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Conserved mechanism of collision dependent mRNA degradation

○Satoshi HASHIMOTO[1], Aoi SATOH[2,3], Jiahuan ZENG[1],
Jennifer SAUERLAND[4], Hideaki TAKEDA[1], Hotaka KOBAYASHI[2,3], Toshifumi INADA[1]

[1] The Institute of Medical Science, The University of Tokyo, Division of RNA and gene regulation,
[2] Institute of Advanced Medical Sciences, Tokushima University, Division of Gene Regulation,
[3] Institute for Quantitative Biosciences, The University of Tokyo, Laboratory of mRNA Translation,
[4] University of Heidelberg

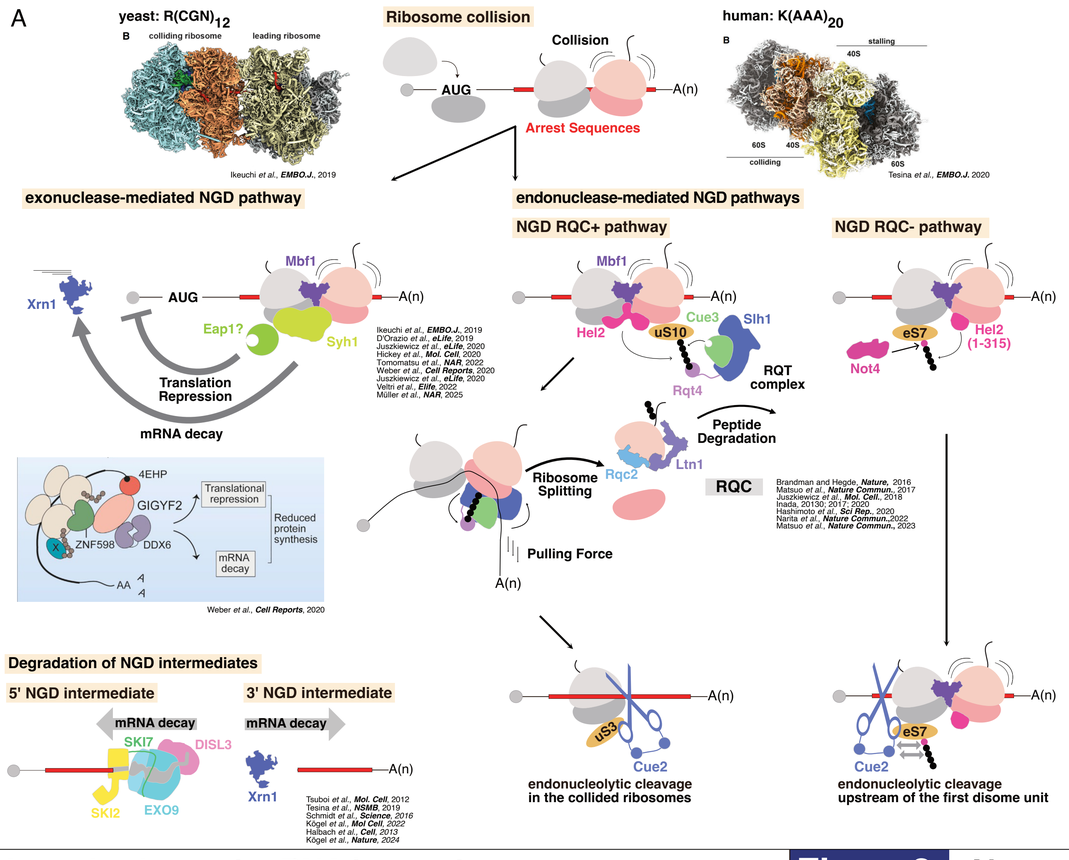
Summary

Accurate gene expression is essential for all organisms, but errors during this process are unavoidable. Cells have evolved multiple quality control mechanisms to handle such errors and maintain cellular homeostasis. Among these, No-Go Decay (NGD) is a well-characterized mRNA surveillance pathway. It prevents the accumulation of problematic mRNAs containing sequences prone to ribosome stalling and collisions, such as tandem rare codons in yeast or poly(A) stretches in mammals.

When a ribosome stalls during translation and collides with a trailing ribosome, a disome is formed (Ikeuchi *et al.*, ***EMBO J.***, 2019). In yeast, the disome is specifically recognized by the E3 ubiquitin ligase Hel2, which polyubiquitinates the ribosomal protein uS10, triggering ribosome-associated quality control (RQC) to degrade the aberrant nascent polypeptide (Matsuo *et al.*, ***Nat. Commun.***, 2017; ***NSMB***, 2020). Ribosome collision also activates No-Go-Decay, and Cue2 is responsible for the endonucleolytic cleavage at the vicinity of the collided ribosome (D'Orazio *et al.*, ***eLife***, 2019), depending on the Hel2-mediated polyubiquitination of uS10 and eS7 (Ikeuchi et al., EMBO J., 2019; Tomomatsu *et al.*, ***NAR***, 2022). In addition, Syh1 is proposed to promote mRNA decay upon ribosome stalling by recruiting the exonuclease Xrn1, thereby triggering the exonuclease-mediated decay pathway (Veltri *et al.*, ***eLife***, 2022). However, it remains unclear how both pathways, Hel2-Cue2-dependent endonucleolytic cleavage, and Syh1-Xrn1-dependent exonucleolytic degradation, contribute to the ribosome collision-mediated mRNA decay.

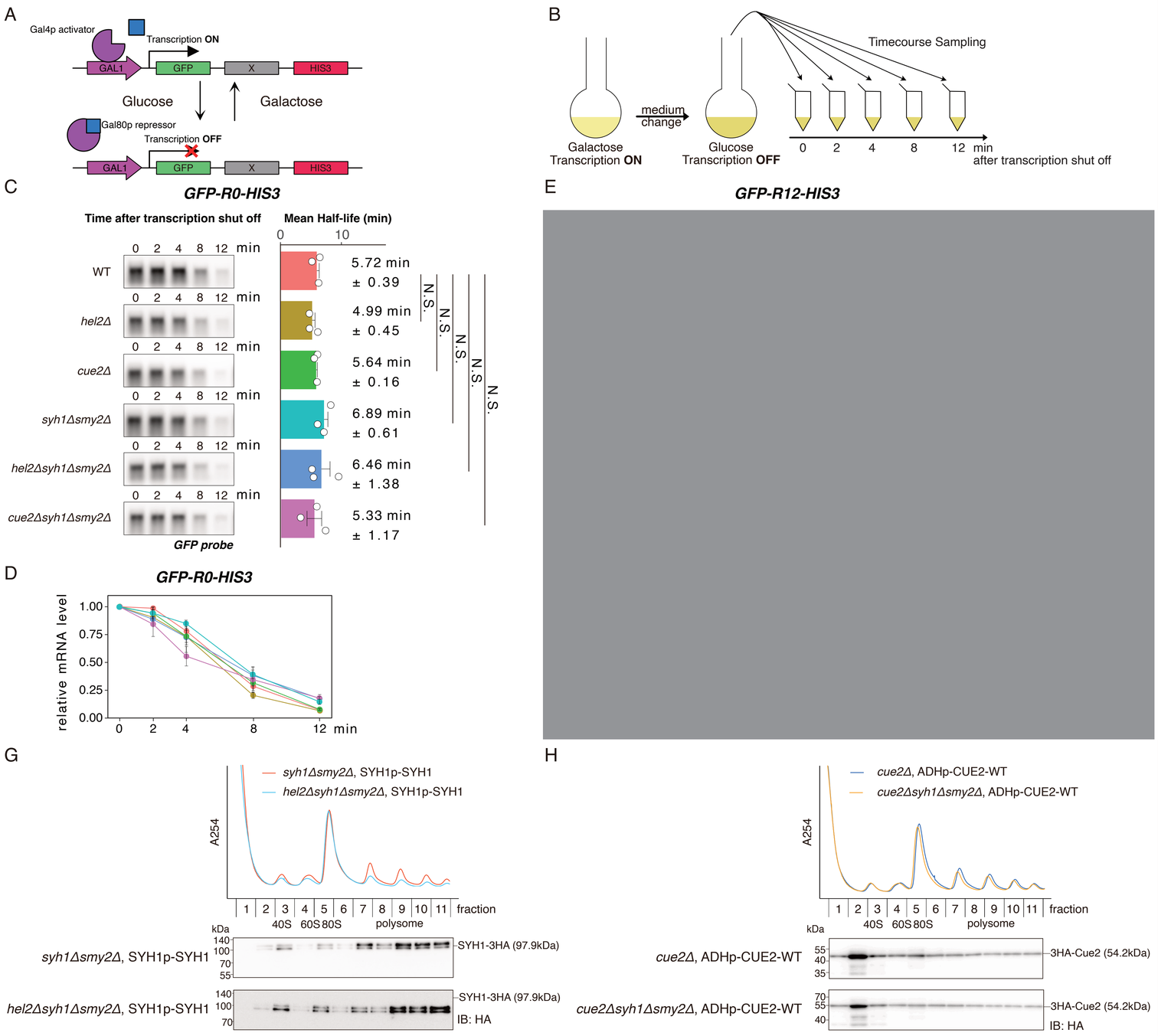
Here, we demonstrate that in yeast, Syh1-Xrn1 and Hel2-Cue2 pathways independently function in the ribosome collision-induced mRNA decay. In addition, we confirm that these mechanisms are conserved in mammals by using a Tet-Off system and single-molecule imaging. These findings deepen our understanding of mRNA quality control and offer the potential for developing mRNA-targeted therapies.

Introduction



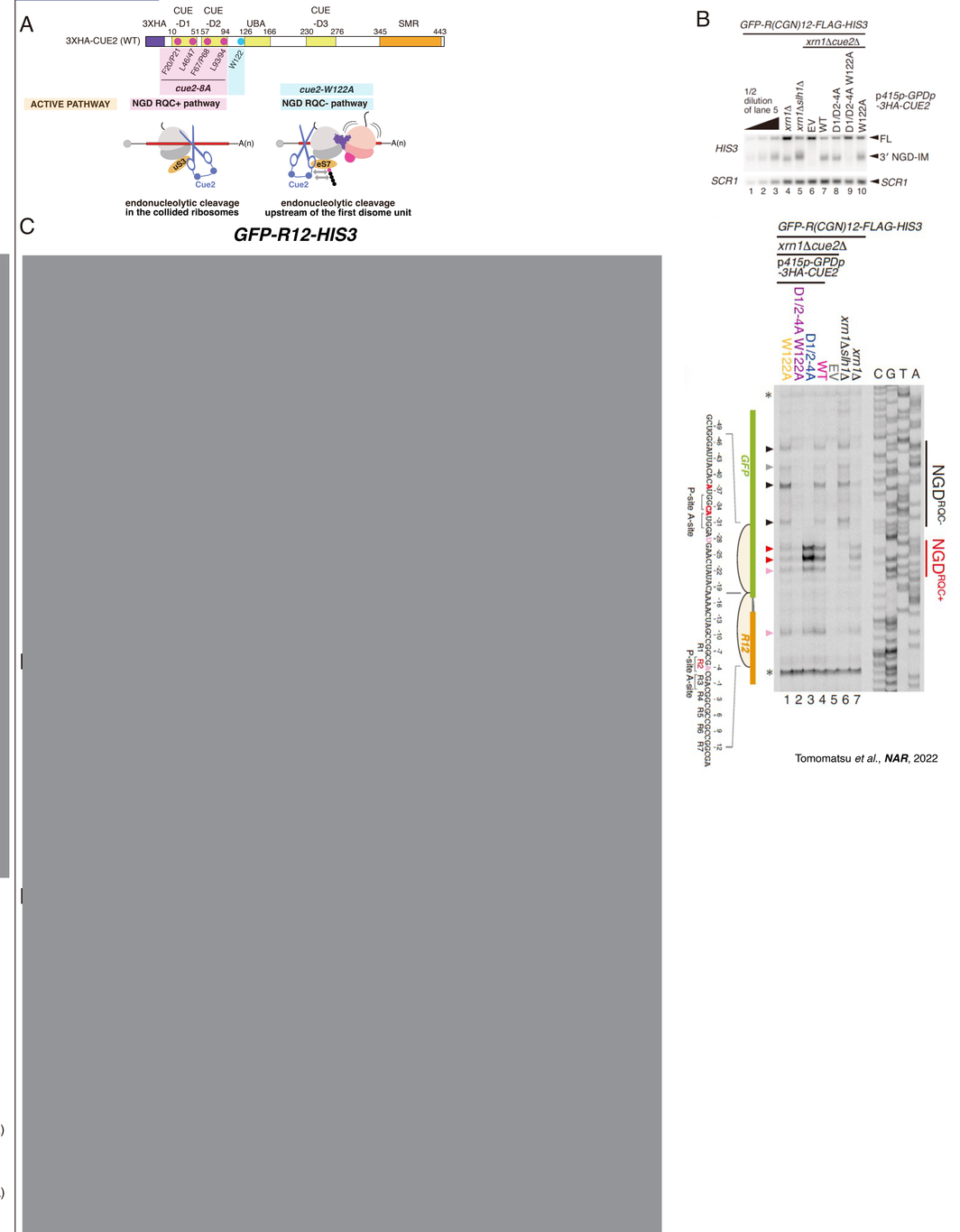
A. Schematic of collision dependent mRNA decay in eukaryotes. When ribosome translates arrest sequences, ribosome collisions occur. There are two pathways to resolve these collisions: Exonuclease-mediated NGD and endo-nuclease mediated NGD (No-Go Decay). In Exonuclease-mediated NGD, the collided ribosome is recognized by Syh1, which recruits the exonuclease Xrn1, triggering mRNA degradation. In endo-nuclease mediated NGD, the collided ribosomes are sensed by Hel2. The leading ribosome is then ubiquitinated, allowing recognition by the RQT complex, which leads to ribosome dissociation into subunits. Subsequently, the mRNA is cleaved by the endonuclease Cue2 and further degraded by the exonucleases Xrn1 and the exosome. The aberrant peptide on 60S subunit is ubiquitinated by Ltn1 and degraded by the proteasome. **B.** Schematic of collision dependent mRNA decay in procaryotes. In proteobacteria, SmrB recognizes the collided ribosome and endonucleolytically cleaves the mRNA, leading to the degradation of both the mRNA and nascent peptides. In contrast, in firmicutes, Muts2 recognizes the collided ribosome and dissociates the leading ribosome, leading to the degradation of both the mRNA and nascent peptides. **C.** Sequence alignment of SMR domains of representative proteins. A conserved DxH motif is important for its catalytic activity and a GxG motif is predicted to interact with the RNA substrate. **D.** Sequence alignment of 4EHP-binding domain and GYF domain of representative proteins.

Figure1 Syh1 and Hel2 works independently to degrade aberrant mRNA in yeast



A. Schematic of the Galactose responsive promoter. Transcription of the reporter is only active under the galactose conditions. When Galactose is removed, transcription of the reporter is inactivated. **B.** Yeast cells were harvested at the indicated time points after transcription shutoff, by switching the medium from galactose to glucose. **C-F.** mRNA levels of ***GFP-R0/R12-HIS3*** reporter was quantified by Northern Blotting. Relative mRNA levels were normalized to that at 0 min and plotted in panels D and F. The half-life was calculated by determining the first-order decomposition rate constant using the nonlinear least-squares method. The mean half-life is shown in panels C and E. Statistical analysis was performed using the Benjamini and Hochberg method. Key; padj < 0.001 : ***, padj < 0.01 : **, padj < 0.05 : *, N.S. : Non-Significant **G and H.** Sucrose density gradient profiling and the corresponding protein distribution for the indicated strains.

Figure2 Aberrant mRNA is mainly degraded by NGD RQC- pathway



A. Schematic of NGD RQC+ and NGD RQC-. For NGD RQC+, W122 of Cue2 is essential. For NGD RQC-, the indicated residues in the CUE domain are essential. **B.** Top: Northern blot analysis of the ***GFP-R12-HIS3*** reporter. ***SCR*** is used as the loading control. Bottom: Primer extension analysis showing the cleavage sites of NGD RQC+ and NGD RQC-. **C and D.** Relative mRNA levels and half-life of the ***GFP-R12-HIS3*** reporter. Statistical analysis was performed using the Benjamini and Hochberg method. Key; padj < 0.001 : ***, padj < 0.01 : **, padj < 0.05 : *, N.S. : Non-Significant **E.** Sucrose density gradient profiling and the corresponding protein distribution for the indicated strains.

Figure3 GIGYF2 and ZNF598 works independently to degrade aberrant mRNA in mammal

A. Schematic of the Tet-Off reporter system. tTA binds to TRE (Tet Response Element) and activates transcription in the absence of doxycycline (Dox). In the presence of Dox, tTA cannot bind to TRE, and transcription is shut off. **B.** Schematic of the arrest sequences in yeast and mammal. **C.** Western blot of KO strains used for half-life measurement. **D-G.** mRNA levels of the GFP-K0/K20-mCherry reporter were measured by RT-qPCR using HEK293T cells. Statistical analysis was performed using the Benjamini and Hochberg method. Key; padj < 0.001 : ***, padj < 0.01 : **, padj < 0.05 : *, N.S. : Non-Significant

Conclusion