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Ribosome Associated Protein Quality Control: Mechanism and Function

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ABSTRACT

Due to numerous reasons, including faulty m RNA, insufficient availability of charged t RNA, genetic errors, ribosomes are failed to synthesize protein sometimes. All organisms develop their machinery to recognize stalled ribosomes. Stalled ribosomes, results in the production of a truncated polypeptide which can affect cells. So, they must be eliminated, by mechanisms known as Ribosome-associated protein quality control (RQC). E3 ubiquitin ligase Ltn1 in RQC promotes clearance of 60S subunit and targets aberrant nascent polypeptides for proteasomal degradation. In eukaryotes, RQC facilitates the ribosomal rescue, where staled m RNAs release and allow to degrade and ribosomal subunits are to be recycled for further use. Ribosome-associated protein quality control in yeast is accomplished by Hel2-dependent ubiquitination of uS10 and RQC-trigger (RQT) complex. RQC in a mammal is done by ZNF598-dependent ubiquitination of collided ribosomes, which also activates signal integrator 3, a component of the ASCC complex. Human RQT (h RQT) is made up of ASCC3, ASCC2, TRIP4, which are orthologs of RNA helicase Slh1, ubiquitin-binding protein Cue3, and ykR023W protein respectively. Ubiquitin-binding activity and ATPase activity of ASCC2 and ASCC3 respectively, are important for RQC. So, it is obvious that the h RQT complex recognizes the ubiquitinated defective ribosome and induces subunit dissociation for RQC. Biogenesis of new polypeptide, folding, correct localization are the fundamental processes to maintain proteostasis, which involve various factors directly attached with ribosomes and chaperones. Ribosome-associated protein biogenesis factors mediate the cellular proteostasis network to form integrity.

Keywords- ASCC3, ASCC2, TRIP4, RQC, collided ribosomes.

I. INTRODUCTION

Distorted proteins can form continuously by gene mutation, error in gene expression, absence of some domains, or chemical changes. For these facts proteins cannot fold properly, their normal functions are interrupted, forming toxic substances. Cells evolve a variety of mechanisms to prevent the production and accumulation of these proteins. (Joazeiro, C. A. 2019). Cells develop the mechanisms to target the polypeptides to degrade, as cells get benefit from removing errors. If the termination codon will not read by ribosomes, the protein product will be truncated and likely to be defective. If the truncated protein will fold further, it may lack key downstream domains and exerts dominant-negative effects. Error proteins are surely arrested by ribosomes, tagging them for degradation, making rapid elimination, and minimizing inappropriate interaction in the cytosol (Brandman et al., 2016).

Proteasomal Degradation of Aberrant Protein:

Three procedures that are used to mediate m RNA degradation are Nonsense m RNA decay (NMD), Nonstop m RNA decay (NSD), no-go-decay (NGD). NMD requires a premature stop codon, whereas NSD targets m RNA lacks stop, NDG senses the obstacles of elongation.

Stalled ribosomes activate NSD & NDG pathway, where 5' ⟶ 3' degradation happens by exonuclease Xrn1 and 3' ⟶ 5' degradation occurs through Ski2-Ski3-Ski8 along with exosome.

Aberrant m RNA produces aberrant peptides, further producing the truncated polypeptide. Bacterial to RNAs are responsible for the degradation of these proteins. Two groups of people show differently that aberrant proteins degrade in a proteasome-dependent manner. Inada group shows steady-state levels of nonstop proteins were reduced, relative to a stop codon-containing control, this reduction is due to at least in part to the instability of non-stop proteins and the non-stop protein degradation should be slowdown by pharmacological inhibition of proteasome (Joazeiro, C. A. 2017).

E3 Ligase Listerine / Ltn 1 & Ribosome Associated Quality Control Pathway:

The exact process of targeting non-stop proteins is unknown. Some illustrations may raise the possibility that new strands of protein can be recognized by cytoplasmic Protein Quality Control Pathway, after being released from the ribosome (Medicherla & Goldberg, 2008).

Here E3 is responsible for the ubiquitylation of non-stop protein. The first suggested E3 ligase responsible for targeting non-stop protein for degradation is Ccr4- Not m RNA deadenylase (Dimitrova et al., 2009).

Studies show that Not4 affects non-stop protein levels by regulating non-stop m RNA level and non-stop protein synthesis (Joazeiro, C. A. 2017). Ltn1 ligase

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mediates ubiquitylation and degradation of non-stop proteins is discovered in 2010 by Bengtson & Joazeiro. Mutation in Ltn1 ortholog, Listerine was reported as the cause of neurodegeneration in a mouse (Chu et al., 2009).

Ltn1 was found to show synthetic lethal interaction with the gene encoding the RNAP II transcription elongation factor Rtf1 and also found that deletion of this Ltn1 increased the steady-state level of non-stop reporter. Bengtson & Joazeiro (2010) explained Ltn1 shows the characteristics of E3 ligase controlling non-stop protein degradation. Distorted protein quality control will emphasize neurodegeneration (Joazeiro, C. A. 2017).

Ribosome Splitting, For Upstream of Ltn1:

Hbs1 and Dom 34 are the components of NGD and are also known to function in NSD for the m-RNA 3' end of the stalled ribosome. Hbs1 is homologous to erf3, translation termination factor, Dom 34 is homologous to eRF1. eRf3 load eRF1 on A site at a stop codon during translation termination. Productive binding occurs through eRF3 dissociation, eRF1 mediated recruitment of ribosomal subunit splitting AAA ATPase Rli1 / ABCE1. Due to this reaction, free t-RNA and nascent polypeptide chains are released and ribosome dissociated.

ERF1 motif responsible for t-RNA hydrolysis is laca king in Dom 34. A study on yeast and mammalian invitro translation systems revealed that ribosomal rescue is initiated with Hbs1 / Hbs1L loading Dom 34/ Pelota on a stalled ribosome in codon independent manner (Joazeiro, C. A. 2017). Dom 34 / Pelota recruit Rli1 / ABCE1 for ribosomal dissociation, without peptidyl t-RNA hydrolysis (Pisareva et al., 2011).

Stalled Translation Elongation Required RQC Pathway Related Substrate: Translation of m-RNA With Nonuniform Speed:-

In translation elongation, transfer of nascent polypeptide chain associated with t-RNA, at ribosomal P site to A site (Aminoacyl t-RNA conjugate position) occurs, this follows A site translocation of acceptor t-RNA attached with nascent chain grown by one residue, from A site by the help of eukaryotic elongation factor 2 (e EF2). A new aminoacyl tRNA into A site with the help of GTPase eukaryotic elongation factor 1 alpha (Eef1 A).

Proteins are synthesized with an average speed of ~ 6 amino acids per second (Ingolia et al., 2011, Bastrom et al., 1986). Elongation of the translation does not proceed in a regular sequence, can be altered by various factors. Proline acts poorly both as a peptidyl donor and a peptidyl acceptor (Muto & Ito 2008, Wohlgemuth et al., 2008).

Cells recognize ribosomal pausing during the elongation stage with the objective of fine-tuning protein production until needed. In some cases, pausing is done by the interaction of nascent polypeptide with ribosomal exit tunnel. An example is the Arginine attenuator peptide (AAP) (Fang et al., 2004).

AAP encoded by an open reading frame upstream of Arg 2. The expression of Arg 2 is repressed by Arg mediated ribosome pausing when it presents insufficient (Joazeiro, C. A. 2017).

Arrest Translation for Ribosome Associated Quality Control:

Translation elongation stalling cancan occur as two Arginine codons CGA are adjacent to each other or when multiple Proline codons are present in adjacent places. Halting of the ribosome can also occur when translation starts with aberrant m RNA, generated as a result of chemical damage, endonuclease action or, errors in gene expression.

Some aberrant mRNA forms because they lack stop codons, called nonstop codons. Nonstop mRNAs are of two classes. Class 1 transcripts generated by cleavage within coding regions. Class 1 transcripts are produced by self-cleaving ribosome sequence, fused to some reporter gene. Class 2 nonstop transcripts have poly (A) tail, so that become accessible to a translation by ribosome.

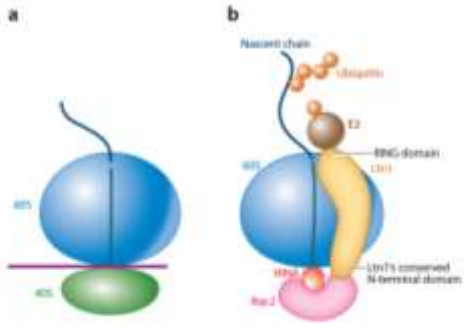

Figure 4

The ribosome-associated quality control complex (RQC). engaged in nascent chain ubiquitylation. (a) Elongating 80S ribosome. (b) Model of ubiquitylation mediated by Ltn1/RQC. based on cryo-EM structures. Dom34-mediated rescue dissociates stalled ribosomes without hydrolyzing the associated peptidyl-tRNA. The tRNA moiety that becomes exposed on the intersubunit surface of the 60S subunit is directly recognized by the RQC. subunit Rqc2/Tae2 (NEMF in mammals), which also functions in stabilizing Ltn1 binding via interaction with Ltn1's conserved N-terminal domain. In turn, the RING domain at the Ltn1 C terminus is properly positioned to directly ubiquitylate the nascent chain coming out of the exit tunnel. Ltn1 does not target nascent chains in normally elongating ribosomes, as steric clashes would prevent it from binding simultaneously with a 60S subunit (see panel a). The ubiquitin signal, along with Rqc1 (which is not visible in the available RQC structures), serves to recruit Cdc48 (VCP in mammals), which extracts and delivers the nascent chain to the proteasome (not represented). 1 Joazeiro, C. A. 2017

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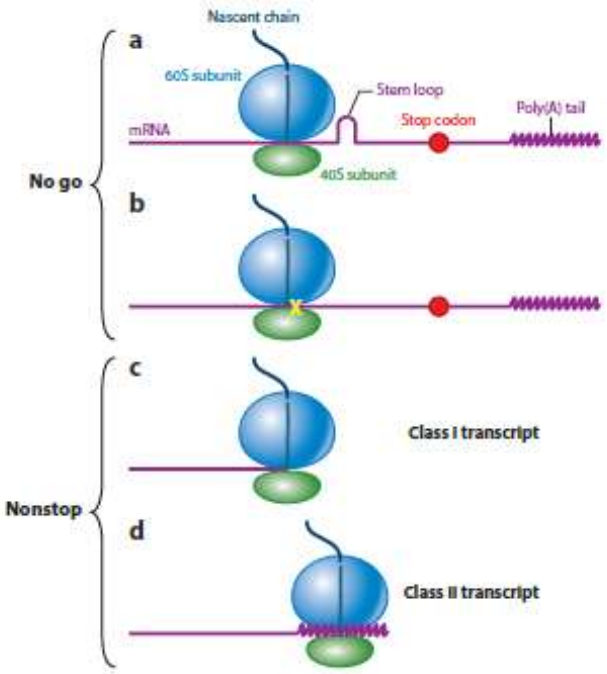


Figure 1

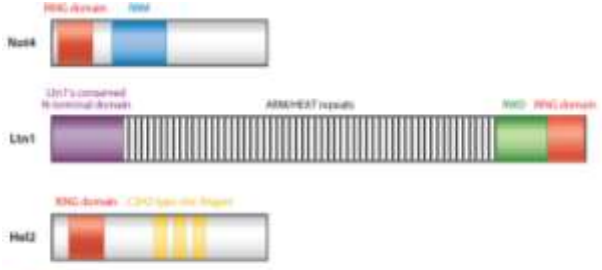
Ribosomes can stall during translation elongation for diverse reasons, including (*a*) inhibitory stem-loop structure, (*b*) suboptimal decoding, (*c*) mRNA truncated within coding sequences (class I nonstop mRNA), and (*d*) mRNA lacking stop codons due to premature cleavage and polyadenylation within coding sequences [class II nonstop mRNA; in class II nonstop mRNA (panel *d*), ribosomes eventually reach and translate the poly(A) tail (*scribble line*)]. Among the several known responses to ribosome stalling are repression of translation initiation, endonucleolytic mRNA cleavage, ribosomal subunit dissociation (ribosomal rescue), activation of the no-go mRNA decay pathway (panels *a* and *b*) or the nonstop mRNA decay pathway (panels *c* and *d*), and ribosome-associated quality control complex–mediated degradation of the encoded nascent polypeptide. 2 Joazeiro, C. A. 2017

Class 2 nonstop mRNA could be generated by errors in gene expression. Class 1 & Class 2 nonstop mRNAs will produce through stalled ribosomes. Ribosome associate protein quality control pathway elicits when a paused ribosome is recognized as in aberrant or, undesirable state.

Poly Adenylated tail & Translation Elongation

Class 2 nonstop transcript can be produced by premature cleavage and polyadenylation at the coding region of mRNA (Frischmeyer et al., 2002). Poly (A) tail translation, causes ribosome stall and starts NSD & RQC. In fungi, stalling on poly (A) is mediated by encoded poly lysine nascent peptide. It was proposed that the negative electrostatic potential of the ribosome exit tunnel cause translation of polybasic sequences, while they are still in the tunnel.

In mammalian cells, ribosomes can stall upon Poly (A) translation; 12 to 20 AAA codons are required to make an effect when fused to the reporter gene. Human poly (A) tails are ranges from 150-250 nucleotide long, so stalling will occur on poly (A) tail mostly. Evidence found that stalling is mostly less efficient with lysine AAG codons. In summary, the subjects remain controversial, mRNA sequence of poly (A) and encoded polylysine may contribute to ribosome stalling, with the contribution of organisms or, cell type (Joazeiro, C. A. 2017).



3 Joazeiro, C. A. 2017

Ribosome Footprinting & Detecting Pauses and Stalls:

Ribosome stalling is needed for the substrates of RQC, obtain from invitro translation or, reporter genes. Ribosome profiling is a complex process of footprinting, which includes steps like isolating the translating ribosomes and identifying deep sequencing, the m RNA fragments must be protected from invitro RNAase digestion. This will provide a map at nucleotide resolution. Ribosome profiling also provides information about ribosome pausing or, stalling, by the accumulation of deep sequencing read at a specific region relative to the transcript's background read levels. We studied strong pauses in translational elongation in mouse to

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yeast cells reported by ribosome profiling. Pause in proline amino acid codon is most represented. Cleavage in m RNA 3' end ribosome stalling generated by stochastic endonuclease cleavage is difficult to document, 3' end of unligated, cleaved exons of the HAC1 m RNA is successfully detected (Guydish & Green 2014).

Ribosome profiling also supports the role of polybasic peptides installed translation in yeast, by 3 basic residues sufficient for detectable pause.

Mapping reads poly (A) tail obtain from profiling experiment to specific transcript is hampered by low complexity sequence, indicating evidence of poly (A) tail stalling.

Gene studies suggest that rare codons activate NGD. The effect documented with reporter genes could have overexpression, causing t-RNA to become limiting (Joazeiro, C. A. 2017).

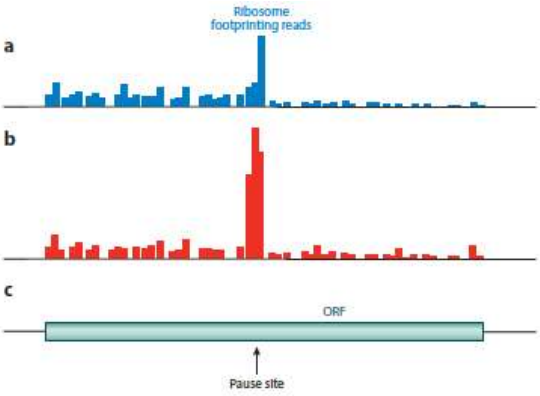
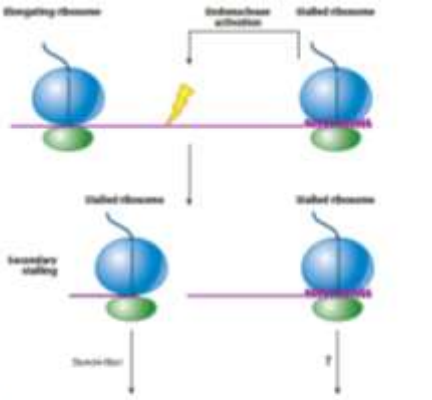


Figure 5
Mapping endogenous pause sites with ribosome profiling. The ribosome profiling approach generates sequencing reads (*vertical blue and red bars* in panels *a* and *b*, respectively) corresponding to mRNA fragments that are protected from RNase digestion due to ribosome binding. Reads can be mapped to the corresponding exon or open reading frame (ORF) (panel *c*). Pause sites are characterized by a greater number of reads at specific positions (*arrow*) relative to the transcript background read levels. With strong pauses or stalls, a reduction in footprint signal can also be observed 3′ of the pause site (panels *a* and *b*). Ribosome occupancy is expected to increase at pause or stall sites that are targeted for ribosome-associated quality control in cells that are defective for the relevant ribosomal rescue factor (panel *b*) relative to control cells (panel *a*). 4 Joazeiro, C. A. 2017

Hbs1 and Dom 34 target 3' m RNA Stalled Ribosome:

Studies analyze that both in yeast and mammalian homologs of Hbs1 and Dam 34 can target and rescue stalled ribosomes at or, 3'end of m RNA. Hbs1 & Dom 34 acts on ribosomes stalled at 3' m RNA end, and cryo-EM work has provided evidence for mechanisms: Dom 34 binding involves both empty ribosomal A site and empty ribosomal tunnel where Dom 34 beta loop is inserted. Further supporting the case for Dom 34-Hbs1 act on ribosome stalled at m RNA 3' end, the Hbs1 N- terminal such that it clashes the m RNA.

Ribosome profiling also reflects the data that ribosomes stalled at 3' end can be rescued in a Dam 34 dependent manner. Dom 34 is required for the nascent chain associated with the 3' end stall ribosome to be targeted by Ltn 1 (Matsudal et al., 2014).



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Mutation in Gtpbp 2, causes one of the two mammalian Hbs 1 homologs to lead to failure to resolve to stall. Gtpbp 2 binds to Pelota (the Dom 34 homolog).

Gtpbp 2 in mammals, there are reported examples in yeast in which Dom 34 deletion appeared to stabilize nascent chain encoded by class 2 nonstop reporter genes.

Like Gtpbp2 mutation, Dom 34 deletion fails to rescue the endogenous pause identified in Ribosome Profiling Study. So far genetic redundancy or, for other reasons, little evidence found that Dom 34 / Pelota generally acts on stalled ribosomes at internal m RNA positions in vivo (Joazeiro, C. A. 2017).

RQC factor Hel 2 / Znf598 stimulates ribosome rescue:

Hel 2 was first discovered in RQC by genetic screening in yeast (Brandman et al., 2012). Yeast cells lacking Hel 2 were collected and screened based on the full-length reporter, we have found a distinct phenotype from LTN 1 deficient cells. Further studies revealed in Hel 2 deficient cells, simultaneous deletion of Ltn 1 fails to rescue the expression of products (Bandman et al., 2012). This study suggests that in absence of Hel 2, ribosomes continue translating and passing stalling signals, and also this Hel 2 effect is found in arginine CGA-mediated stalling.

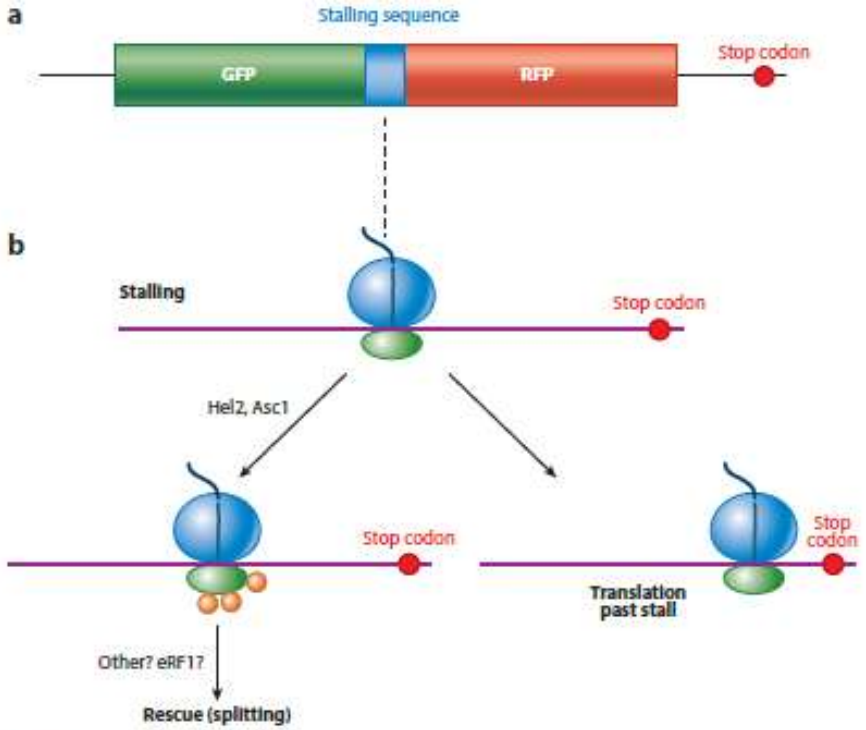

Figure 7

Hel2 (Znf598 in mammals) and Asc1 (RACK1 in mammals) may sense stalled ribosomes and stimulate rescue downstream. (*a*) A reporter gene with GFP (*green*) and RFP (*red*) coding sequences and a stalling signal in between (*blue*). (*b*) A ribosome translating the reporter and stalling in an internal mRNA position before reaching the RFP coding sequence. In wild-type cells (*left arrow*), the Hel2 E3 ligase binds to stalled ribosomes and mediates ubiquitylation of ribosomal proteins in the small (40S) subunit. The Asc1 protein, together with an unidentified E3 ligase, mediates ubiquitylation of a partially overlapping set of targets. 40S subunit ubiquitylation in turn promotes ribosomal rescue. Defects in Hel2 or Asc1 function (*right arrow*) result in the failure to trigger rescue, allowing a greater fraction of stalled ribosomes to proceed with translation past the stalling signal.

In the RQC pathway, Hel 2 and Znf598 are found in the 80s and polysome fractions of the sucrose gradient. There is a total of 2000 Hel 2 pre-haploid yeast cells, whereas there are 100 folds excess translating ribosomes, when Met is the signal, the Hel 2 can not pre associated with the ribosome to mediate stalling. Experiments of ribosome foot-printing confirm that Hel 2 was not required for the ribosome to stall when translating the GIP-Arg 12- RFP receptor (Joazeiro, C. A. 2017).

Defective Rescue of Ribosome Pause and Neurodegeneration:

Listening mutation causes GTPBP2 mutation, which causes neurodegeneration in mice (Ischimura et al., 2014). For listening & GTPBP2 mutant mice, neurodegeneration is frequent. In lister ENU mice, the neuronal loss has been observed primarily in the spinal cord and in Gtpbp2 mutant mice cortex, retina and cerebellum make disorders.

Neurodegeneration caused by Gtpbp2 mutation requires a respected mutation in n-Tr20. Due to Gtpbp2

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& n-Tr20 mutation, ribosomal spelling increases in arginine AGA (Joazeiro, C. A. 2017).

Ribosome Associated Protein Quality Control Mechanism:

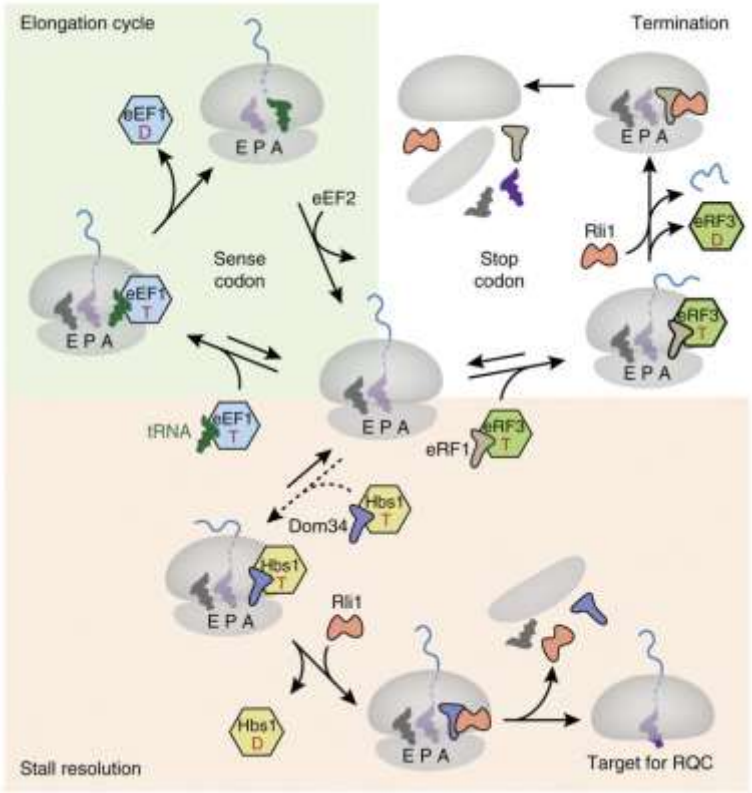
Recognition of Stalled Ribosome: -

Stalling can occur due to various reasons, conformation of the nascent chain–ribosome–mRNA complex can differ in key ways. It involves the presence or absence of mRNA in the ribosomal aminoacyl (A) site, the codon identity of the A site, and the conformational state of the ribosome. It is unclear whether each of these states is recognized and split by a unified mechanism or whether different states require specific factors.

An important advance toward dissection of the RQC pathway was the serendipitous observation of polyubiquitinated nascent chains on stalled ribosomes produced by in vitro translation in reticulocyte lysates47. The protein translocation field had long used the trick of translating a truncated mRNA to produce stalled ribosome–nascent chain complexes48. This permits the generation of defined-length translation complexes, depending on the point of truncation, to trap putative intermediates in a cotranslational process such as protein translocation into an organelle. The realization that ubiquitination of a small proportion of these nascent chains might represent a physiologically relevant degradation pathway suggested an experimentally tractable route to its mechanistic study.

Present days, the best-studied system that mediates preferential splitting of stalled ribosomes is the Hbs1–Dom34–Rli1 pathway, and its best-characterized target is a ribosome stalled at the 3′ end of an aberrant mRNA. Hbs1 is a member of the translational GTPase family, whose members bind at the GTPase center near the A site of the ribosome. The family includes eukaryotic elongation factor 1 (eEF1A), which delivers tRNAs to the ribosome, and eRF3, which delivers eRF1 to the ribosome for termination; another member, eEF2, mediates ribosome translocation. These proteins, either alone (eEF2) or in complex with a partner (eEF1A with tRNA; eRF3 with eRF1; and Hbs1 with Dom34) bind in a GTP-dependent manner to distinct states of the ribosome: eEF2 probably prefers the hybrid state of the ribosome, whereas the other complexes favor the nonrotated state. In addition, the binding partners (tRNA and eRF1) impart further specificity based on the mRNA codon in the A site. Thus, the translational GTPases can be conceptualized as monitoring the state of the ribosome and initiating the appropriate downstream reaction (Brandman & Hegde, 2016).

The situation in which the A-site codon has a suitable aminoacyl-tRNA is less clear now. It will occur with stalling at a polybasic coding sequence, such as the poly(A) tail. So the competition with aminoacyl-tRNA–eEF1A does occur in these situations, but elongation is not possible, owing to unfavorable architecture around the peptidyl transferase center, as has been observed in other cases of peptide-induced stalling. In this case, Dom34–Hbs1 would eventually act, albeit more slowly.



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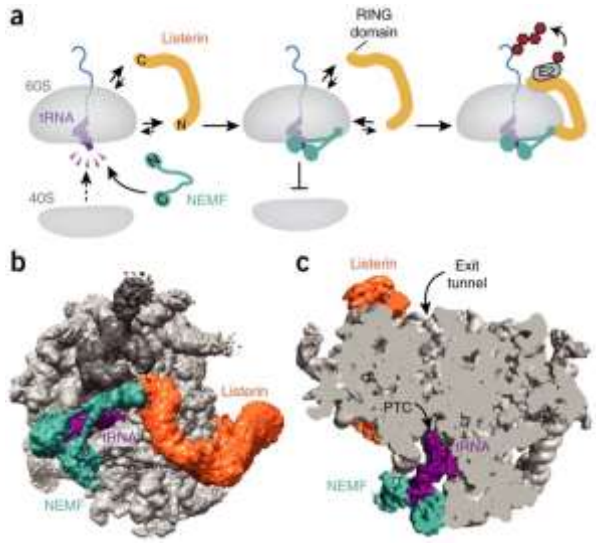
Assembly and Structure of Ubiquitination Complex:

To remove the small subunit from a nascent chain–80S complex is the exposure of the intersubunit interface of the nascent chain–60S complex. A short nascent chain, can drop off and leave behind an empty 60S, that presumably can re-enter the translation cycle. Longer nascent chains would be trapped, thereby exposing the attached peptidyl (P)-site tRNA at the interface side of the 60S. It has therefore been speculated that the interface and/or tRNA might provide the cue for Ltn1 recruitment, thereby explaining why Ltn1 is seen only on 60S complexes and does not promiscuously target translating ribosomes. Indeed, the initial low-resolution structure of a reconstituted nascent chain–60S–Listerin complex has shown that Listerin's position substantially clashes with the 40S (Shao et al., 2015).

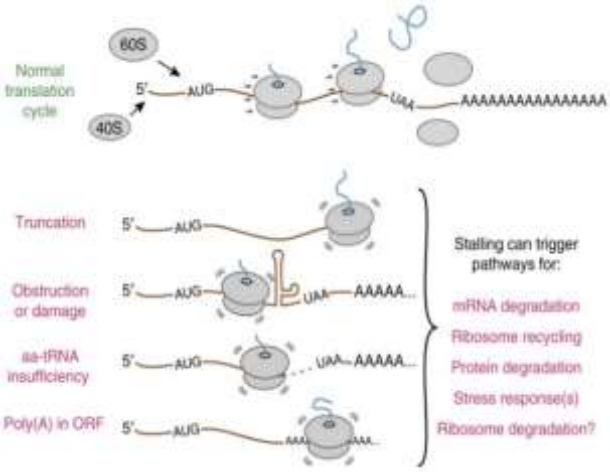
The structures of the yeast and mammalian 60S–RQC by cryo-EM have not only corroborated these biochemical conclusions but also defined the binding sites and locations of these factors. To isolate the yeast 60S–RQC, the complex was assembled *in vivo* and stabilized by use of a strain in which the RING ligase domain of Ltn1 is deleted (thereby precluding progression beyond the step of RQC assembly; the complex was then affinity isolated via an epitope-tagged Rqc1 or Ltn1. The mammalian complex was instead assembled *in vitro* by incubation of a purified stalled 80S ribosome–nascent chain complex with recombinant splitting factors (Pelota, Hbs1 and ABCE1), NEMF, and Listerine. Thus, both the yeast and mammalian complexes represent the step immediately preceding nascent-chain ubiquitination (Brandman & Hegde, 2016).



CAT Tail and Introduction of Stress Response:

Stress responses, facilitate the restoration of homeostasis. Prominent examples include the unfolded-protein responses of the endoplasmic reticulum and mitochondria and the heat-shock response in the cytosol. Perhaps similarly, disruption of Ltn1 or Rqc1 induces activation of heat-shock factor 1 (Hsf1) in yeast; however, disruption of Rqc2 not only fails to induce Hsf1 but also abrogates the response induced by deleting Ltn1 or Rqc1. Although the RQC-dependent mechanism of induction of Hsf1 remains unknown, the observation of an A-site tRNA bound to Rqc2 in the cryo-EM structure has led to new insights that might hold the key to understanding this stress response.

Stress and translational stalling may be related in the reverse direction also. Ribosome profiling experiments show that heat shock can lead to pervasive translation stalling ~60 codons from the initiation site. This position is noteworthy because it is the point at which chaperones are likely to first engage the nascent polypeptide. Thus, some mechanisms may be there by which chaperone availability is communicated to the translation apparatus to reduce synthesis. Similarly, the stress in the endoplasmic reticulum has recently been observed to trigger regulatory ubiquitination on the ribosome, perhaps to modulate translation. Whether or how these stress-triggered effects on translation initiate mRNA or polypeptide degradation remains to be studied in-depth, but the observations highlight the intimate and emerging relationships between translation elongation and protein homeostasis pathways (Brandman & Hegde, 2016).



Nascent Chain Extraction and Degradation:

Genetic studies suggest that the Cdc48 complex is required for nascent-chain extraction. Because neither Rqc1 nor Cdc48 is needed for nascent-chain ubiquitination *in vitro*, both appear to act after Ltn1. This scenario is supported by the dependence of Cdc48 recruitment on both Ltn-mediated polyubiquitination and Rqcl. Because Rqc1 is not needed for Ltn1-mediated ubiquitination, the current model posits that the Cdc48 complex is recruited by a bivalent interaction with Rqc1 and polyubiquitin, after which it uses its ATP-powered activity to drive downstream steps. The established 'separase' activity of Cdc48 may apply force to the nascent chain and or, RQC and hence facilitate extraction of the ubiquitinated substrate from the 60S ribosome.

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When the ester bond between the tRNA and nascent chain is broken, there may be relatively little impedance to polypeptide release. Thus, hydrolysis of this bond is likely to be the key step in freeing both the nascent chain and the t RNA. One model suggests that Cdc48 pulls the nascent chain, via its polyubiquitin, from the mouth of the exit tunnel. This might reposition the ester bond slightly to a location that is more favorable for its hydrolysis. Perhaps the ability of Rqc2 to bind tRNA in the A site allows uncharged tRNA or eRF1 to somehow act in this hydrolysis reaction. It is noteworthy that eRF1 has the approximate shape of a tRNA and could conceivably interact with Rqc2 or NEMF in the same way as alanyl- or threonyl-tRNA. The main advantage of coupling tRNA hydrolysis with Cdc48 activity is that the released nascent chain can be delivered promptly to the proteasome by Cdc48 (Brandman & Hegde, 2016).

In the cell, stalled ribosomes are rescued through ribosome-associated protein quality-control (RQC)pathways. After splitting of the stalled ribosome, a C-terminal polyalanine 'tail' is added to the unfinished polypeptide attached to the tRNA on the 50S ribosomal subunit. In Bacillus subtilis, polyalanine tailing is catalyzed by the NEMF family protein RqcH, in cooperation with RqcP. However, the mechanistic details of this process remain unclear. Here we demonstrate that RqcH is responsible for tRNAAla selection during RQC elongation, whereas RqcP lacks any tRNA specificity. The ribosomal protein uL11 is crucial for RqcH, but not RqcP, recruitment to the 50S subunit, and B. subtilis lacking uL11 are RQC deficient. Through mutational mapping, we identify critical residues within RqcH and RqcP that are important for interaction with the P-site tRNA and the 50S subunit (Brandman & Hegde, 2016).

II. DISCUSSION

Two important findings in that study were, Ltn 1 exists in predominantly 60s ribosome subunit bound form, and the protein not only accumulates but also stably attaches to the ribosome in Ltn 1 deficient extracts. Ltd 1 targets proteins on the ribosome, before releasing them in the cytosol, analogous to that of the bacterial tmRNA system, this explains the translation couple protein quality control which is known as RQC.

Dom 34 mediates rescue of ribosomal stalled subunits 80s and remaining 60s subunits along with peptidyl t RNA could remain jammed. This, in turn, enlightens the finding that Ltn 1 is associated with the 60s predominantly and links between ribosome dissociation and Ltn 1 binding.

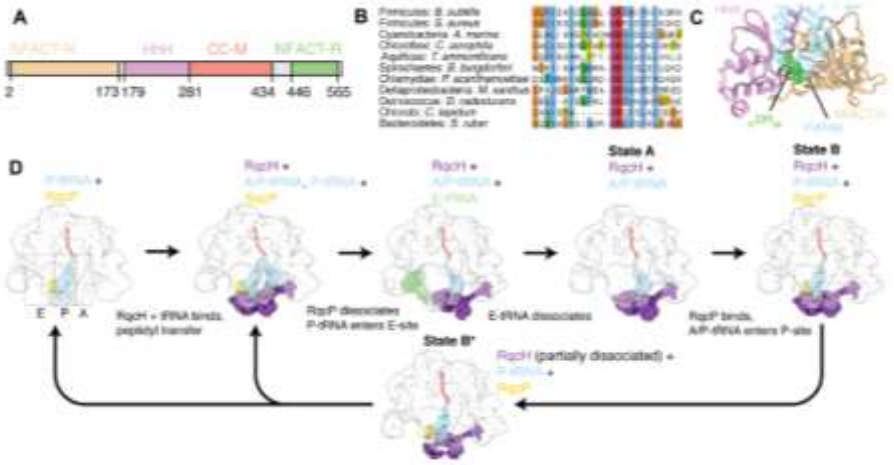

Figure 1. Bacterial ribosome-associated RQC is mediated by RqcH and RqcP. (A) RqcH domain composition. Amino acid positions are numbered as per *B. subtilis* RqcH. (B) Sequence alignment of RqcH homologs from diverse bacteria. (C) Interaction between RqcH (HhH domain in lilac, NFACT-N domain in tan, α1DR$_{66}$ motif in green) and P-tRNA (transparent cyan) in state B (PDB 7ASA (42)). (D) Overview of proposed RQC elongation cycle mediated by RqcH and RqcP (42). Beginning with a 50S with trapped P-tRNA, binding and dissociation cycles of RqcH and RqcP, accompanied by movement of tRNAs through the A-, P- and E-sites, results in mRNA- and 30S-independent elongation.

III. CONCLUSION

In less than a decade after the discovery, we have learned a great deal about the RQC pathway. Among the pathway's remarkable features is the strong degree of conservation of its components among eukaryotes, encompassing the early steps following ribosome stalling orchestrated by Asc1/RACK1 and Hel2/Znf592, the rescue of stalled ribosomes by Dom34/Pelota and Hbs1/Hbs1L/Gtpbp2 (and perhaps eRF1–eRF3), and the degradation of associated mRNA and nascent chains by the exosome and RQC, respectively. However, much has yet to be learned about RQC mechanisms. About early events upstream of the RQC, the picture that now emerges is that there appear to be alternative mechanisms to sense and rescue stalled ribosomes, consistent with the known structural diversity among the latter. Several future directions for research in the RQC pathway, particularly regarding these early steps, have been pointed out above.

The folding of nascent polypeptide sequences outside the ribosomal exit tunnel can affect ribosomal elongation rates under both normal and stress conditions (Gloge et al. 2014), a future direction of interest will be to examine whether defective folding of nascent chains can serve as yet another source of RQC substrates. RQC field should aim to continue generating much-needed mechanistic and structural information and to further integrate the acquired knowledge with the information generated by ribosome footprinting and proteomic analyses in physiologically relevant settings. An expected product of the work is that one should ultimately be able to enumerate sources of RQC substrates and specify the relative prevalences of such substrates. RQC pathway may provide a better understanding of RQC biology and may serve as the basis to explain how neurodegeneration in response to RQC pathway mutations may take place at the molecular level (Joazeiro, C. A. 2017).

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