No.	K23-1050	
研究課題名	Structural basis of ribosome associated quality control	
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(Form 2)

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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual)

Annual Report

Report

Report on Proposal Number K23-1050

Project Title: Structural basis of ribosome associated quality control

Target period: April 1, 2024 to March 31, 2025

Ribosome stalling induces quality control pathways targeting the nascent polypeptide (RQC: Ribosome associated Quality Control) and the mRNA (NGD: No-Go Decay). The RQC pathway monitors translation and ensures efficient elimination of aberrant nascent protein products. Collided ribosomes consisting of the leading ribosome and the following colliding ribosome(s) serve as a proxy for translation problems in the cell.

For the structural and mechanistic analysis of ubiquitination-mediated recognition and dissociation of the collided ribosomes we established and together with the Inada lab further improved the in vitro reconstituted human system. To that end, we could build on the insights from the yeast system for which we published the structure of the RQT-ribosome complexes recently (Best et al., 2023). As suggested, the ubiquitylation reaction was optimized and wt as well as ATPase deficient mutant forms of hRQT were used for the hRQT-ribosome reconstitution in order to yield stable complexes for the structural analysis. However, initial cryo-EM studies did not yield any clear 3D information on the hRQT interaction with the collided human ribosome, therefore indicating the need for more optimization and stabilization of the complexes. These efforts have been continued, yet, have not yielded novel structural results.

The Inada lab found that the deubiquitinating enzymes Ubp2/3 facilitate the formation of highly specific K63-linked polyubiquitin chains on uS10 of the collided ribosomes. Here, collided ribosomes with Ubp3 in the catalytic-dead form were prepared, however, not yielding clear 3D reconstructions by cryo-EM in first attempts. Yet, our results on the eS7 de-ubiquitinating enzyme Otu2 could already be published (Ikeuchi et al., 2023).

The second step of RQC, the proteasomal-degradation of the aberrant polypeptide on 60S subunit, involves first the Ltn1 and NEMF (Rqc2 in yeast) recognition of the 60S subunit with the aberrant polypeptide. Here, in collaboration with the Inada lab we determined all steps of the CAT-tail formation catalyzed by Rqc2 in yeast, thereby providing a comprehensive structural-functional analysis of the yeast CAT-tail formation which has recently been published (Tesina et al., 2023). Now, the Inada lab has established the system to assay the CAT-tailing formation in humans, including NEMF, LTN1-KO cells and comprehensive construction of the plasmids expressing the mutants of human RQC factors, thereby preparing the structural analysis of the human system. Here, we have been successful in isolation and structural analysis of human ribosomal RQC complexes. Similar to the situation in yeast, many distinct states of the human CAT tailing cycle and new intermediates could be resolved at molecular resolution including complete models of NEMF and Listerin. We also observed for the first time the RQC component TCF25 and can explain it guides together with Listerin the specificity of ubiquitin K48 linkage targeting the nascent chain for proteasomal degradation. Analysis of (patient derived) mutants of NEMF have been

completed and help to rationalize the observations of the biochemical analysis of human CAT tails. These results are currently prepared for publication.

The Inada lab has discovered that the mono-ubiquitination of eS7 contributes the UPR-mediated translation of HAC1 mRNA, a transcription factor in UPR. The novel decoder for the monoubiquitinated eS7 was also recently identified by the Inada lab. These insights have set the stage for the structural analysis by my lab that uncovered the recognition mechanism of this novel UPR pathway that depends on the specific modification of ribosomes. Here, we are awaiting last genetic analysis for completion to proceed with the preparation of a manuscript which combines the functional data by the Inada lab and our cryo-EM results.

Both labs have contributed together to a study on the Mbf1/EDF1 protein that recognizes and binds to collided stalled ribosomes (lead by the lab of Hani Zaher). These results have been published recently (Kim et al., 2024).

In addition, another joint review article on the ribosome as a hub to coordinate mRNA decay has been published recently (Inada & Beckmann, 2024).

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