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. Molecular mechanism of RQC and NGD

Ribosome-associated Quality Control (RQC) is crucial for monitoring faulty translation and removing abnormal proteins during their synthesis. It plays a key role in protein homeostasis early on. We have identified the E3 ubiquitin ligase Hel2 and its mammalian homolog ZNF598 as vital for RQC and discovered a novel RQT complex that dissociates ubiquitinated ribosomes into subunits.

In yeast, RQT complex components Cue3 and Rqt4 bind to K63-linked ubiquitin chains, facilitating recruitment to colliding ribosomes. High-speed atomic force microscopy has revealed that the disordered regions of Rqt4 enhance interaction with the ubiquitin chain. Meanwhile, in mammals, ZNF598 is essential for polyubiquitinating uS10, necessary for initiating RQC. The human RQT (hRQT) complex, comprised of ASCC3, ASCC2, and TRIP4, relies on K63-linked polyubiquitination of uS10 for ribosome dissociation. Additionally, RQC works in tandem with No-Go Decay (NGD), leading to mRNA cleavage in collided ribosomes. We identified two NGD pathways: NGDRQC-, which involves mRNA cleavage linked to RQC, and NGDRQC+, which operates independently. The ubiquitin-binding activity of Cue2 is necessary for NGDRQC-, while Mbf1 and uS3 interactions are crucial for NGDRQC+.

NEMF, the homolog of Rqc2 in yeast, interacts with 60S ribosome-nascent chain complexes and recruits Ltn1/Listerin for ubiquitination. Rqc2 extends stalled tRNA-bound peptides with alanine and threonine residues (CAT tails) through a non-canonical reaction, which helps degrade substrates lacking accessible ubiquitination sites. Our recent findings suggest that failure to degrade CAT-tailed proteins disrupts neuronal morphogenesis and cell survival. In mammals, NEMF modifies nonstop mRNAs by adding C-terminal tails predominantly of alanine.

1.1. Editing of the polyubiquitin architecture on the collided ribosome maintains persistent RQC activity

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In Ribosome-associated Quality Control (RQC), K63-linked polyubiquitination of uS10 on the stalled ribosome is crucial for recruiting the RQC-trigger (RQT) complex. However, the mechanisms governing the maintenance and recycling of polyubiquitin architecture on colliding ribosomes remain unclear. Here we demonstrate that two deubiquitinating enzymes (DUBs), Ubp2 and Ubp3, play key roles in editing and recycling polyubiquitin chains on uS10, thereby contributing to the promotion of RQC activity. Specifically, Ubp2 eliminates the K63-linked polyubiquitin chain from uS10 on the free 40S subunit for recycling, while Ubp3 predominantly cleaves the K48-linked di-ubiquitin and the K48/K63-mixed-linkage poly-ubiquitin chain from uS10 on the translating ribosomes. Notably, we further demonstrate that the ubiquitin chains containing K48-linkage of the uS10 on the colliding ribosome act as a negative signal for the RQT-mediated ribosome dissociation process. Collectively, our findings clarify the ubiquitin code in RQC and define the positive functions of these two DUBs in maintaining the persistent RQC activity.

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.

2.1. Collision-induced ribosome degradation driven by ribosome competition and translational perturbations

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Individual stalling of catalytically inactive ribosomes at the start codon triggers ubiquitination of ribosomal protein uS3 and subsequent 18S rRNA decay. While collisions between ribosomes during translation elongation represent a more widespread form of translation perturbation, their impact on ribosome stability remains unknown. Here, we clarify a bifurcation in ubiquitination-mediated ribosome turnover, identifying a collision-induced branch of uS3 ubiquitination and small subunit destabilization in yeast. This pathway eliminates not only non-functional ribosomes but also translationally active ones with a prokaryotic-like decoding center, driven by competition with wild-type ribosomes due to differing translation rates. We further show that endogenous ribosomal subunit stoichiometry shifts toward a small-subunit-shortage state via ubiquitination upon perturbed translation triggered by the anti-cancer drug cisplatin and the growth phase transition. These findings reveal a mechanism by which ribosome dynamics generally affects ribosome stability, implicating ribosome dysfunction, heterogeneity, and stress-related translational disturbances in small subunit degradation.

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*, the membrane protein Ire1 is activated by ER stress, leading to the splic-

ing 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.

3.1. Crucial roles of Grr1 in splicing and translation of *HAC1* mRNA upon unfolded stress response

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In the process of the unfolded protein response (UPR), the Hac1p protein is induced through a complex regulation of the *HAC1* mRNA. This includes the mRNA localization on the endoplasmic reticulum (ER) membrane and stress-triggered splicing. In yeast, a specific ribosome ubiquitination process, the monoubiquitination of eS7A by the E3 ligase Not4, facilitates the translation of *HAC1*^{i}, a spliced form of the *HAC1* mRNA. Upon UPR, the mono-ubiquitination of eS7A increases due to the downregulation of Ubp3, a deubiquitinating enzyme of eS7A. However, the exact mechanisms behind these regulations have remained unknown. In this study, an E3 ligase, Grr1, an F-box protein component of the SCF ubiquitin ligase complex, which is responsible for Ubp3 degradation, has been identified. Grr1-mediated Ubp3 degradation is required to maintain the level of eS7A monoubiquitination that facilitates Hac1p translation depending on the ORF of *HAC1*^{i}. Grr1 also facilitates the splicing of *HAC1*^{u} mRNA independently of Ubp3 and eS7A ubiquitination. Finally, we propose distinct roles of Grr1 upon UPR, *HAC1*^{u} splicing, and *HAC1*^{i} mRNA translation. Grr1-mediated Ubp3 degradation is crucial for *HAC1*^{i} mRNA translation, highlighting the crucial role of ribosome ubiquitination in translational during UPR.

3.2. Structure of a Gcn2 dimer in complex with the large 60S ribosomal subunit

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The integrated stress response (ISR) is a central signalling network that enables eukaryotic cells to respond to a variety of different environmental stresses. Such stresses cause ribosome collisions that lead to activation of the kinase Gcn2, resulting in the phosphorylation and inactivation of eukaryotic initiation factor 2 and thereby promoting selective translation of mRNAs to restore homeostasis. Despite the importance of the ISR and intensive study over the past decades, structural insight into how Gcn2 interacts with ribosomal particles has been lacking. Using ex vivo affinity purification approaches, we have obtained a cryo-electron microscopy structure of a yeast Gcn2 dimer in complex with the ribosomal 60S subunit. The Gcn2 dimer is formed by dimerization of the histidine tRNA synthetase-like domains, which establish extensive interactions with the stalk-base and sarcin-ricin loop of the 60S subunit. The C-terminal domain of Gcn2 is also dimerized and occupies the A- and P-site tRNA binding sites at the peptidyl-transferase center of the 60S subunit. Complementary functional studies indicate that binding of Gcn2 to the 60S subunit does not require the co-activators Gcn1 or Gcn20, nor does it lead to phosphorylation of eIF2a. Instead, upon stress, we observe a shift of Gcn2 from the 60S subunit into the colliding ribosome fraction, suggesting that the Gcn2-60S complex represents an inactive stand-by state to enable a rapid redistribution to collided ribosomes, and thereby facilitating a quick and efficient response to stress.

Publication list

1. Sato, N., Nakano, Y., Matsuki, Y., Tomomatsu, S., Li, S., Matsuo. Y., Inada, T.
   Crucial roles of Grr1 in splicing and translation of HAC1 mRNA upon unfolded stress response.
   Nat Commun. 416(1):2172. (2025)
2. Paternoga, H., Xia, L., Dimitrova-Paternoga, L., Li, S., Yan, LL., Oestereich, M., Kasvandik, S., Nanjaraj, Urs AN., Beckert, B., Tenson, T., Zaher, H., In-

ada, T., Wilson, DN.
Structure of a Gcn2 dimer in complex with the large 60S ribosomal subunit.
Proc Natl Acad Sci USA. 122(15):e2415807122. doi: 10.1073/pnas.2415807122. (2025)

3. Tomomatsu, S., Matsuo. Y., Ohtake, F., Tomita, T., Saeki,Y., Inada, T.*
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EMBO J. DOI: 10.1038/s44318-025-00568-0 (2025)
4. Li, S., Shounai, O., Kato, M., Ikeuchi, K. Inada, T.
Collision-induced ribosome degradation driven by ribosome competition and translational perturbations.
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