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. Ribosome-associated Quality Control (RQC) for translation abnormalities

Ribosome-associated Quality Control (RQC) monitors aberrant translation and decomposes and removes abnormal proteins during synthesis. RQC plays an important role in maintaining protein homeostasis at a very early stage. We have previously reported a molecular mechanism that recognizes and dissociates stagnant ribosomes during translation elongation, the early stage of RQC. In the last several years, we have reported that the E3 ubiquitin ligase Hel2 and its mammalian homolog ZNF598 are required for RQC, and that the novel RQT complex is involved in the dissociation of ubiquitinated ribosomes into subunits. We and Hegde lab have reported that E3 ubiquitin ligase recognizes collided ribosomes (Disome/Trisome) and the specific structure of collided ribosomes. We previously reported that the ubiquitination of uS10 by Hel2 was reconstituted. Next, we identified an RQT complex that specifically dissociated the ubiquitinated ribosomes into subunits and reconstituted the reaction in vitro. In yeast, the RQT complex components Cue3 and Rqt4 interact with the K63-linked ubiquitin chain and accelerate the recruitment of the RQT complex to the ubiquitinated colliding ribosomes. The CUE domain of Cue3 and N-terminal domain of Rqt4 bind independently to the K63-linked ubiquitin chain. Their deletion abolished the ribosomal dissociation mediated by the RQT complex. High-speed atomic force microscopy (HS-AFM) revealed that the intrinsically disordered regions of Rqt4 enabled expansion of the searchable area for interaction with the ubiquitin chain. These findings provide mechanistic insights into decoding the ubiquitin code for the clearance of colliding ribosomes by the RQT complex.

In mammals, uS10 is polyubiquitinated, whereas 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-EM analysis of a human-collided disome revealed a distinct composite interface, with substantial differences that from of yeast collided disomes. Biochemical analysis of collided ribosomes showed that ZNF598 forms K63-linked polyubiquitin chains on uS10, which are crucial for mammalian RQC initiation. The human RQT (hRQT) complex, composed only of ASCC3, ASCC2,

and TRIP4, dissociates collided ribosomes depending on the ATPase activity of ASCC3 and the ubiquitin-binding capacity of ASCC2. The hRQT-mediated subunit dissociation requires K63-linked polyubiquitination of uS10, while monoubiquitination of eS10 or uS10 is not sufficient. Therefore, we conclude that ZNF598 functionally marks collided mammalian ribosomes via K63-linked polyubiquitination of uS10 for trimeric hRQT complex-mediated subunit dissociation.

The collision of ribosomes also induces No-Go Decay (NGD) quality controls in conjugation with RQC and triggers endonucleolytic cleavage of mRNA in the collided ribosome. We also reported two pathways of NGD: mRNA cleavage coupled to the dissociation of the collided ribosome response in RQC and mRNA cleavage independent of RQC in the vicinity of the collided ribosomes. The ubiquitin-binding activity of Cue2 is required for NGDRQC-, but not NGDRQC+, and it involves the first two N-terminal Cue domains. In contrast, Trp122 of Cue2 was crucial for NGDRQC+. Moreover, the colliding ribosome association factor Mbf1 and its interaction with uS3 are crucial for NGDRQC+ via the SDD1-staller. We propose that in Cue2-dependent cleavage upstream of collided ribosomes (NGDRQC-), polyubiquitination of eS7a is recognized by two N-terminal Cue domains of Cue2. In contrast, for cleavage within collided ribosomes (NGDRQC+), the UBA domain, Trp122, and the interaction between Mbf1 and uS3 are critical.

NEMF (Rqc2 in yeast) interacts with 60S RNCs and recruits Ltn1/Listerin, which ubiquitinates peptidyl-tRNA on dissociated 60S subunits. In the 60S subunit, Rqc2 catalyzes the C-terminal extension of stalled tRNA-bound peptides with alanine and threonine residues (CAT-tails) in a non-canonical mRNA-independent elongation reaction. CAT tailing enables the degradation of substrates that lack an Ltn1p-accessible ubiquitination site by exposing a lysine residue that is normally sequestered in the ribosome exit tunnel. In the context of nascent chain degradation in budding yeast, CAT tailing is a fail-safe mechanism that expands the range of RQC-degradable substrates. However, the physiological functions of CAT-tailing remain elusive. We recently found that Failure to Degrade CAT-Tailed Proteins Disrupts Neuronal Morphogenesis and Cell Survival. NEMF, a mammalian RQC2 homolog, modifies the translation products of nonstop mRNAs, which are major erroneous mRNAs in mammals, with a C-terminal tail mainly composed of alanine and several other amino acids. Overproduction of non-stop mRNAs induces NC aggregation and caspase-3-dependent apoptosis and impairs neuronal morphogenesis, which is ameli

1.1. Decoding of the ubiquitin code for clearance of colliding ribosomes by the RQT complex.

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The collision sensor Hel2 specifically recognizes colliding ribosomes and ubiquitinates the ribosomal protein uS10, leading to noncanonical subunit dissociation by the ribosome-associated quality control trigger (RQT) complex. Although uS10 ubiquitination is essential for rescuing stalled ribosomes, its function and recognition steps are not fully understood. Here, we showed that the RQT complex components Cue3 and Rqt4 interacted with the K63-linked ubiquitin chain and accelerated the recruitment of the RQT complex to the ubiquitinated colliding ribosome. The CUE domain of Cue3 and the N-terminal domain of Rqt4 bound independently to the K63-linked ubiquitin chain. Their deletion abolished ribosomal dissociation mediated by the RQT complex. High-speed atomic force microscopy (HS-AFM) reveals that the intrinsically disordered regions of Rqt4 enabled the expansion of the searchable area for interaction with the ubiquitin chain. These findings provide mechanistic insight into the decoding of the ubiquitin code for clearance of colliding ribosomes by the RQT complex.

1.2. Structural basis for clearing of ribosome collisions by the RQT complex.

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Translation of aberrant messenger RNAs can cause stalling of ribosomes resulting 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 dissociation of the stalled ribosomes. A central event is therefore the splitting of collided ribosomes by the ribosome quality control trigger complex, RQT, by an unknown mechanism. Here we show that RQT requires accessible mRNA and the presence of a neighboring ribosome. Cryo-

genic electron microscopy of RQT-ribosome complexes reveals that RQT engages the 40S subunit of the lead 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, causing destabilizing conformational changes of the small ribosomal subunit, ultimately resulting in subunit dissociation. Our findings provide the conceptual framework for a helicase-driven ribosomal splitting mechanism.

1.3. Molecular basis of eIF5A-dependent CAT tailing in eukaryotic ribosome-associated quality control.

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Ribosome-associated quality control (RQC) is a conserved process degrading potentially toxic truncated nascent peptides whose malfunction underlies neurodegeneration and proteostasis decline in aging. During RQC, dissociation of stalled ribosomes is followed by 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 CAT tails (carboxy-terminal tails) and ubiquitination by Ltn1 mark nascent peptides for proteasomal degradation. Here we present ten cryogenic electron microscopy (cryo-EM) structures, revealing the mechanistic basis of individual steps of the CAT tailing cycle covering initiation, decoding, peptidyl transfer, and tRNA translocation. We discovered eIF5A as a crucial eukaryotic RQC factor enabling peptidyl transfer. Moreover, we observed dynamic behavior of RQC factors and tRNAs allowing for the processivity of the CAT tailing cycle without additional energy input. Together, these results elucidate key differences as well as common principles between CAT tailing and canonical translation.

1.4. Molecular basis for recognition and deubiquitination of 40S ribosomes by Otu2.

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In actively translating 80S ribosomes the ribosomal protein eS7 of the 40S subunit is monoubiquitinated by the E3 ligase Not4 and deubiquitinated by Otu2 upon ribosomal subunit recycling. Despite its importance for translation efficiency the exact role and structural basis for this translational reset is poorly understood. Here, structural analysis by cryo-electron microscopy of native and reconstituted Otu2-bound ribosomal complexes reveals that Otu2 engages 40S subunits mainly between ribosome recycling and initiation stages. Otu2 binds to several sites on the intersubunit surface of the 40S that are not occupied by any other 40S-binding factors. This binding mode explains the discrimination against 80S ribosomes via the largely helical N-terminal domain of Otu2 as well as the specificity for mono-ubiquitinated eS7 on 40S. Collectively, this study reveals mechanistic insights into the Otu2-driven deubiquitination steps for translational reset during ribosome recycling/(re)initiation.

1.5. Mechanistic insights into the roles of the UFM1 E3 ligase complex in ufmylation and ribosome-associated protein quality control.

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Ubiquitin-fold modifier 1 (UFM1) is a ubiquitin-like protein covalently conjugated with intracellular proteins through ufmylation, similar to ubiquitylation. Ufmylation is involved in processes such as endoplasmic reticulum (ER)-associated protein degradation, ribosome-associated protein quality control

(RQC) at the ER (ER-RQC), and ER-phagy. However, it remains unclear how ufmylation regulates such distinct ER-related functions. Here, we provide insights into the mechanism of the UFM1 E3 complex in not only ufmylation but also ER-RQC. The E3 complex consisting of UFL1 and UFBP1 interacted with UFC1, UFM1 E2, and, subsequently, CDK5RAP3, an adaptor for ufmylation of ribosomal subunit RPL26. Upon disome formation, the E3 complex associated with ufmylated RPL26 on the 60*S* subunit through the UFM1-interacting region of UFBP1. Loss of E3 components or disruption of the interaction between UFBP1 and ufmylated RPL26 attenuated ER-RQC. These results provide insights into not only the molecular basis of the ufmylation but also its role in proteostasis.

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 function of ribosome dynamic modification in stress response

The synthesis and modification of secretory proteins in the endoplasmic reticulum is essential for cells. The accumulation of abnormal proteins in the endoplasmic reticulum is harmful to cells and therefore responds by inducing the UPR pathway. In Saccharomyces cerevisiae, the membrane protein Ire1, activated by endoplasmic reticulum stress, splices the precursor mRNA of the transcription factor Hac1, and Hac1 is synthesized to induce transcription of chaperones. In higher eukaryotes, PARK phosphorylates eIF2α and suppresses the initiation of cell-wide translation. To elucidate the physiological function of ribosomal ubiquitination, we discovered a novel translational regulator in the endoplasmic reticulum stress response. We discovered a novel translational control mechanism during endoplasmic reticulum stress in S. cerevisiae and clarified that ubiquitination of ribosomal protein eS7 by E3 ubiquitin ligase Not4 is essential.

Publication list

1. Matsuo, Y.*, Uchihashi, T. & Inada, T.*
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2. Best, K., Ikeuchi, K., Kater, L., Best, D., Musial, J., Matsuo, Y., Berninghausen, O., Becker, T., Inada, T.* and Beckmann, R.*
   Structural basis for clearing of ribosome collisions by the RQT complex. ***Nat. Commun***. doi:https://doi.org/10.1038/s41467-023-36230-8. (2023)
3. Tesina, P.#,*, Ebine, S.#, Buschauer, R.#, Thoms, M., Matsuo, Y., Inada, T.* and Beckmann, R.* (#Equal contribution)
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4. Ikeuchi, K., Ivic, N., Buschauer, R., Cheng, J., Fröhlich, T., Matsuo, Y., Berninghausen, O., Inada, T., Becker, T.* and Beckmann, R.*Molecular basis for recognition and deubiquitination of 40S ribosomes by Otu2.
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5. Ishimura, R.#, Ito, S.#, Mao, G., Komatsu-Hirota, S., Inada, T.*, Noda, N.*, Komatsu, M.* (#Equal contribution)
   Mechanistic insights into the roles of the UFM1 E3 ligase complex in ufmylation and ribosome-associated protein quality control.
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