Department of Basic Medical Sciences

Division of RNA and Gene Regulation
RNA 制御学分野

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Quality control of translation eliminates aberrant proteins and maintains protein homeostasis and normal cell function. Improving the accuracy of translation and preventing the production of abnormal proteins is a practical approach for suppressing a series of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. We analyzed the molecular mechanism of quality control mechanisms that suppress abnormal proteins and clarified the molecular basis of drug discovery. We propose that the increase in translation accuracy and enhancement of translation quality control mechanisms are possible strategies to prevent abnormal protein production and prolong healthy life expectancy.

1. Quality control for translation abnormalities Molecular mechanism of RQC and NGD

Ribosome-associated Quality Control (RQC) is responsible for monitoring aberrant translation and for decomposing and removing abnormal proteins during their synthesis. RQC plays a crucial role in maintaining protein homeostasis at an early stage. We have previously reported a molecular mechanism that recognizes and dissociates stagnant ribosomes during translation elongation, which is an early stage of RQC. In recent years, we have identified the E3 ubiquitin ligase Hel2 and its mammalian homolog ZNF598 as essential for RQC. Additionally, we discovered a novel RQT complex involved in the dissociation of ubiquitinated ribosomes into subunits. Our work, along with that of the Hegde lab, has shown that the E3 ubiquitin ligase can recognize collided ribosomes (Disomes/Trisomes) and their specific structural features. We have previously reconstituted the ubiquitination of uS10 by Hel2 and identified an RQT complex that specifically dissociates ubiquitinated ribosomes into subunits, achieving in vitro reconstitution of this reaction.

In yeast, RQT complex components Cue3 and Rqt4 interact with K63-linked ubiquitin chains, facilitating the recruitment of the RQT complex to the ubiquitinated colliding ribosomes. The CUE domain of Cue3 and the N-terminal domain of Rqt4 bind independently to the K63-linked ubiquitin chain, and their deletion abolishes ribosomal dissociation mediated by the RQT complex. High-speed atomic force microscopy (HS-AFM) has revealed that the intrinsically disordered regions of Rqt4 allow for an expanded searchable area for interaction with the ubiquitin chain. These findings provide mechanistic insights into how the ubiquitin code is decoded for the clearance of colliding ribosomes by the RQT complex.

In mammals, uS10 is polyubiquitinated, while eS10 is preferentially mono-ubiquitinated by ZNF598. We characterized the ubiquitination activity of ZNF598 and its importance in human RQT-mediated subunit dissociation, using endogenous XBP1u and poly(A) translation stallers. Cryo-electron microscopy (Cryo-EM) analysis of human-collided disomes revealed a distinct composite interface with substantial differences from those of yeast collided disomes. Biochemical analysis showed that ZNF598 forms K63-linked polyubiquitin chains on uS10, which are crucial for initiating mammalian RQC. The human RQT

(hRQT) complex, consisting only of ASCC3, ASCC2, and TRIP4, dissociates collided ribosomes depending on the ATPase activity of ASCC3 and the ubiquitin-binding capacity of ASCC2. For the hRQT to mediate subunit dissociation, K63-linked polyubiquitination of uS10 is required; however, monoubiquitination of eS10 or uS10 alone is insufficient. Thus, we conclude that ZNF598 functionally marks collided mammalian ribosomes through K63-linked polyubiquitination of uS10, allowing for the trimeric hRQT complex to mediate subunit dissociation.

The collision of ribosomes also triggers No-Go Decay (NGD) quality controls in conjunction with RQC, which leads to endonucleolytic cleavage of mRNA in the collided ribosome. We reported two NGD pathways: one involving mRNA cleavage coupled to the dissociation of collided ribosomes in RQC (NGDRQC-) and another independent of RQC occurring near collided ribosomes (NGDRQC+). The ubiquitin-binding activity of Cue2 is necessary for NGDRQC-, but not for NGDRQC+. This activity involves the first two N-terminal Cue domains, while Trp122 of Cue2 is critical for NGDRQC+. Additionally, the colliding ribosome association factor Mbf1 and its interaction with uS3 are vital for NGDRQC+ via the SDD1-staller. We propose that for Cue2-dependent cleavage upstream of collided ribosomes (NGDRQC-), polyubiquitination of eS7a is recognized by the two N-terminal Cue domains of Cue2. Conversely, for cleavage within collided ribosomes (NGDRQC+), the UBA domain, Trp122, and the interaction between Mbf1 and uS3 are critical.

NEMF (the homolog of Rqc2 in yeast) interacts with 60S ribosome-nascent chain complexes (RNCs) and recruits Ltn1/Listerin, which ubiquitinates peptidyl-tRNA on dissociated 60S subunits. Within the 60S subunit, Rqc2 catalyzes the C-terminal extension of stalled tRNA-bound peptides with alanine and threonine residues (CAT tails) through a non-canonical, mRNA-independent elongation reaction. CAT tailing enables the degradation of substrates lacking an Ltn1p-accessible ubiquitination site by exposing a lysine residue that is typically sequestered in the ribosome exit tunnel. In the context of nascent chain degradation in budding yeast, CAT tailing acts as a fail-safe mechanism that broadens the range of substrates degradable by RQC. However, the physiological functions of CAT tailing remain elusive. We recently discovered that failure to degrade CAT-tailed proteins disrupts neuronal morphogenesis and cell survival. In mammals, NEMF modifies the translation products of nonstop mRNAs, which are a major type of erroneous mRNA, by adding a C-terminal tail composed mainly of alanine and several other amino acids.

1.1. Mechanisms of Translation-coupled Quality Control.

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The collision sensor Hel2 specifically recognizes colliding ribosomes and ubiquitinates the ribosomal protein uS10. This process leads to the noncanonical dissociation of ribosomal subunits through the ribosome-associated quality control trigger (RQT) complex. While the ubiquitination of uS10 is essential for rescuing stalled ribosomes, its exact function and recognition mechanisms are not fully understood. In this study, we demonstrated that the RQT complex components Cue3 and Rqt4 interact with the K63-linked ubiquitin chain, which accelerates the recruitment of the RQT complex to the ubiquitinated colliding ribosome. The CUE domain of Cue3 and the N-terminal domain of Rqt4 independently bind to the K63-linked ubiquitin chain. Deleting these domains abolished the ribosomal dissociation mediated by the RQT complex. High-speed atomic force microscopy (HS-AFM) revealed that the intrinsically disordered regions of Rqt4 enhance the interaction area with the ubiquitin chain. These findings offer mechanistic insight into how the ubiquitin code is decoded for the clearance of colliding ribosomes by the RQT complex.

Translation of aberrant messenger RNAs can cause ribosomal stalling, which results in ribosomal collisions. Collided ribosomes are specifically recognized to initiate stress responses and quality control pathways. Ribosome-associated quality control facilitates the degradation of incomplete translation products and requires the dissociation of stalled ribosomes. A central event in this process is the splitting of collided ribosomes by the ribosome quality control trigger complex (RQT), though the mechanism remains unclear. We show that RQT requires accessible mRNA and the presence of a neighboring ribosome. Cryogenic electron microscopy imaging of RQT-ribosome complexes reveals that RQT engages the 40S subunit of the leading ribosome and can switch between two conformations. We propose that the Ski2-like helicase 1 (Slh1) subunit of RQT applies a pulling force on the mRNA, leading to destabilizing conformational changes in the small ribosomal subunit, ultimately resulting in subunit dissociation. Our findings provide a conceptual framework for a helicase-driven mechanism of ribosomal splitting.

Ribosome-associated quality control (RQC) is a conserved process that degrades potentially toxic truncated nascent peptides. Malfunctions in this process contribute to neurodegeneration and proteostasis decline in aging. During RQC, the dissociation of stalled ribosomes is followed by the elongation of the nascent peptide with alanine and threonine residues, driven by Rqc2 independently of mRNA, the small

ribosomal subunit, and guanosine triphosphate (GTP)-hydrolyzing factors. The resulting carboxy-terminal (CAT) tails and subsequent ubiquitination by Ltn1 mark nascent peptides for proteasomal degradation. In this study, we present ten cryogenic electron microscopy (cryo-EM) structures that reveal the mechanistic basis for the individual steps of the CAT tailing cycle, including initiation, decoding, peptidyl transfer, and tRNA translocation. We identified eIF5A as a crucial eukaryotic RQC factor that facilitates peptidyl transfer. Additionally, we observed the dynamic behavior of RQC factors and tRNAs, which allows the CAT tailing cycle to occur without additional energy input. Together, these results elucidate key differences and common principles between CAT tailing and canonical translation.

1.2. Multiprotein bridging factor 1 is required for robust activation of the integrated stress response on collided ribosomes

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In yeast multiprotein bridging factor 1 (Mbf1) has been proposed to function in the integrated stress response (ISR) as a transcriptional coactivator by mediating a direct interaction between general transcription machinery and the process's key effector Gcn4. However mounting evidence has demonstrated that Mbf1 (and its human homolog EDF1) is recruited to collided ribosomes a known activator of the ISR. In this study we connect these otherwise seemingly disparate functions of Mbf1. Our biochemical and structural analyses reveal that Mbf1 functions as a core ISR factor by interacting with collided ribosomes to mediate Gcn2 activation. We further show that Mbf1 serves no role as a transcriptional coactivator of Gcn4. Instead Mbf1 is required for optimal stress-induced eukaryotic initiation factor 2α (eIF2α) phosphorylation and downstream de-repression of GCN4 translation. Collectively our data establish that Mbf1 functions in ISR signaling by acting as a direct sensor of stress-induced ribosome collisions.

1.3. The UFM1 system: Working principles cellular functions and pathophysiology

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Ubiquitin-fold modifier 1 (UFM1) is a ubiquitin-like protein covalently conjugated with intracellular proteins through UFMylation, a process similar to ubiquitylation. Growing lines of evidence regarding not only the structural basis of the components essential for UFMylation but also their biological properties shed light on crucial roles of the UFM1 system in the endoplasmic reticulum (ER), such as ER-phagy and ribosome-associated quality control at the ER, although there are some functions unrelated to the ER. Mouse genetics studies also revealed the indispensable roles of this system in hematopoiesis, liver development, neurogenesis, and chondrogenesis. Of critical importance, mutations of genes encoding core components of the UFM1 system in humans cause hereditary developmental epileptic encephalopathy and Schohat-type osteochondrodysplasia of the epiphysis. Here, we provide a multidisciplinary review of our current understanding of the mechanisms and cellular functions of the UFM1 system as well as its pathophysiological roles and discuss issues that require resolution.

2. Molecular mechanism of quality control NRD for deficient ribosomes.

The ribosome is the central machinery for protein synthesis and is responsible for accurate codon recognition and highly efficient peptide-bond formation. Ribosomes interact with various factors to perform essential functions in gene expression. Since abnormal ribosomes generated during the synthesis cause various expression abnormalities, cells have a quality control mechanism Nonfunctional Ribosomal RNA Decay (NRD) recognizes and eliminates functionally defective ribosomes. We recently analyzed the quality control of ribosomes deficient in function due to base substitution mutations conserved in all species, which are essential for accurate codon recognition in 18S

rRNA and ubiquitin at the K212 residue of ribosomal protein uS3 in yeast. We identified E3 ubiquitin ligases that are both essential and involved. We identified Fap1 as a stalling sensor that triggers 18S nonfunctional rRNA decay via polyubiquitination of uS3. Ribosome profiling revealed the enrichment of Fap1 at the translation initiation site and an association with elongating individual ribosomes. Cryo-EM structures of Fap1-bound ribosomes revealed that Fap1 probes the mRNA simultaneously at both the entry and exit channels, suggesting an mRNA stasis sensing activity and Fap1 sterically hinders the formation of canonical collided di-ribosomes. Our findings indicate that individual stalled ribosomes are the potential signal for ribosome dysfunction, leading to accelerated turnover of the ribosome itself. It was also revealed that the ubiquitinated stagnant 80S ribosome was dissociated into its subunits by Slh1, and then the abnormal 40S was degraded.

3. The Role of Ribosomal Dynamic Modification in Stress Response

The synthesis and modification of secretory proteins in the endoplasmic reticulum (ER) are essential functions for cells. When abnormal proteins accumulate in the ER, they can be harmful, prompting the cell to activate the unfolded protein response (UPR) pathway. In *Saccharomyces cerevisiae* (yeast), the membrane protein Ire1 is activated by ER stress, leading to the splicing of precursor mRNA for the transcription factor Hac1. The resultant Hac1 protein then induces the transcription of chaperones, which assist in protein folding. In higher eukaryotes, the protein PARK phosphorylates eIF2α, resulting in the suppression of global protein translation initiation. Our investigation into the physiological role of ribosomal ubiquitination revealed a novel translational regulator involved in the ER stress response. We identified a new mechanism of translational control during ER stress in *S. cerevisiae* and established that the ubiquitination of the ribosomal protein eS7, carried out by the E3 ubiquitin ligase Not4, is essential for this process.

Publication list

1. Inada, T.* and Beckmann, R.*Mechanisms of Translation-coupled Quality Control.
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2. Kim, K., Li, J., Urs, A. N., Pacheco, M.E., Lasehinde, V., Denk, T., Tesina, P., Tomomatsu, S., Matsuo, Y., McDonald E., Beckmann, R., Inada, T., Green, R., Zaher, H.Multiprotein bridging factor 1 is required for robust activation of the integrated stress response on collided ribosomes.
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3. Komatsu, K., Inada, T. and Noda, N. The UFM1 system: Working principles cellular functions and pathophysiology
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