Title

A ubiquitin language communicates ribosomal distress.

Journal

Seminars in Cell and Developmental Biology, 154(Pt B)

Authors

Monem, Parissa

Arribere, Joshua

Publication Date

2024-02-15

DOI

10.1016/j.semcdb.2023.03.009

Peer reviewed

HHS Public Access

Author manuscript

Semin Cell Dev Biol. Author manuscript; available in PMC 2024 February 20.

Published in final edited form as:

Semin Cell Dev Biol. 2024 February 15; 154(Pt B): 131–137. doi:10.1016/j.semcdb.2023.03.009.

A ubiquitin language communicates ribosomal distress

Parissa C. Monem,

Joshua A. Arribere*

Department of Molecular, Cell, and Developmental Biology, University of California at Santa Cruz, Santa Cruz, CA, USA

Abstract

Cells entrust ribosomes with the critical task of identifying problematic mRNAs and facilitating their degradation. Ribosomes must communicate when they encounter and stall on an aberrant mRNA, lest they expose the cell to toxic and disease-causing proteins, or they jeopardize ribosome homeostasis and cellular translation. In recent years, ribosomal ubiquitination has emerged as a central signaling step in this process, and proteomic studies across labs and experimental systems show a myriad of ubiquitination sites throughout the ribosome. Work from many labs zeroed in on ubiquitination in one region of the small ribosomal subunit as being functionally significant, with the balance and exact ubiquitination sites determined by stall type, E3 ubiquitin ligases, and deubiquitinases. This review discusses the current literature surrounding ribosomal ubiquitination during translational stress and considers its role in committing translational complexes to decay.

Keywords

Translation; Ribosome; Ubiquitin; No-Go mRNA Decay (NGD); Nonstop mRNA Decay (NSD)

1. Introduction

Translation is an essential and tightly regulated process, with much of its regulation necessarily hinging on the ribosome. Considering that translation gives rise to all proteins in the cell, it is no surprise that faulty translation contributes to the aggregation of toxic proteins and can elicit disease phenotypes such as neurodegeneration [4,33]. To mitigate this, eukaryotes have evolved translational surveillance mechanisms that ensure ribosomes accurately synthesize proteins from full-length mRNAs in a timely fashion. A key functional intermediate that communicates translation status is a ubiquitinated ribosome, and the precise sites of ubiquitination vary depending on what the ribosome has to "say" about its translation experience.

Ubiquitination is typically considered a hallmark of proteasomal degradation, but ubiquitination also plays a part in non-degradative signaling responses [12,43]. This difference in protein fate lies in the number of ubiquitins and their linkages, the pattern

*Corresponding author. jarriber@ucsc.edu (J.A. Arribere).

of which creates molecular interfaces that are recognized by effectors [30,61]. In its non-degradative capacity, ubiquitin functions as a signaling molecule in much the same way that phosphorylation is used in protein signaling cascades throughout biology. Covalent attachment of ubiquitin to substrates is the culmination of a ubiquitin cascade involving ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-ligating (E3) enzymes [15,59]. While E1 and E2 enzymes interact with each other and ubiquitin, E3 ubiquitin ligases interact with ubiquitination targets, imparting substrate specificity. To enable this vital precision, organisms encode hundreds of unique E3 ligases [7,79]. Among the pool of proteins targeted by E3 ligases are ribosomal proteins [44,46,50,65,70].

In recent years, there has been a particular focus on cotranslational ribosomal ubiquitination as a mechanism to rapidly alter the outcome of translation. Much of the literature on ribosomal ubiquitination comes from the study of two translational surveillance pathways: No-Go mRNA Decay (NGD) and Nonstop mRNA Decay (NSD) [23,37,72]. NGD occurs on mRNAs bearing elongation-inhibiting features, such as secondary structures, rare codons, polybasic amino acid-encoding sequences, or damaged nucleotides [17]. NSD is triggered by mRNAs lacking stop codons, generated by premature polyadenylation or mRNA cleavage [20]. While prompted by different mRNA species, NGD and NSD are defined by the recruitment of several of the same effectors, ultimately leading to mRNA decay, ribosome rescue, and nascent peptide degradation. A molecular understanding of the biochemical steps of mRNA repression and ribosome rescue have been reviewed elsewhere and will not be covered here [19,32,36,66].

Here, we review the current knowledge of ribosomal ubiquitination during translation and highlight future directions for its study. First, we collect known ribosomal protein sites subject to ubiquitination in the context of surveillance and translational distress. We then discuss structural, genetic, and biochemical data suggesting target specificity mechanisms used by distinct E3 ubiquitin ligases and deubiquitinases. Lastly, we consider downstream consequences of ribosomal ubiquitination, and suggest models in which effectors recognize ubiquitinated aberrant translational complexes and commit such complexes to decay.

2. An array of ubiquitination targets on the ribosome

2.1. Similar and distinct ubiquitination sites across organisms

Several studies have exploited mass spectrometry to characterize the ubiquitinated proteome in a process known as ubiquitin remnant profiling [9,42,44,58,77]. The selective profiling of ubiquitin-modified peptides is made possible due to a unique di-glycine tag left on ubiquitin conjugates following trypsin digestion. This di-glycine tag is the remnant of ubiquitin on its covalently-bound substrate and thus allows for the precise identification of the substrate's participating lysine residue.

Initial studies from several groups suggested that site-specific ubiquitination of ribosomal proteins could influence or be influenced by translation. Yeast RPS7A/eS7A was identified as a target of ribosomal ubiquitination in polysomes [56]. The Bennet lab identified 40S small ribosomal subunit proteins (RPS2/uS5, RPS3/uS3, and RPS20/uS10) which were ubiquitinated upon treatment with translation inhibitors such as cycloheximide, anisomycin,

and harringtonine [27]. Further in support of a link to active translation, later studies revealed a requirement for RPS10/eS10 and RPS20/uS10 ubiquitination in the resolution of ribosomal stalling in human cells [37,72] and *C. elegans* [54], and work in yeast demonstrated a requirement for ubiquitination of RPS20/uS10 [48] and RPS3/uS3 [67].

Work across many groups over the last several years uncovered ubiquitinated 40S proteins and their targeted lysine residue(s) in different organisms (Table 1). Notably, some ubiquitination targets are unique to metazoans or yeast, indicating divergence in ubiquitination targeting mechanisms or ribosomal protein accessibility. Additionally, while most of the literature has focused on monoubiquitination events, work in yeast has demonstrated a requirement for polyubiquitination in translational surveillance [62], providing support for K63-linked chains, namely on the yeast-specific RPS7/eS7 as well as RPS3/uS3 [31]. Whether polyubiquitination occurs *in vivo* in metazoans is currently unclear.

2.2. Ubiquitination sites reside between collided ribosomes

An important outstanding question in translational surveillance was how cells distinguish problematic stalls from transient pauses in translation. A model emerged to solve this problem: ribosome collisions. Work from several groups supports an important role for ribosome collisions in both ubiquitination and surveillance [31,38,49,67]. As we describe below, ubiquitination sites coincide with contact sites between collided ribosomes, suggesting a direct relationship between collisions and ubiquitination.

To study discretely stalled ribosomes, the Hegde lab utilized a rabbit reticulocyte lysate translation system supplemented with a mutant release factor, stalling ribosomes at stop codons [38,64]. Poly-ribosomes were analyzed by cryo-EM, and the resultant structure revealed the stalled/leading and collided/trailing ribosomes in distinct orientations, producing two 40S inter-ribosomal interfaces (Fig. 1A). Interface 1 consists of the mRNA exit channel of the stalled ribosome and the mRNA entry channel of the collided ribosome. Notably, this interface lacks ubiquitination sites. Interestingly, interface 2 involves all identified ubiquitination sites on the collided ribosome and RACK1, a ribosomal protein required for stall detection (Fig. 1B) [72].

A trio of structural studies in yeast visualized structures of disomes stalled on an arginine reporter mRNA [31], trisomes stalled on an endogenous stalling mRNA (*SDD1*) [49], and disomes stalled on endogenous mRNAs [78]. While these structures largely agree with one another, there are some key differences (*e.g.*, the relative positioning of RACK1 in [78] compared to [31,49]). It is unclear whether such differences represent distinct ribosome collision types, steps along a ribosome collision pathway, and/or distinct sample preparations.

In addition to revealing the orientations of stalled ribosomes, these structural studies provided information regarding the locations of lysine residues targeted for ubiquitination. These lysines are found on the flexible tails of their respective proteins on the solvent face of the 40S subunit (Fig. 1 A, Fig. 1B). Due to the flexible nature of these tails, they are often missing from structures, thus preventing visualization of conjugated ubiquitin (if present). Future work optimizing the capture of ubiquitinated ribosomes and visualization

of ubiquitin itself on ribosomes will provide important insights into ubiquitination states in different mutant backgrounds. The potential ubiquitination sites of the trailing/collided ribosomes are much closer to the interface than the same ubiquitination sites on the stalled/leading ribosome. Whether this asymmetry yields a differential outcome with respect to ubiquitination of each ribosome and downstream effects remains unexplored. For more on the emerging understanding of collisions and their effects in the cell, see a recent review [52].

3. E3 ubiquitin ligases involved in ribosomal ubiquitination

Several E3 ubiquitin ligases respond to aberrant translation. While information exists suggesting their ribosomal protein targets (Table 2), the specificity of these ligases for their targets is poorly understood. As every ribosome would be expected to have each of these ribosomal proteins, how each E3 ligase differentiates between ribosomes and sites remains an open question.

One E3 ligase (ZNF598) specifically engages ribosomes upon collision, suggesting that ZNF598 recognizes the collision interface [38]. It remains unclear whether this is true of the other E3 ligases; it is possible that different stalls generate different collisions, each with its own distinct interface (Fig. 2). This model is supported by work suggesting unique E3 ligase dependencies in distinct pathways [1,31,54,62]. Additionally, evidence exists supporting a combinatorial and hierarchical ubiquitination system [21,24,31]. Furthermore, there are differences between some disome structures at the ubiquitination sites in the 40S-40S interface [31,49,78]. It is possible that ribosomes stalled in different conformations display targets for an E3 ligase specialized for that collision type. Future work will hopefully tease apart the specificity and ordering of each ribosomal ubiquitination event.

Below, we discuss the current understanding of each E3 ligase that acts on stalled ribosomes.

3.1. ZNF598/Hel2

In yeast, Hel2 was first identified as a RING-domain-containing E3 ubiquitin ligase required for histone regulation [68]. It is unclear whether this histone regulatory function for Hel2 is connected to translational control. Hel2 was later found to repress the full-length product of a ribosomal stalling reporter with an internal polybasic stretch [5,45]. Knockdown or deletion of ZNF598, the metazoan homolog of Hel2, in humans, worms, and zebrafish generally agrees with the stalling reporter stabilization phenotypes seen in yeast, providing evidence for ZNF598 function during ribosomal stalling in higher organisms [23,37,54,55,72].

Multiple groups identified ubiquitination targets of ZNF598 via ubiquitin remnant profiling (see description in 2.1), uncovering ZNF598-dependent ubiquitination of RPS10/eS10 and RPS20/uS10, and to a lesser extent RPS3/uS3 [23,37,72]. Genetic and biochemical studies verified the functional importance of ZNF598-dependent modification of RPS10/eS10, RPS20/uS10, RPS3/uS3, and RPS2/uS5 in metazoans [21-24,37,54,72]. Work in yeast revealed Hel2-dependent modification of RPS20/uS10, RPS7A/eS7A, and RPS3/uS3 during ribosome stalling [31,48,67] (see Table 2 for proteins and Table 1 for targeted residues).

Given that ZNF598/Hel2 preferentially ubiquitinated ribosomes upon multi-ribosome collisions [38] and that multiple ribosomes were required for NGD [67], an interest in "disomes" was born. Many studies emerged utilizing ribosome footprint profiling, or Ribo-seq, to map the positions of translating disomes genome-wide [2,26,51,78]. One such study performed Ribo-seq on yeast disomes in a Hel2 mutant and observed differences in the distributions of disomes genome-wide, thus implicating Hel2 in functioning on the disome unit [51]. Similar disome datasets have yet to be generated in mammalian ZNF598 mutants, nor has robust NGD stalling been observed in metazoan Ribo-seq [2,26]. Future work will help understand the intricacies of disome Ribo-seq outside of yeast, and will inform endogenous targets of ZNF598.

While ZNF598/Hel2 is widely regarded as a major regulator of stalled ribosomes, evidence exists challenging its prominence in various stall situations, such as NSD [31,54,62]. Much of the field's understanding of ribosomal ubiquitination by ZNF598/Hel2 relies on internally stalling NGD reporters exclusively [23,37,72]. Given that NSD and NGD arise on distinct mRNA substrates, a more complete illustration of ribosomal ubiquitination will require a consideration of a variety of stalling substrates. Additionally, work in yeast saw a requirement for Hel2-mediated ubiquitination of RPS3/uS3 at K212 in nonfunctional rRNA decay (NRD) [71], but found it dispensable in NGD and ribosome quality control (RQC) of nascent peptides [31;48]. Taken together, these works highlight the distinct functions of ZNF598/Hel2 during a variety of translational stresses.

3.2. RNF10/Mag2

Prior work in human cells found ZNF598-enhanced ubiquitination of RPS2/uS5 and RPS3/uS3; however, much of these ubiquitinated species persisted in ZNF598 knockouts [38,53]. This finding prompted a consideration of different E3 ubiquitin ligases acting during NGD, leading to work focused on RNF10/Mag2 [22,24].

Known as RNF10 in humans and Mag2 in yeast, RNF10/Mag2 is a RING-domain-containing E3 ubiquitin ligase initially linked to translational surveillance during 18S NRD in yeast [71]. In this work from the Inada lab, Mag2 was found to be required for ubiquitination of RPS3/uS3 on non-functional 80S ribosomes to enable their dissociation and to facilitate 18S rRNA degradation.

Additional groups observed a requirement for human RNF10 to ubiquitinate RPS2/uS5 and RPS3/uS3 in the context of treatment with elongation and initiation inhibitors [22,24] (see Table 2 for proteins and Table 1 for targeted residues). These results support a function for RNF10 in ubiquitinating RPS2/uS5 and RPS3/uS3 [24]. It is also possible that E3 ligases collaborate to bring about repression, as is supported by data showing ZNF598 affecting ubiquitination of RPS2/uS5 and RPS3/uS3 during elongation stalls [24,38,53]. It thus remains a possibility that ZNF598 and RNF10 work together to ubiquitinate ribosomes at RPS2/uS5 and RPS3/uS3. NRD data in yeast are consistent with an E3-collaboration model, suggesting that Mag2 initially monoubiquitinates RPS3/uS3, followed by K63-polyubiquitination by Hel2 and an additional E3 ligase Rsp5 [71].

3.3. Not4

The conserved Ccr4-Not complex is critical for RNA metabolism, regulating various steps from transcription to decay [14,57]. Ccr4-Not is key to initiating mRNA decay via deadenylation and promotion of decapping [63,75]. One component of the Ccr4-Not complex is the RING finger E3 ubiquitin ligase Not4. While Not4 is conserved in humans as CNOT4, it is only a stable subunit of the Ccr4-Not complex in yeast [14] and has only been characterized in translational surveillance in yeast and flies [76].

Multiple groups have found Not4 to be required for a variety of translational roles in yeast, including the polysome association of Not4 during stalling with cycloheximide [56,60], the degradation of NGD stalling peptides [1,16,47], and cotranslational decay of stalling reporter mRNAs and mRNAs with suboptimal codons [1,8] Notably, Not4 is also required for the ubiquitination of RPS7/eS7, a yeast-specific ribosomal ubiquitination target [1,31,56] (see Table 2 for proteins and Table 1 for targeted residues).

Recent works point toward potential downstream consequences of Not4-mediated ribosomal ubiquitination in yeast. One study found that Not4 is required for mRNA cleavages upstream of a stall site, possibly acting through RPS7/eS7 monoubiquitination at K84 [31]. While this model illustrates a downstream function for Not4, effectors which directly recognize Not4-mediated ubiquitination remain poorly understood. Another recent study teased apart the functions of Otu2 and Ubp3 as two deubiquitinases (DUBs) potentially working with Not4 to modulate ribosomal ubiquitination events on RPS7/eS7 at K83 [73]. Specifically, knockout of Otu2 or Ubp3 resulted in increased RPS7/eS7 ubiquitination on 40S subunits or 80S ribosomes and polysomes, respectively. This study saw reduced protein synthesis as a consequence of perturbing the deubiquitination cycle of RPS7/eS7, consistent with prior work showing decreased polysomes in a Not4 knockout [56]. Together these works link Not4 with a pair of DUBs controlling translation efficiency. Further work is needed to understand the biological contexts in which ribosomes are affected by RPS7/eS7 ubiquitination, as well as the direct function of these ubiquitin marks.

3.4. MKRN1/2

MKRN1 and MKRN2 are two paralogs of a conserved RNA-binding RING finger E3 ubiquitin ligase. Previously implicated in a variety of roles such as regulating telomere length and interacting with poly(A)-binding protein (PABPC1) [10,41], MKRN1/2 was more recently linked to translational surveillance.

Given its history with PABPC1, a recent study validated the interaction between MKRN1/2 and PABPC1, and proposed a model for MKRN1/2 mRNA binding and function during translation of poly(A) sequences [29]. Upon MKRN1/2 knockdown, this work observed increased full-length translation of an internal poly(A)-stalling reporter. Notably, ubiquitin remnant profiling (see description in 2.1) of untreated cells uncovered MKRN1/2-dependent ubiquitination of RPS10/eS10 at K107 (Table 2), which is a distinct site from the commonly-studied ZNF598-dependent RPS10/eS10 ubiquitination at K138 and K139 [29] (Table 1). The function of ubiquitinating RPS10/eS10 at these various sites remains a question to be addressed in future studies.

4. Deubiquitinases functioning during translational stress

Deubiquitinases (DUBs) are enzymes responsible for removing ubiquitin peptides from substrates [13]. Here, we discuss DUBs that function alongside E3 ubiquitin ligases as an additional avenue to affect translation (Fig. 3).

4.1. 40S subunit homeostasis: OTUD3, USP21, and USP10

The DUBs OTUD3 and USP21 were recently linked to translational surveillance via an overexpression screen selecting for readthrough of NGD poly(A) stalls [21]. This work uncovered roles for OTUD3 and USP21 in deubiquitinating 40S proteins (Fig. 3 A) targeted by ZNF598 (namely RPS20/uS10 and RPS10/eS10), as well as a role for USP21 in deubiquitinating RPS3/uS3 and RPS2/uS5. Both of these functions were enhanced upon UV-induced RQC, suggesting a role for DUBs in maintaining the 40S pool during cellular stresses.

Interestingly, ZNF598 protein was previously found to be in vast excess compared to OTUD3 and USP21 in human cells [34], suggesting a model in which cells entrust ZNF598 with the power to ubiquitinate 40S proteins which are slowly deubiquitinated. Subsequent work supports this model, revealing that 40–100-fold overexpression of OTUD3 and USP2 is required to suppress ZNF598 function [21]. Taken together, these works point toward a necessity for ZNF598 (and other E3 ligases) to accurately target aberrant ribosomes to maintain 40S subunit levels.

Additionally, the DUB USP10 was also identified in the same overexpression screen selecting for readthrough of NGD poly(A) stalls [21]. Further work observed increased RPS3/uS3 and RPS2/uS5 ubiquitination in USP10 knockout cells, and ultimately 40S subunit degradation upon constitutive, drug-induced ubiquitination of these sites by the E3 ligase RNF10 [22,24,53].

4.2. E3 ubiquitin ligase abundance: USP9X

A recent proteomic study linked the DUB USP9X and translational surveillance, identifying USP9X as a ZNF598-interacting protein [23]. USP9X has since been shown to regulate protein abundance of both MKRN1/2 and ZNF598 (Fig. 3B), with work observing lower levels of these E3 ligases and reduced NGD function upon USP9X knockdown [11]. These data support a model where MKRN1/2 and ZNF598 auto-ubiquitinate, as is common for RING E3 ligases, and thus require USP9X for stability. Further work focused on E3 ligase regulation, at the DUB level and beyond, will prove useful in understanding how cells keep ubiquitination of ribosomes under tight control.

5. Ubiquitin as a signal for downstream repression

While ubiquitination is required for NGD and NSD, it remains unclear how ubiquitin commits translational complexes to decay and recycling. That is, ubiquitin in and of itself does not elicit mRNA repression nor ribosome rescue. A hint comes from the observation that key repressive effectors required for NGD and NSD contain ubiquitin-binding domains (Table 3). It thus is plausible that ubiquitin serves to localize effectors to problematic

mRNAs, which use their effector domain(s) to alter translational complexes. Distinct ubiquitination sites, in combination with nearby ribosomal proteins and rRNA, could feasibly provide unique contact sites, allowing for accurate differentiation between aberrant stalls and innocuous pauses.

For example, recruitment of CUE2/NONU-1 [18,25] via CUE domains was recently discovered to link ribosomal ubiquitination with mRNA decay [54,74]. Additionally, recruitment of HBS-1 and CUE3/ASCC could link ribosomal ubiquitination with ribosome rescue, therefore allowing for subunit recycling and downstream nascent chain degradation [39,49,54].

A major challenge is the identification of ubiquitin-binding domains. Some effectors (*e.g.*, CUE2/NONU-1, CUE3/ASCC) have ubiquitin-binding domains recognizable at the primary sequence level. However, among the many known ubiquitin-interacting domains in biology, there is little sequence conservation even among homologs in different organisms, which is a hallmark of the nature of the often hydrophobic interaction between a protein and ubiquitin [30,61]. As ubiquitin-binding motifs can be encoded by as little as a few alpha helices or beta sheets, our ability to identify them from sequence alone is expected to remain poor. While prediction of ubiquitin-binding domains from sequence may improve, until then we expect that ubiquitin-binding assays will remain critical to learn whether a downstream effector is recruited via ubiquitin or by some other means. Given the relatively poor identification of ubiquitin-binding domains from sequence alone, we expect that many more effectors are ubiquitin-dependent than is currently appreciated.

6. Conclusions and future perspectives

The work discussed above highlights the major focus placed on regulatory ribosomal ubiquitination in recent years. Reports of ubiquitination on many 40S subunit proteins have since been linked to a group of E3 ubiquitin ligases responsible for their modification, with deubiquitinases regulating levels of 40S subunits and E3s themselves. These studies have laid a groundwork of molecular interactions to help understand NGD and NSD.

Taken together, ribosomal ubiquitination can function to recruit a group of effectors which carry out commitment steps toward repression. However, it remains unclear how ubiquitin-binding effectors actually bind ubiquitin, especially considering that some effectors contain multiple ubiquitin-binding domains and that they are presented with a constellation of potential ubiquitin sites to bind. We anticipate future studies will reveal direct functions of individual ubiquitination sites, characterize the ubiquitin-binding domains within effectors, and identify novel ubiquitin-binding effectors.

Lastly, much of the study of ribosomal ubiquitination comes from situations in which a large number of ribosomes experience the same stress. Whether subsets of endogenous mRNAs have their ribosomes ubiquitinated is poorly understood, in part because only a small number of ribosomes would be on such an mRNA at once, thus complicating a clear measure of their ubiquitination status. In this way, we are currently only “listening” to the ubiquitin language of the “loudest” ribosomes communicating an assault on translation.

Considering that the cell is essentially a crowded room of ribosomes, each with their own ubiquitin story to tell, we are currently missing what the “quieter” ribosomes are saying. This gap in knowledge is exemplified by the finding of ~60 metazoan ribosomal proteins ubiquitinated at a total of ~268 residues, while we only have hints at function for ~10 residues on the small subunit (Fig. 4) [27,44]. There is little understanding of the biological significance and/or functional consequences of ribosomal ubiquitination outside of these handful of sites. We look forward to works delving into the meaning of these largely uncharacterized ubiquitination events and expect these contributions to amplify the voices of translation.

Acknowledgments

This work was supported in part by a T32 Training Grant Fellowship from the National Institute of General Medical Sciences (NIGMS) (5T32GM133391-02) to P.C.M., an R01 grant from the NIGMS (1R01GM131012-01) to J.A.A., a Searle Scholars award to J.A.A., and start-up funds from UCSC to J.A.A.

Abbreviations:

NGD	No-Go mRNA Decay
NSD	Nonstop mRNA Decay
RING	Really Interesting New Gene
CUE	Coupling of Ubiquitin conjugation to ER degradation
NRD	Non-functional rRNA Decay
DUB	Deubiquitinase

References

[1]. Allen GE, Panasenko OO, Villanyi Z, Zagatti M, Weiss B, Pagliazzo L, Huch S, Polte C, Zahoran S, Hughes CS, Pelechano V, Ignatova Z, Collart MA, Not4 and Not5 modulate translation elongation by Rps7A ubiquitination, Rli1 moonlighting, and condensates that exclude eIF5A, Cell Rep. 36 (9) (2021), 109633. [PubMed: 34469733]

[2]. Arpat A, Liechti A, De Matos M, Dreos R, Janich P, Gatfield D, Transcriptomewide sites of collided ribosomes reveal principles of translational pausing, Genome Res. 30 (7) (2020) 985–999. [PubMed: 32703885]

[3]. Becker T, Armache JP, Jarasch A, Anger AM, Villa E, Sieber H, Motaal BA, Mielke T, Berninghausen O, Beckmann R, Structure of the no-go mRNA decay complex Dom34-Hbs1 bound to a stalled 80S ribosome, Nat. Struct. Mol. Biol 18 (6) (2011) 715–720. [PubMed: 21623367]

[4]. Bengtson MH, Joazeiro CAP, Role of a ribosome-associated E3 ubiquitin ligase in protein quality control, Nature 467 (7314) (2010) 470–473. [PubMed: 20835226]

[5]. Brandman O, Stewart-Ornstein J, Wong D, Larson A, Williams CC, Li GW, Zhou S, King D, Shen PS, Weibezahn J, Dunn JG, Rouskin S, Inada T, Frost A, Weissman JS, A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress, Cell 151 (5) (2012) 1042–1054. [PubMed: 23178123]

[6]. Brickner JR, Soll JM, Lombardi PM, Vågbø CB, Mudge MC, Oyeniran C, Rabe R, Jackson J, Sullender ME, Blazosky E, Byrum AK, Zhao Y, Corbett mark A., Gécz J, Field M, Vindigni A, Slupphaug G, Wolberger C, Mosammaparast N, A ubiquitin-dependent signalling axis specific

for ALKBH-mediated DNA dealkylation repair, Nature 551 (7680) (2017) 389–393. [PubMed: 29144457]

[7]. Buetow L, Huang DT, Structural insights into the catalysis and regulation of E3 ubiquitin ligases, Nat. Rev. Mol. Cell Biol 7 (10) (2016) 626–642.

[8]. Buschauer R, Matsuo Y, Sugiyama T, Chen YH, Alhusaini N, Sweet T, Ikeuchi K, Cheng J, Matsuki Y, Nobuta R, Gilmozzi A, Berninghausen O, Tesina P, Becker T, Coller J, Inada T, Beckmann R, The Ccr4-Not complex monitors the translating ribosome for codon optimality, Science 368 (6488) (2020).

[9]. Bustos D, Bakalarski CE, Yang Y, Peng J, Kirkpatrick DS, Characterizing ubiquitination sites by peptide-based immunoaffinity enrichment, Mol. Cell. Proteom 11 (12) (2012) 1529–1540.

[10]. Cassar PA, Carpenedo RL, Samavarchi-Tehrani P, Olsen JB, Park CJ, Chang WY, Chen Z, Choey C, Delaney S, Guo H, Guo H, Tanner RM, Perkins TJ, Tenenbaum SA, Emili A, Wrana JL, Gibbings D, Stanford WL, Integrative genomics positions MKRN 1 as a novel ribonucleoprotein within the embryonic stem cell gene regulatory network, EMBO Rep. 16 (10) (2015) 1334–1357. [PubMed: 26265008]

[11]. Clancy A, Heride C, Pinto-Fernández A, Elcocks H, Kallinos A, Kayser-Bricker KJ, Wang W, Smith V, Davis S, Fessler S, McKinnon C, Katz M, Hammonds T, Jones NP, O'Connell J, Follows B, Mischke S, Caravella JA, Ioannidis S, Clague MJ, The deubiquitylase USP9X controls ribosomal stalling, J. Cell Biol 220 (2021) 3.

[12]. Clague MJ, Urbé S, Ubiquitin: same molecule, different degradation pathways, Cell 143 (5) (2010) 682–685. [PubMed: 21111229]

[13]. Clague MJ, Urbé S, Komander D, Breaking the chains: deubiquitylating enzyme specificity begets function, Nat. Rev. Mol. Cell Biol 20 (6) (2019) 338–352. [PubMed: 30733604]

[14]. Collart MA, The Ccr4-Not complex is a key regulator of eukaryotic gene expression, Wiley Interdiscip. Rev. RNA 7 (4) (2016) 438–454. [PubMed: 26821858]

[15]. Deshaies RJ, Joazeiro CAP, RING domain E3 ubiquitin ligases, Annu. Rev. Biochem 78 (2009) 399–434. [PubMed: 19489725]

[16]. Dimitrova LN, Kuroha K, Tatematsu T, Inada T, Nascent peptide-dependent translation arrest leads to Not4p-mediated protein degradation by the proteasome, J. Biol. Chem 284 (16) (2009) 10343–10352. [PubMed: 19204001]

[17]. Doma MK, Parker R, Endonucleolytic cleavage of eukaryotic mRNAs with stalls in translation elongation, Nature 440 (7083) (2006) 561–564. [PubMed: 16554824]

[18]. D'Orazio KN, Wu CCC, Sinha N, Loll-Krippleber R, Brown GW, Green R, The endonuclease cue2 cleaves mRNAs at stalled ribosomes during no go decay, eLife (2019) 8.

[19]. D'Orazio KN, Green R, Ribosome states signal RNA quality control, Mol. Cell 81 (7) (2021) 1372–1383. [PubMed: 33713598]

[20]. Frischmeyer PA, Hoof A, van K, O'Donnell, Guerrerio AL, Parker R, Dietz HC, An mRNA surveillance mechanism that eliminates transcripts lacking termination codons, Science 295 (5563) (2002) 2258–2261. [PubMed: 11910109]

[21]. Garshott DM, Sundaramoorthy E, Leonard M, Bennett EJ, Distinct regulatory ribosomal ubiquitylation events are reversible and hierarchically organized, eLife 9 (2020) 1–22.

[22]. Garshott DM, An H, Sundaramoorthy E, Leonard M, Vicary A, Harper JW, Bennett EJ, iRQC, a surveillance pathway for 40S ribosomal quality control during mRNA translation initiation, Cell Rep. 36 (9) (2021), 109642. [PubMed: 34469731]

[23]. Garzia A, Jafarnejad SM, Meyer C, Chapat C, Gogakos T, Morozov P, Amiri M, Shapiro M, Molina H, Tuschl T, Sonenberg N, The E3 ubiquitin ligase and RNA-binding protein ZNF598 orchestrates ribosome quality control of premature polyadenylated mRNAs, Nat. Commun (2017) 8.

[24]. Garzia A, Meyer C, Tuschl T, The E3 ubiquitin ligase RNF10 modifies 40S ribosomal subunits of ribosomes compromised in translation, Cell Rep. 36 (5) (2021), 109468. [PubMed: 34348161]

[25]. Glover ML, Burroughs AM, Monem PC, Egelhofer TA, Pule MN, Aravind L, Arribere JA, NONU-1 encodes a conserved endonuclease required for mRNA translation surveillance, Cell Rep. 30 (13) (2020) 4321–4331, e4. [PubMed: 32234470]

[26]. Han P, Shichino Y, Schneider-Poetsch T, Mito M, Hashimoto S, Udagawa T, Kohno K, Yoshida M, Mishima Y, Inada T, Iwasaki S, Genome-wide survey of ribosome collision, Cell Rep. 31 (5) (2020), 107610. [PubMed: 32375038]

[27]. Higgins R, Gendron JM, Rising L, Mak R, Webb K, Kaiser SE, Zuzow N, Riviere P, Yang B, Fenech E, Tang X, Lindsay SA, Christianson JC, Hampton RY, Wasserman SA, Bennett EJ, The unfolded protein response triggers site-specific regulatory ubiquitylation of 40s ribosomal proteins, Mol. Cell 59 (1) (2015) 35–49. [PubMed: 26051182]

[28]. Hilal T, Yamamoto H, Loerke J, Bürger J, Mielke T, Spahn CMT, Structural insights into ribosomal rescue by Dom34 and Hbs1 at near-atomic resolution, Nat. Commun 7 (2016) 1–8.

[29]. Hildebrandt A, Brüggemann M, Rücklé C, Boerner S, Heidelberger JB, Busch A, Hänel H, Voigt A, Möckel MM, Ebersberger S, Scholz A, Dold A, Schmid T, Ebersberger I, Roignant JY, Zarnack K, König J, Beli P, The RNA-binding ubiquitin ligase MKRN1 functions in ribosome-associated quality control of poly(A) translation, Genome Biol. 20 (1) (2019) 1–20. [PubMed: 30606230]

[30]. Husnjak K, Dikic I, Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions, Annu. Rev. Biochem 81 (2012) 291–322. [PubMed: 22482907]

[31]. Ikeuchi K, Tesina P, Matsuo Y, Sugiyama T, Cheng J, Saeki Y, Tanaka K, Becker T, Beckmann R, Inada T, Collided ribosomes form a unique structural interface to induce Hel2–driven quality control pathways, EMBO J. 38 (5) (2019) 1–21.

[32]. Inada T, Quality controls induced by aberrant translation, Nucleic Acids Res. 48 (3) (2020) 1084–1096. [PubMed: 31950154]

[33]. Ishimura R, Nagy G, Dotu I, Zhou H, Yang XL, Schimmel P, Senju S, Nishimura Y, Chuang JH, Ackerman SL, Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration, Science 345 (6195) (2014) 455–459. [PubMed: 25061210]

[34]. Itzhak DN, Tyanova S, Cox J, Borner GHH, Global, quantitative and dynamic mapping of protein subcellular localization, eLife 5 (2016) 1–36.

[35]. Jia J, Absmeier E, Holton N, Pietrzyk-Brzezinska AJ, Hackert P, Bohnsack KE, Bohnsack MT, Wahl MC, The interaction of DNA repair factors ASCC2 and ASCC3 is affected by somatic cancer mutations, Nat. Commun 11 (1) (2020) 1–13. [PubMed: 31911652]

[36]. Joazeiro CAP, Ribosomal stalling during translation: providing substrates for ribosome-associated protein quality control, Annu. Rev. Cell Dev. Biol 33 (2017) 343–368. [PubMed: 28715909]

[37]. Juszkiewicz S, Hegde RS, Initiation of quality control during poly(A) translation requires site-specific ribosome ubiquitination, Mol. Cell 65 (4) (2017) 743–750, e4. [PubMed: 28065601]

[38]. Juszkiewicz S, Chandrasekaran V, Lin Z, Kraatz S, Ramakrishnan V, Hegde RS, ZNF598 is a quality control sensor of collided ribosomes, Mol. Cell 72 (3) (2018) 469–481, e7. [PubMed: 30293783]

[39]. Juszkiewicz S, Speldewinde SH, Wan L, Svejstrup JQ, Hegde RS, The ASC-1 complex disassembles collided ribosomes, Mol. Cell 79 (4) (2020) 603–614, e8. [PubMed: 32579943]

[40]. Kang RS, Daniels CM, Francis SA, Shih SC, Salerno WJ, Hicke L, Radhakrishnan I, Solution structure of a CUE-ubiquitin complex reveals a conserved mode of ubiquitin binding, Cell 113 (5) (2003) 621–630. [PubMed: 12787503]

[41]. Kim JH, Park SM, Kang MR, Oh SY, Lee TH, Muller MT, Chung IK, Ubiquitin ligase MKRN1 modulates telomere length homeostasis through a proteolysis of hTERT, Genes Dev. 19 (7) (2005) 776–781. [PubMed: 15805468]

[42]. Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, Sowa ME, Rad R, Rush J, Comb MJ, Harper JW, Gygi SP, Systematic and quantitative assessment of the ubiquitin-modified proteome, Mol. Cell 44 (2) (2011) 325–340. [PubMed: 21906983]

[43]. Komander D, Rape M, The ubiquitin code, Annu. Rev. Biochem 81 (2012) 203–229.

[44]. Koyuncu S, Loureiro R, Lee HJ, Wagle P, Krueger M, Vilchez D, Rewiring of the ubiquitinated proteome determines ageing in *C. elegans*, Nature 596 (7871) (2021) 285–290. [PubMed: 34321666]

[45]. Letzring DP, Wolf AS, Brule CE, Grayhack EJ, Translation of CGA codon repeats in yeast involves quality control components and ribosomal protein L1, RNA 19 (9) (2013) 1208–1217. [PubMed: 23825054]

[46]. Martínez-Férriz A, Ferrando A, Fathinajafabadi A, Farràs R, Ubiquitin-mediated mechanisms of translational control, Semin. Cell Dev. Biol (2021).

[47]. Matsuda R, Ikeuchi K, Nomura S, Inada T, Protein quality control systems associated with no-go and nonstop mRNA surveillance in yeast, Genes Cells 19 (1) (2014) 1–12. [PubMed: 24261871]

[48]. Matsuo Y, Ikeuchi K, Saeki Y, Iwasaki S, Schmidt C, Udagawa T, Sato F, Tsuchiya H, Becker T, Tanaka K, Ingolia NT, Beckmann R, Inada T, Ubiquitination of stalled ribosome triggers ribosome-associated quality control, Nat. Commun 8 (1) (2017).

[49]. Matsuo Y, Tesina P, Nakajima S, Mizuno M, Endo A, Buschauer R, Cheng J, Shounai O, Ikeuchi K, Saeki Y, Becker T, Beckmann R, Inada T, RQT complex dissociates ribosomes collided on endogenous RQC substrate SDD1, Nat. Struct. Mol. Biol 27 (4) (2020) 323–332. [PubMed: 32203490]

[50]. Mayor T, Lipford JR, Graumann J, Smith GT, Deshaies RJ, Analysis of polyubiquitin conjugates reveals that the Rpn10 substrate receptor contributes to the turnover of multiple proteasome targets, Mol. Cell. Proteom 4 (6) (2005) 741–751.

[51]. Meydan S, Guydosh NR, Disome and trisome profiling reveal genome-wide targets of ribosome quality control, Mol. Cell 79 (4) (2020) 588–602, e6. [PubMed: 32615089]

[52]. Meydan S, Guydosh NR, A cellular handbook for collided ribosomes: surveillance pathways and collision types, Curr. Genet 67 (1) (2021) 19–26. [PubMed: 33044589]

[53]. Meyer C, Garzia A, Morozov P, Molina H, Tuschl T, The G3BP1-Family-USP10 deubiquitinase complex rescues ubiquitinated 40s subunits of ribosomes stalled in translation from lysosomal degradation, Mol. Cell 77 (6) (2020) 1193–1205, e5. [PubMed: 31981475]

[54]. Monem PC, Vidyasagar N, Piatt AL, Sehgal E, Arribere JA, Ubiquitination of stalled ribosomes enables mRNA decay via HBS-1 and NONU-1 in vivo, PLoS Genet. 19 (1) (2023), e1010577. [PubMed: 36626369]

[55]. Mishima Y, Han P, Ishibashi K, Kimura S, Iwasaki S, Ribosome slowdown triggers codon-mediated mRNA decay independently of ribosome quality control, EMBO J. 41 (5) (2022), e109256. [PubMed: 35040509]

[56]. Panasenko OO, Collart MA, Presence of Not5 and ubiquitinated Rps7A in polysome fractions depends upon the Not4 E3 ligase, Mol. Microbiol 83 (3) (2012) 640–653. [PubMed: 22243599]

[57]. Panasenko OO, The role of the E3 ligase Not4 in cotranslational quality control, Front. Genet (2014) 5.

[58]. Peng J, Schwartz D, Elias JE, Thoreen CC, Cheng D, Marsischky G, Roelofs J, Finley D, Gygi SP, A proteomics approach to understanding protein ubiquitination, Nat. Biotechnol 21 (8) (2003) 921–926. [PubMed: 12872131]

[59]. Pickart CM, Eddins MJ, Ubiquitin: structures, functions, mechanisms, Biochim. Biophys. Acta Mol. Cell Res 1695 (1–3) (2004) 55–72.

[60]. Preissler S, Reuther J, Koch M, Scior A, Bruderek M, Frickey T, Deuerling E, Not4–dependent translational repression is important for cellular protein homeostasis in yeast, EMBO J. 34 (14) (2015) 1905–1924. [PubMed: 25971775]

[61]. Randles L, Walters KJ, Ubiquitin and its binding domains, Front. Biosci 17 (2012) 2140–2157.

[62]. Saito K, Horikawa W, Ito K, Inhibiting K63 polyubiquitination abolishes No-Go type stalled translation surveillance in Saccharomyces cerevisiae, PLoS Genet. 11 (4) (2015) 1–18.

[63]. Sandler H, Kreth J, Timmers HTM, Stoecklin G, Not1 mediates recruitment of the deadenylase Caf1 to mRNAs targeted for degradation by tristetraprolin, Nucleic Acids Res. 39 (10) (2011) 4373–4386. [PubMed: 21278420]

[64]. Shao S, Murray J, Brown A, Taunton J, Ramakrishnan V, Hegde RS, Decoding mammalian ribosome-mRNA states by translational GTPase complexes, Cell 167 (5) (2016) 1229–1240, e15. [PubMed: 27863242]

[65]. Shcherbik N, Pestov DG, Ubiquitin and ubiquitin-like proteins in the nucleolus: multitasking tools for a ribosome factory, Genes Cancer 1 (7) (2010) 681–689. [PubMed: 21113400]

[66]. Shoemaker CJ, Green R, Translation drives mRNA quality control, Nat. Struct. Mol. Biol 19 (6) (2012) 594–601. [PubMed: 22664987]

[67]. Simms CL, Yan LL, Zaher HS, Ribosome collision is critical for quality control during No-Go decay, Mol. Cell 68 (2) (2017) 361–373, e5. [PubMed: 28943311]

[68]. Singh RK, Gonzalez M, Kabbaj M-HM, Gunjan A, Novel E3 ubiquitin ligases that regulate histone protein levels in the budding yeast Saccharomyces cerevisiae, PLoS One 7 (5) (2012) 36295.

[69]. Sitron CS, Park JH, Brandman O, Asc1, Hel2, and Slh1 couple translation arrest to nascent chain degradation, RNA 23 (5) (2017) 798–810. [PubMed: 28223409]

[70]. Spence J, Rayappa RG, Dittmar G, Sherman F, Karin M, Finley D, Cell cycle-regulated modification of the ribosome by a variant multiubiquitin chain, Cell 102 (2000) 67–76. [PubMed: 10929714]

[71]. Sugiyama T, Li S, Kato M, Ikeuchi K, Ichimura A, Sequential ubiquitination of ribosomal protein us3 triggers the degradation of non-functional 18S rRNA, Cell Rep. 26 (2019) 3400–3415. [PubMed: 30893611]

[72]. Sundaramoorthy E, Leonard M, Mak R, Liao J, Fulzele A, Bennett EJ, ZNF598 and RACK1 regulate mammalian ribosome-associated quality control function by mediating regulatory 40s ribosomal ubiquitylation, Mol. Cell 65 (4) (2017) 751–760, e4. [PubMed: 28132843]

[73]. Takehara Y, Yashiroda H, Matsuo Y, Zhao X, Kamigaki A, Matsuzaki T, Kosako H, Inada T, Murata S, The ubiquitination-deubiquitination cycle on the ribosomal protein eS7A is crucial for efficient translation, IScience 24 (3) (2021), 102145. [PubMed: 33665564]

[74]. Tomomatsu S, Watanabe A, Tesina P, Hashimoto S, Ikeuchi K, Li S, Matsuo Y, Beckmann R, Inada T, Two modes of Cue2-mediated mRNA cleavage with distinct substrate recognition initiate no-go decay, Nucleic Acids Res. (2022) 1–18. [PubMed: 34268577]

[75]. Tucker M, Valencia-Sanchez MA, Staples RR, Chen J, Denis CL, Parker R, The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in Saccharomyces cerevisiae, Cell 104 (3) (2001) 377–386. [PubMed: 11239395]

[76]. Wu Z, Wang Y, Lim J, Liu B, Li Y, Vartak R, Stankiewicz T, Montgomery S, Lu B, Ubiquitination of ABCE1 by NOT4 in response to mitochondrial damage links co-translational quality control to PINK1-directed mitophagy, Cell Metab. 28 (1) (2018) 130–144, e7. [PubMed: 29861391]

[77]. Yan LL, Simms CL, McLoughlin F, Vierstra RD, Zaher HS, Oxidation and alkylation stresses activate ribosome-quality control, Nat. Commun 10 (2019) 1. [PubMed: 30602773]

[78]. Zhao T, Chen Y-M, Li Y, Wang J, Chen S, Gao N, Qian W, Disome-seq reveals widespread ribosome collisions that promote cotranslational protein folding, Genome Biol. 22 (1) (2021) 1–35. [PubMed: 33397451]

[79]. Zheng N, Shabek N, Ubiquitin ligases: structure, function, and regulation, Annu. Rev. Biochem 86 (2017) 129–157. [PubMed: 28375744]

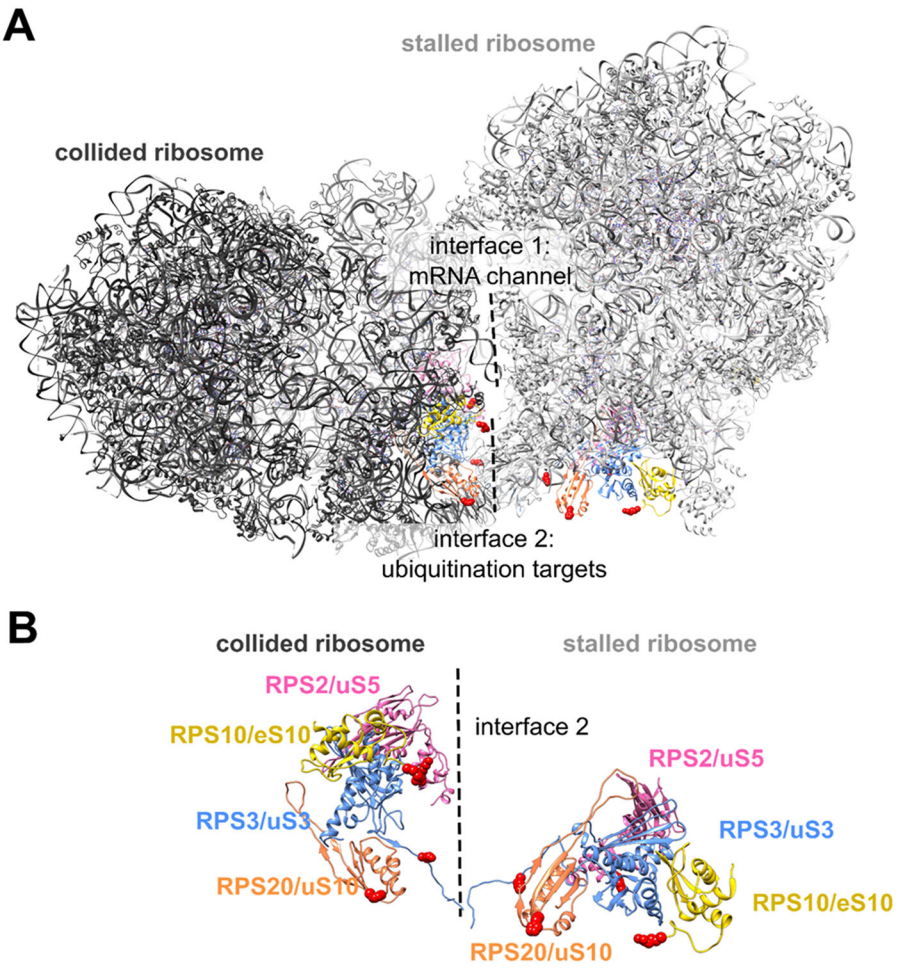

Fig. 1.

Ribosomal ubiquitination sites localize to the disome interface. (A) Cryo-EM structure of rabbit reticulocyte ribosomes from [38] (PDB 6HCQ and 6HCM). The 5'-most, collided/trailing ribosome is shown in dark gray, while the 3'-most, stalled/leading ribosome is shown in light gray. Ribosomal proteins ubiquitinated in metazoans are colored: RPS2/uS5 in pink, RPS3/uS3 in light blue, RPS10/eS10 in yellow, and RPS20/uS10 in orange. Labeled with red spheres are side chains of ubiquitination target lysines of each protein. In cases where the target lysine was not modeled due to its flexibility, the closest visible residue is highlighted. Interfaces 1 and 2 are labeled and defined as regions where the 40S subunits of each ribosome interact. (B) Zoom in on the ribosomal proteins indicated in part (A). Identities of ribosomes containing each set of proteins are labeled above.

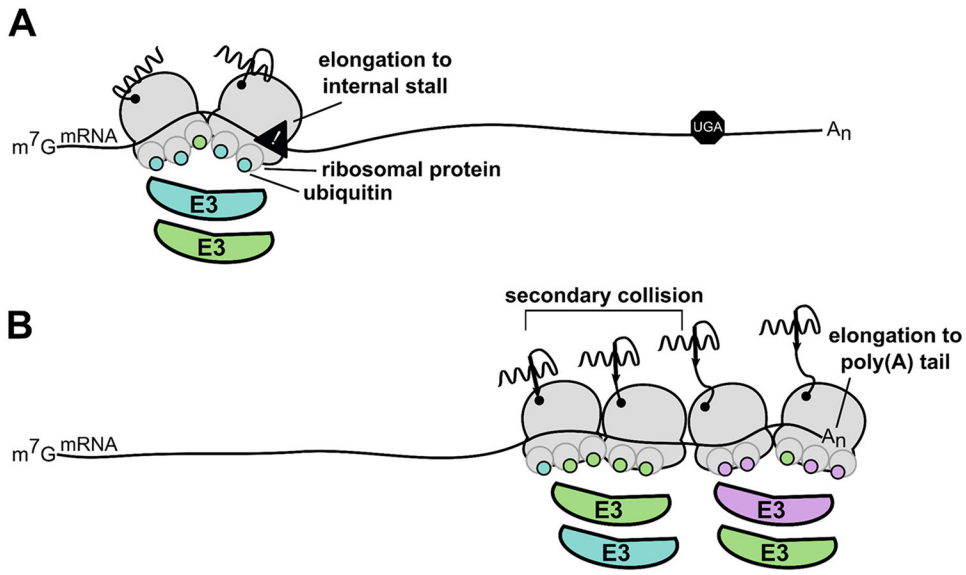

Fig. 2.

Distinct stalls generate substrates for different E3 ubiquitin ligases. (A) Model for E3 ligase targeting during an internal ribosomal stall. Black triangle with an exclamation point represents a stalling feature. Gray circles represent ribosomal proteins experiencing ubiquitination. Colored circles represent ubiquitin, colored according to the E3 ligase responsible for their placement. E3 ligases are shown in their respective colors. (B) Model for E3 ubiquitin ligase targeting during a ribosomal stall within the poly(A) tail at the 3' edge of an mRNA. Features shown as in part (A).

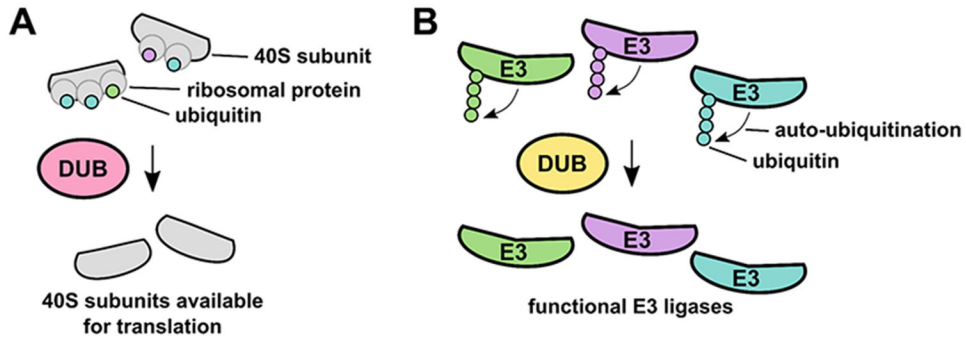

Fig. 3.

DUBs control the ubiquitination status of 40S subunits and E3 ubiquitin ligases. (A) Model for deubiquitinase (DUB) function during ribosomal ubiquitination. Gray ovals represent 40S subunits, with gray circles as ribosomal proteins experiencing ubiquitination. Ubiquitins are indicated with colored circles. DUB acting on 40S subunits is shown in pink. (B) Model for DUB function during E3 ubiquitin ligase auto-ubiquitination. Poly-ubiquitin chains are shown as strings of colored circles. E3 ligases are shown in their respective colors. DUB acting on E3 ligases is shown in yellow.

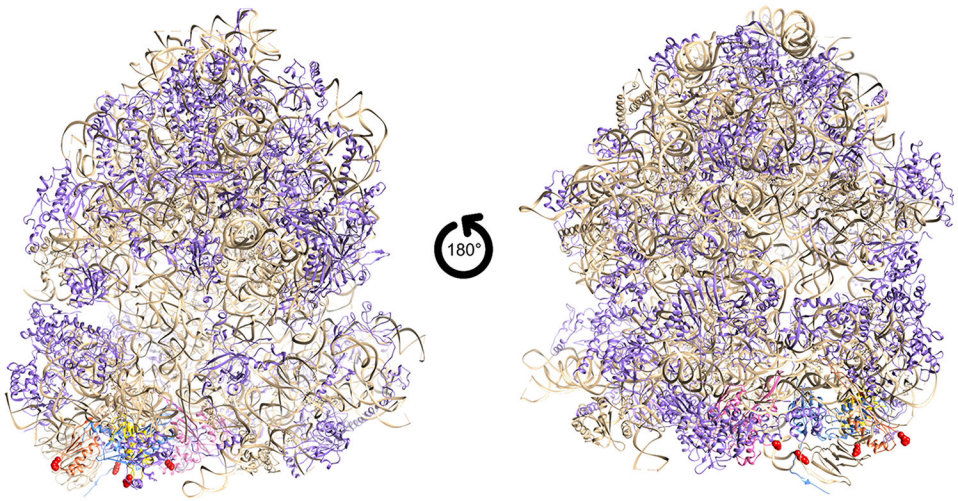

Fig. 4.

Ribosomal ubiquitination sites with unclear consequences. Cryo-EM structure collided/trailing ribosome from [38] (PDB 6HCQ). Characterized ribosomal ubiquitination protein targets and lysine residues (Table 1) are colored as in Fig. 1. Uncharacterized ribosomal ubiquitination protein targets from [27] and [44] are colored in lavender. Other ribosomal proteins and rRNA are colored in beige.

Table 1

Ribosomal proteins ubiquitinated during translational stress.

Ribosomal protein	Organism & lysine residue	Source
RPS2/uS5	*H. sapiens:* K54 [*], K58 [*,†]	[21,24,27]
RPS3/uS3	*S. cerevisiae:* K212 [*]; *H. sapiens:* K214 [*,†], K249 [†]	[21,23,24,27,31,48,67,72]
RPS7/eS7	*S. cerevisiae:* K83 [*], K84 [*]	[1,31,48,56,73]
RPS10/eS10	*H. sapiens:* K138 [*,†], K139 [*,†], K107 [†]; *C. elegans:* K125 [*,†]	[21,23,29,37,44,54,72]
RPS20/uS10	*S. cerevisiae:* K6 [*], K8 [*]; *H. sapiens:* K4 [*,†], K8 [*,†]; *C. elegans:* K6 [*,†], K9 [*,†]	[21,23,27,31,37,44,48,54,72]

Table of ribosomal proteins reported to be ubiquitinated upon ribosomal stalling, via treatment with translation drugs and/or expression of stalling reporters. Lysine residues shown are supported by *genetic evidence, [†]biochemical evidence, or both in their respective organisms.

Table 2

E3 ubiquitin ligases and their ribosomal protein targets.

E3 ubiquitin ligase	Ribosomal protein target
ZNF598/Hel2	RPS10/eS10; RPS20/uS10; RPS2/uS5; RPS3/uS3; RPS7/eS7
RNF10/Mag2	RPS2/uS5; RPS3/uS3
Not4	RPS7/eS7
MKRN1/2	RPS10/eS10

Table of E3 ubiquitin ligases functioning during ribosomal stalling. Ribosomal proteins displaying a change in ubiquitination status in deletions or knockdowns of each E3 ligase are shown.

Table 3

Translational surveillance effectors recognizing altered ribosomes.

Effector	Effector domain	Recruitment domain	Source
CUE2/NONU-1	Smr nuclease	CUE*,†	[18,25,40,54,74]
HBS-1	eEF1A-like GTPase	Uba-like triple-helix bundle^	[3,28,54]
CUE3/ASCC	RecA-like helicase	CUE*,†	[6,35,39,48,49,69]

Table of known translational surveillance effectors suspected to act downstream of and dependent on ribosomal ubiquitination. Effector domains shown are defined by the established function in translational surveillance pathways. Recruitment domain is supported by *genetic evidence demonstrating its requirement in surveillance, †biochemical evidence displaying its ubiquitin-binding ability, or ^structural homology with other ubiquitin-binding domains.