 couple translation arrest to nascent chain degradation. RNA 23, 798–810. 10.1261/rna.060897.117. [PubMed: 28223409]
38. Stoneley M, Harvey RF, Mulroney TE, Mordue R, Jukes-Jones R, Cain K, Lilley KS, Sawarkar R, and Willis AE (2022). Unresolved stalled ribosome complexes restrict cell-cycle progression after genotoxic stress. Mol Cell 82, 1557–1572 e1557. 10.1016/j.molcel.2022.01.019. [PubMed: 35180429]
39. Chu J, Hong NA, Masuda CA, Jenkins BV, Nelms KA, Goodnow CC, Glynne RJ, Wu H, Masliah E, Joazeiro CA, and Kay SA (2009). A mouse forward genetics screen identifies LISTERIN as an E3 ubiquitin ligase involved in neurodegeneration. Proc Natl Acad Sci U S A 106, 2097–2103. 10.1073/pnas.0812819106. [PubMed: 19196968]
40. Shao S, Brown A, Santhanam B, and Hegde RS (2015). Structure and assembly pathway of the ribosome quality control complex. Mol Cell 57, 433–444. 10.1016/j.molcel.2014.12.015. [PubMed: 25578875]
41. Shen PS, Park J, Qin Y, Li X, Parsawar K, Larson MH, Cox J, Cheng Y, Lambowitz AM, Weissman JS, et al. (2015). Protein synthesis. Rqc2p and 60S ribosomal subunits mediate mRNA-independent elongation of nascent chains. Science 347, 75–78. 10.1126/science.1259724. [PubMed: 25554787]
42. Defenouillère Q, Yao Y, Mouaikel J, Namane A, Galopier A, Decourty L, Doyen A, Malabat C, Saveanu C, Jacquier A, and Fromont-Racine M (2013). Cdc48-associated complex bound to 60S particles is required for the clearance of aberrant translation products. Proc Natl Acad Sci U S A 110, 5046–5051. 10.1073/pnas.1221724110. [PubMed: 23479637]
43. Osuna BA, Howard CJ, Kc S, Frost A, and Weinberg DE (2017). In vitro analysis of RQC activities provides insights into the mechanism and function of CAT tailing. eLife 6, e27949. 10.7554/eLife.27949. [PubMed: 28718767]
44. Verma R, Reichermeier KM, Burroughs AM, Oania RS, Reitsma JM, Aravind L, and Deshaies RJ (2018). Vms1 and ANKZF1 peptidyl-tRNA hydrolases release nascent chains from stalled ribosomes. Nature 557, 446–451. 10.1038/s41586-018-0022-5. [PubMed: 29632312]

45. Su T, Izawa T, Thoms M, Yamashita Y, Cheng J, Berninghausen O, Hartl FU, Inada T, Neupert W, and Beckmann R (2019). Structure and function of Vms1 and Arb1 in RQC and mitochondrial proteome homeostasis. Nature 570, 538–542. 10.1038/s41586-019-1307-z. [PubMed: 31189955]
46. Kuroha K, Zinoviev A, Hellen CUT, and Pestova TV (2018). Release of Ubiquitinated and Non-ubiquitinated Nascent Chains from Stalled Mammalian Ribosomal Complexes by ANKZF1 and Ptrh1. Mol Cell 72, 286–302.e288. 10.1016/j.molcel.2018.08.022. [PubMed: 30244831]
47. Yip MCJ, Keszei AFA, Feng Q, Chu V, McKenna MJ, and Shao S (2019). Mechanism for recycling tRNAs on stalled ribosomes. Nat Struct Mol Biol 26, 343–349. 10.1038/s41594-019-0211-4. [PubMed: 31011209]
48. Verma R, Oania RS, Kolawa NJ, and Deshaies RJ (2013). Cdc48/p97 promotes degradation of aberrant nascent polypeptides bound to the ribosome. Elife 2, e00308. 10.7554/eLife.00308. [PubMed: 23358411]
49. Fedry J, Silva J, Vanevic M, Fronik S, Mechulam Y, Schmitt E, des Georges A, Faller WJ, and Forster F (2024). Visualization of translation reorganization upon persistent ribosome collision stress in mammalian cells. Mol Cell. 10.1016/j.molcel.2024.01.015.
50. Wu CC, Peterson A, Zinshteyn B, Regot S, and Green R (2020). Ribosome Collisions Trigger General Stress Responses to Regulate Cell Fate. Cell 182, 404–416 e414. 10.1016/j.cell.2020.06.006. [PubMed: 32610081]
51. Vind AC, Snieckute G, Blasius M, Tiedje C, Krogh N, Bekker-Jensen DB, Andersen KL, Nordgaard C, Tollenaere MAX, Lund AH, et al. (2020). ZAKalpha Recognizes Stalled Ribosomes through Partially Redundant Sensor Domains. Mol Cell 78, 700–713 e707. 10.1016/j.molcel.2020.03.021. [PubMed: 32289254]
52. Yan LL, and Zaher HS (2021). Ribosome quality control antagonizes the activation of the integrated stress response on colliding ribosomes. Mol Cell 81, 614–628 e614. 10.1016/j.molcel.2020.11.033. [PubMed: 33338396]
53. Ishimura R, Nagy G, Dotu I, Chuang JH, and Ackerman SL (2016). Activation of GCN2 kinase by ribosome stalling links translation elongation with translation initiation. Elife 5. 10.7554/eLife.14295.
54. Harding HP, Ordonez A, Allen F, Parts L, Inglis AJ, Williams RL, and Ron D (2019). The ribosomal P-stalk couples amino acid starvation to GCN2 activation in mammalian cells. Elife 8. 10.7554/eLife.50149.
55. Meydan S, and Guydosh NR (2020). Disome and Trisome Profiling Reveal Genome-wide Targets of Ribosome Quality Control. Mol Cell 79, 588–602 e586. 10.1016/j.molcel.2020.06.010. [PubMed: 32615089]
56. Hashem Y, and Frank J (2018). The Jigsaw Puzzle of mRNA Translation Initiation in Eukaryotes: A Decade of Structures Unraveling the Mechanics of the Process. Annu Rev Biophys 47, 125–151. 10.1146/annurev-biophys-070816-034034. [PubMed: 29494255]
57. Hinnebusch AG (2014). The scanning mechanism of eukaryotic translation initiation. Annu Rev Biochem 83, 779–812. 10.1146/annurev-biochem-060713-035802. [PubMed: 24499181]
58. Brito Querido J, Diaz-Lopez I, and Ramakrishnan V (2024). The molecular basis of translation initiation and its regulation in eukaryotes. Nat Rev Mol Cell Biol 25, 168–186. 10.1038/s41580-023-00624-9. [PubMed: 38052923]
59. Krzyzosiak A, Pitera AP, and Bertolotti A (2022). An Overview of Methods for Detecting eIF2alpha Phosphorylation and the Integrated Stress Response. Methods Mol Biol 2428, 3–18. 10.1007/978-1-0716-1975-9_1. [PubMed: 35171470]
60. Higgins R, Gendron JM, Rising L, Mak R, Webb K, Kaiser SE, Zuzow N, Riviere P, Yang B, Fenech E, et al. (2015). The Unfolded Protein Response Triggers Site-Specific Regulatory Ubiquitylation of 40S Ribosomal Proteins. Mol Cell 59, 35–49. 10.1016/j.molcel.2015.04.026. [PubMed: 26051182]
61. Garshott DM, An H, Sundaramoorthy E, Leonard M, Vicary A, Harper JW, and Bennett EJ (2021). iRQC, a surveillance pathway for 40S ribosomal quality control during mRNA translation initiation. Cell Rep 36, 109642. 10.1016/j.celrep.2021.109642. [PubMed: 34469731]

62. Garzia A, Meyer C, and Tuschl T (2021). The E3 ubiquitin ligase RNF10 modifies 40S ribosomal subunits of ribosomes compromised in translation. Cell Rep 36, 109468. 10.1016/j.celrep.2021.109468. [PubMed: 34348161]

63. Meyer C, Garzia A, Morozov P, Molina H, and Tuschl T (2020). The G3BP1-Family-USP10 Deubiquitinase Complex Rescues Ubiquitinated 40S Subunits of Ribosomes Stalled in Translation from Lysosomal Degradation. Mol Cell 77, 1193–1205 e1195. 10.1016/j.molcel.2019.12.024. [PubMed: 31981475]

64. Lopez AR, Jorgensen MH, Havelund JF, Arendrup FS, Kolapalli SP, Nielsen TM, Pais E, Beese CJ, Abdul-Al A, Vind AC, et al. (2023). Autophagy-mediated control of ribosome homeostasis in oncogene-induced senescence. Cell Rep 42, 113381. 10.1016/j.celrep.2023.113381. [PubMed: 37930887]

65. Parker MD, Collins JC, Korona B, Ghalei H, and Karbstein K (2019). A kinase-dependent checkpoint prevents escape of immature ribosomes into the translating pool. PLoS Biol 17, e3000329. 10.1371/journal.pbio.3000329. [PubMed: 31834877]

66. Huang H, Ghalei H, and Karbstein K (2020). Quality control of 40S ribosome head assembly ensures scanning competence. J Cell Biol 219. 10.1083/jcb.202004161.

67. Bussiere C, Hashem Y, Arora S, Frank J, and Johnson AW (2012). Integrity of the P-site is probed during maturation of the 60S ribosomal subunit. J Cell Biol 197, 747–759. 10.1083/jcb.201112131. [PubMed: 22689654]

68. Lebaron S, Schneider C, van Nues RW, Swiatkowska A, Walsh D, Bottcher B, Granneman S, Watkins NJ, and Tollervey D (2012). Proofreading of pre-40S ribosome maturation by a translation initiation factor and 60S subunits. Nat Struct Mol Biol 19, 744–753. 10.1038/nsmb.2308. [PubMed: 22751017]

69. Ferreira-Cerca S, Kiburu I, Thomson E, LaRonde N, and Hurt E (2014). Dominant Rio1 kinase/ATPase catalytic mutant induces trapping of late pre-40S biogenesis factors in 80S-like ribosomes. Nucleic Acids Res 42, 8635–8647. 10.1093/nar/gku542. [PubMed: 24948609]

70. Belhabich-Baumas K, Joret C, Jady BE, Plisson-Chastang C, Shayan R, Klopp C, Henras AK, Henry Y, and Mougin A (2017). The Rio1p ATPase hinders premature entry into translation of late pre-40S pre-ribosomal particles. Nucleic Acids Res 45, 10824–10836. 10.1093/nar/gkx734. [PubMed: 28977579]

71. Plassart L, Shayan R, Montellese C, Rinaldi D, Larburu N, Pichereaux C, Froment C, Lebaron S, O'Donohue MF, Kutay U, et al. (2021). The final step of 40S ribosomal subunit maturation is controlled by a dual key lock. Elife 10. 10.7554/eLife.61254.

72. Powers T, and Noller HF (1990). Dominant lethal mutations in a conserved loop in 16S rRNA. Proc Natl Acad Sci U S A 87, 1042–1046. 10.1073/pnas.87.3.1042. [PubMed: 2405392]

73. Thompson J, Kim DF, O'Connor M, Lieberman KR, Bayfield MA, Gregory ST, Green R, Noller HF, and Dahlberg AE (2001). Analysis of mutations at residues A2451 and G2447 of 23S rRNA in the peptidyltransferase active site of the 50S ribosomal subunit. Proc Natl Acad Sci U S A 98, 9002–9007. 10.1073/pnas.151257098. [PubMed: 11470897]

74. Paier A, Leppik M, Soosaar A, Tenson T, and Maiväli Ü (2015). The effects of disruptions in ribosomal active sites and in intersubunit contacts on ribosomal degradation in Escherichia coli. Sci Rep 5, 7712. 10.1038/srep07712. [PubMed: 25578614]

75. LaRiviere FJ, Cole SE, Ferullo DJ, and Moore MJ (2006). A late-acting quality control process for mature eukaryotic rRNAs. Mol Cell 24, 619–626. 10.1016/j.molcel.2006.10.008. [PubMed: 17188037]

76. Cole SE, LaRiviere FJ, Merrikh CN, and Moore MJ (2009). A convergence of rRNA and mRNA quality control pathways revealed by mechanistic analysis of nonfunctional rRNA decay. Mol Cell 34, 440–450. 10.1016/j.molcel.2009.04.017. [PubMed: 19481524]

77. Sugiyama T, Li S, Kato M, Ikeuchi K, Ichimura A, Matsuo Y, and Inada T (2019). Sequential Ubiquitination of Ribosomal Protein uS3 Triggers the Degradation of Non-functional 18S rRNA. Cell Rep 26, 3400–3415.e3407. 10.1016/j.celrep.2019.02.067. [PubMed: 30893611]

78. Li S, Ikeuchi K, Kato M, Buschauer R, Sugiyama T, Adachi S, Kusano H, Natsume T, Berninghausen O, Matsuo Y, et al. (2022). Sensing of individual stalled 80S ribosomes by Fap1

for nonfunctional rRNA turnover. Mol Cell 82, 3424–3437.e3428. 10.1016/j.molcel.2022.08.018. [PubMed: 36113412]

79. Bottorff TA, Park H, Geballe AP, and Subramaniam AR (2022). Translational buffering by ribosome stalling in upstream open reading frames. PLoS Genet 18, e1010460. 10.1371/journal.pgen.1010460. [PubMed: 36315596]
80. Oltion K, Carelli JD, Yang T, See SK, Wang HY, Kampmann M, and Taunton J (2023). An E3 ligase network engages GCN1 to promote the degradation of translation factors on stalled ribosomes. Cell 186, 346–362 e317. 10.1016/j.cell.2022.12.025. [PubMed: 36638793]
81. Pochopien AA, Beckert B, Kasvandik S, Berninghausen O, Beckmann R, Tenson T, and Wilson DN (2021). Structure of Gcn1 bound to stalled and colliding 80S ribosomes. Proc Natl Acad Sci U S A 118. 10.1073/pnas.2022756118.
82. Sharma J, Du M, Wong E, Mutyam V, Li Y, Chen J, Wangen J, Thrasher K, Fu L, Peng N, et al. (2021). A small molecule that induces translational readthrough of CFTR nonsense mutations by eRF1 depletion. Nat Commun 12, 4358. 10.1038/s41467-021-24575-x. [PubMed: 34272367]
83. Gurzeler LA, Link M, Ibig Y, Schmidt I, Galuba O, Schoenbett J, Gasser-Didierlaurant C, Parker CN, Mao X, Bitsch F, et al. (2023). Drug-induced eRF1 degradation promotes readthrough and reveals a new branch of ribosome quality control. Cell Rep 42, 113056. 10.1016/j.celrep.2023.113056. [PubMed: 37651229]
84. Coelho JPL, Yip MCJ, Oltion K, Taunton J, and Shao S (2024). The eRF1 degrader SRI-41315 acts as a molecular glue at the ribosomal decoding center. Nat Chem Biol. 10.1038/s41589-023-01521-0.
85. Zhao S, Cordes J, Caban KM, Gotz MJ, Mackens-Kiani T, Veltri AJ, Sinha NK, Weickert P, Kaya S, Hewitt G, et al. (2023). RNF14-dependent atypical ubiquitylation promotes translation-coupled resolution of RNA-protein crosslinks. Mol Cell 83, 4290–4303 e4299. 10.1016/j.molcel.2023.10.012. [PubMed: 37951216]
86. Suryo Rahmanto A, Blum CJ, Scalera C, Heidelberger JB, Mesitov M, Horn-Ghetko D, Graf JF, Mikicic I, Hobrecht R, Orekhova A, et al. (2023). K6-linked ubiquitylation marks formaldehyde-induced RNA-protein crosslinks for resolution. Mol Cell 83, 4272–4289 e4210. 10.1016/j.molcel.2023.10.011. [PubMed: 37951215]
87. Wu Q, and Bazzini AA (2023). Translation and mRNA Stability Control. Annu Rev Biochem 92, 227–245. 10.1146/annurev-biochem-052621-091808. [PubMed: 37001134]
88. Wu Q, Medina SG, Kushawah G, DeVore ML, Castellano LA, Hand JM, Wright M, and Bazzini AA (2019). Translation affects mRNA stability in a codon-dependent manner in human cells. Elife 8. 10.7554/eLife.45396.
89. Narula A, Ellis J, Taliaferro JM, and Rissland OS (2019). Coding regions affect mRNA stability in human cells. RNA 25, 1751–1764. 10.1261/rna.073239.119. [PubMed: 31527111]
90. Harigaya Y, and Parker R (2016). Analysis of the association between codon optimality and mRNA stability in Schizosaccharomyces pombe. BMC Genomics 17, 895. 10.1186/s12864-016-3237-6. [PubMed: 27825301]
91. Bae H, and Coller J (2022). Codon optimality-mediated mRNA degradation: Linking translational elongation to mRNA stability. Mol Cell 82, 1467–1476. 10.1016/j.molcel.2022.03.032. [PubMed: 35452615]
92. Panasenko OO, and Collart MA (2012). Presence of Not5 and ubiquitinated Rps7A in polysome fractions depends upon the Not4 E3 ligase. Mol Microbiol 83, 640–653. 10.1111/j.1365-2958.2011.07957.x. [PubMed: 22243599]
93. Allen GE, Panasenko OO, Villanyi Z, Zagatti M, Weiss B, Pagliazzo L, Huch S, Polte C, Zahoran S, Hughes CS, et al. (2021). Not4 and Not5 modulate translation elongation by Rps7A ubiquitination, Rli1 moonlighting, and condensates that exclude eIF5A. Cell Rep 36, 109633. 10.1016/j.celrep.2021.109633. [PubMed: 34469733]
94. Matsuki Y, Matsuo Y, Nakano Y, Iwasaki S, Yoko H, Udagawa T, Li S, Saeki Y, Yoshihisa T, Tanaka K, et al. (2020). Ribosomal protein S7 ubiquitination during ER stress in yeast is associated with selective mRNA translation and stress outcome. Sci Rep 10, 19669. 10.1038/s41598-020-76239-3. [PubMed: 33184379]

95. Buschauer R, Matsuo Y, Sugiyama T, Chen YH, Alhusaini N, Sweet T, Ikeuchi K, Cheng J, Matsuki Y, Nobuta R, et al. (2020). The Ccr4-Not complex monitors the translating ribosome for codon optimality. Science 368. 10.1126/science.aay6912.

96. Absmeier E, Chandrasekaran V, O'Reilly FJ, Stowell JAW, Rappsilber J, and Passmore LA (2023). Specific recognition and ubiquitination of translating ribosomes by mammalian CCR4-NOT. Nat Struct Mol Biol 30, 1314–1322. 10.1038/s41594-023-01075-8. [PubMed: 37653243]

97. Brito Querido J, Sokabe M, Kraatz S, Gordiyenko Y, Skehel JM, Fraser CS, and Ramakrishnan V (2020). Structure of a human 48S translational initiation complex. Science 369, 1220–1227. 10.1126/science.aba4904. [PubMed: 32883864]

98. Mishima Y, Han P, Ishibashi K, Kimura S, and Iwasaki S (2022). Ribosome slowdown triggers codon-mediated mRNA decay independently of ribosome quality control. EMBO J 41, e109256. 10.15252/embj.2021109256. [PubMed: 35040509]

99. Ugajin N, Imami K, Takada H, Ishihama Y, Chiba S, and Mishima Y (2023). Znf598-mediated Rps10/eS10 ubiquitination contributes to the ribosome ubiquitination dynamics during zebrafish development. RNA 29, 1910–1927. 10.1261/rna.079633.123. [PubMed: 37751929]

100. Geng J, Li S, Li Y, Wu Z, Bhurtel S, Rimal S, Khan D, Ohja R, Brandman O, and Lu B (2024). Stalled translation by mitochondrial stress upregulates a CNOT4-ZNF598 ribosomal quality control pathway important for tissue homeostasis. Nat Commun 15, 1637. 10.1038/s41467-024-45525-3. [PubMed: 38388640]

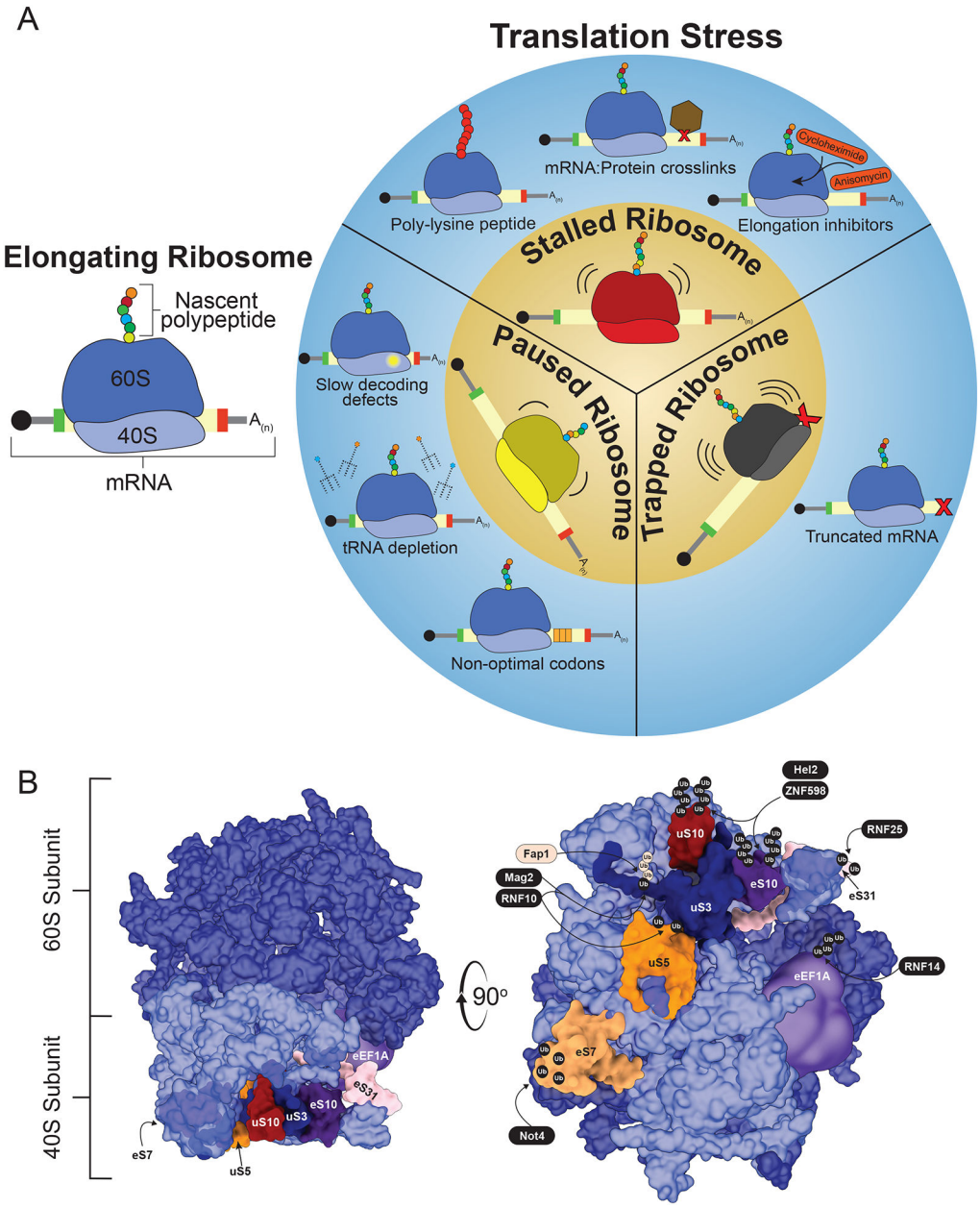

Figure 1 –.

Ribosome classification and ubiquitylation events.

A) (Left) Simplified schematic of a typical elongating ribosome on an example mRNA. mRNA color scheme: black – 5' cap, grey – UTRs, green – start codon, beige – coding region, red – stop codon. (Right) Overview of possible barriers to ribosome progression (inner circle) with common examples of stresses that may result in each barrier (outer circle).

B) Structural overview of the ribosome with highlighted individual ribosomal proteins known to be ubiquitylated. Modified from PDB:8G6J. Side view (left) and bottom (40S) view (right). Bottom view documents RQC pathway ubiquitin ligases and indicates their target sites.

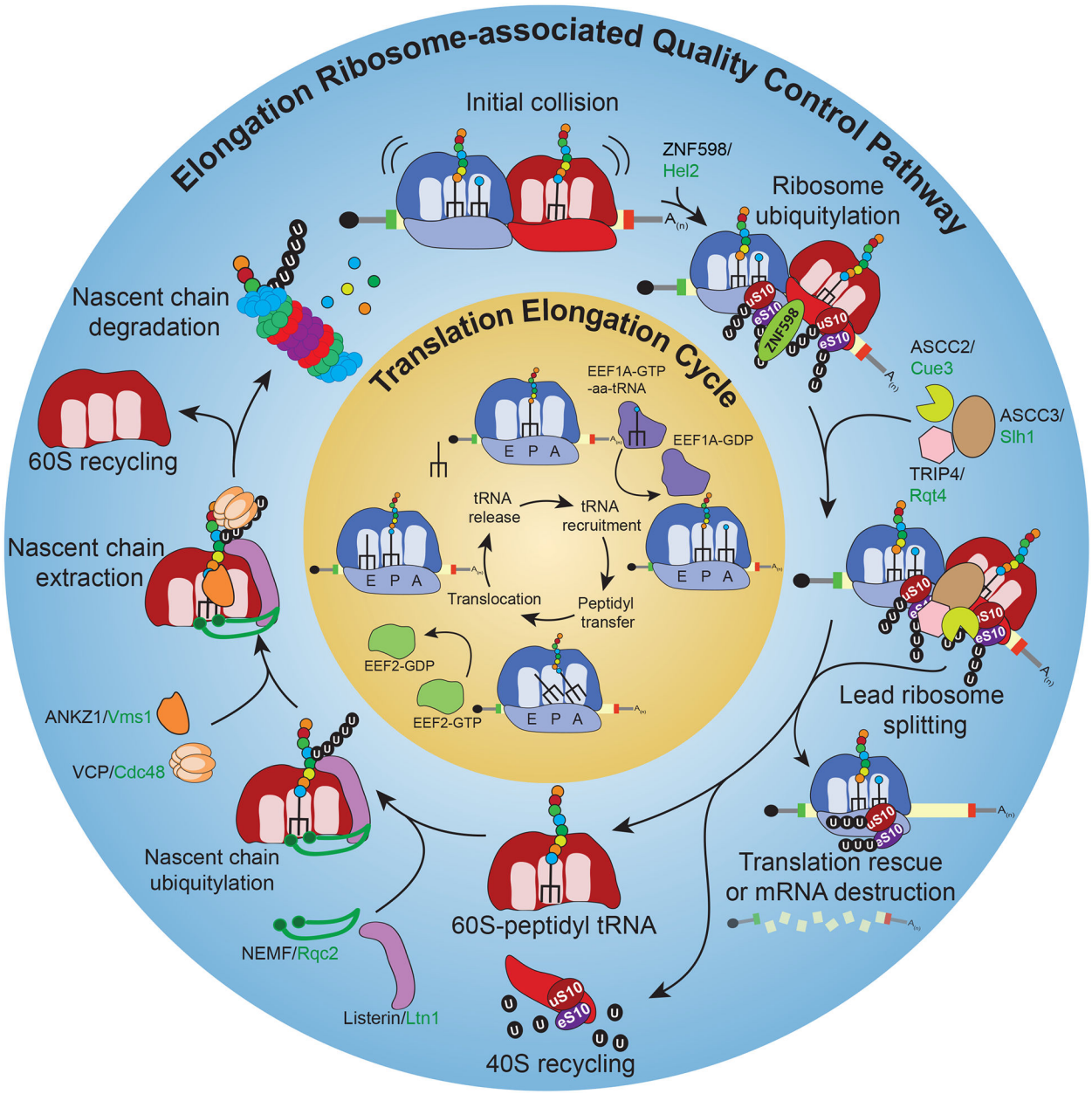

Figure 2 –.

Overview of canonical ribosome-associated quality control pathway.

Disruptions to the normal translation elongation cycle (center) that result in ribosome collisions trigger the canonical elongation RQC pathway (outer circle). The individual steps of this pathway and its component factors are shown clockwise proceeding from the initial collision (top center). Mammalian factors listed in black, yeast orthologs in green.

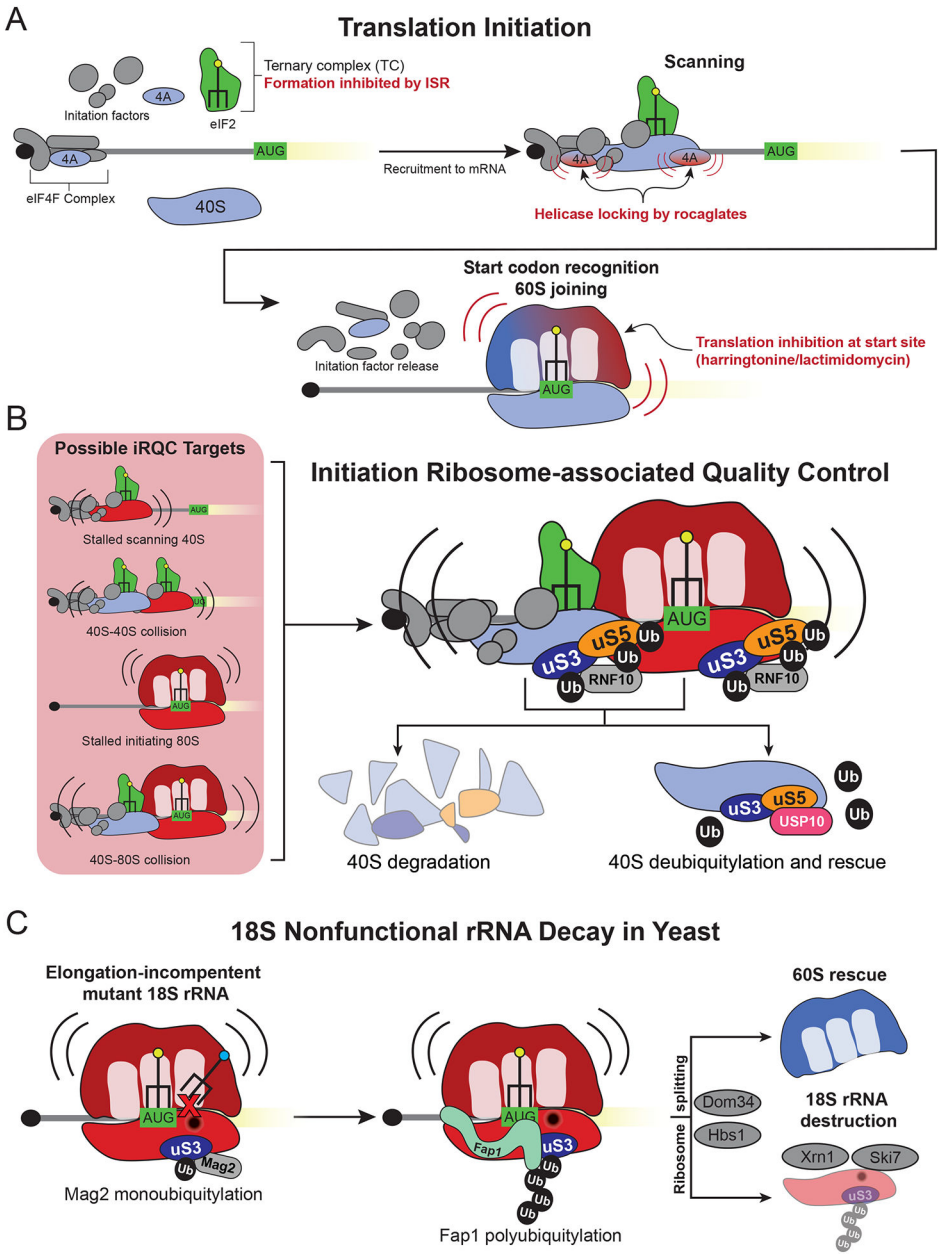

Figure 3 –.

Overview of initiation ribosome-associated quality control (iRQC).

A) Simplified outline of the steps involved in translation initiation. Agents that inhibit each step and lead to iRQC activation are shown in red.

B) Schematic of the mammalian iRQC pathway. Possible ribosomal species that would result in iRQC activation are outlined on the left. RNF10-mediated ubiquitylation and pathway outcomes are shown on the right.

C) Overview of the 18S nonfunctional rRNA decay pathway (NRD) in yeast.

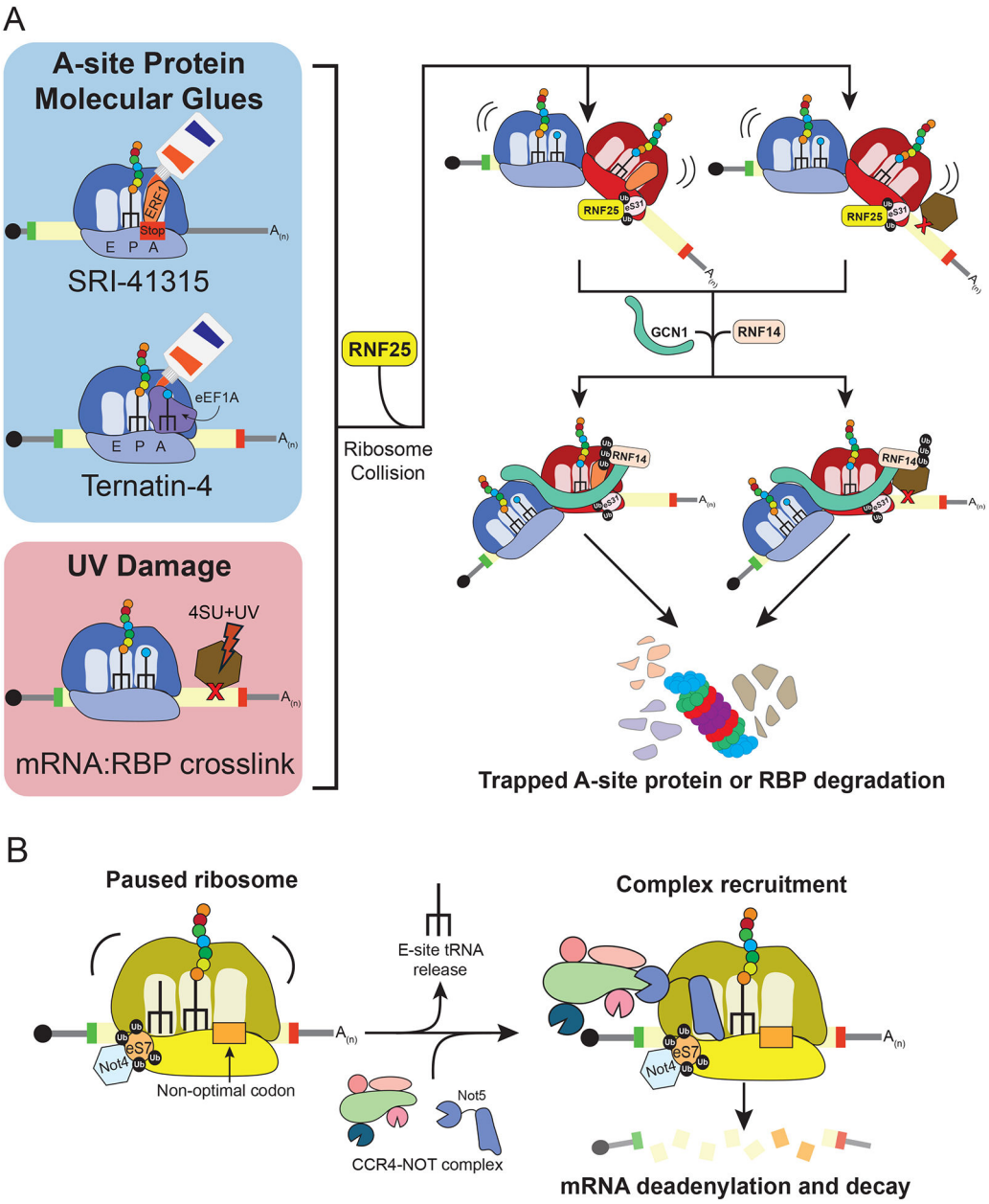

Figure 4 –.

Overview of RQC-dependent resolution of trapped or paused ribosomal complexes.

A) Molecular glues that trap proteins in the A-site (left, top) or crosslinking of RNA-binding proteins to mRNA (left, bottom) block ribosome progression. Upon ribosome collision and ubiquitylation of eS31 by RNF25, GCN1 and RNF14 are recruited, leading to the polyubiquitylation and degradation of the protein preventing ribosome progression (right).

B) Not4-dependent eS7 ubiquitylation occurs on ribosomes paused while decoding rare codons. This ubiquitylation event recruits the CCR4-NOT complex and results in mRNA decay.