No.	K23-1050	
Project Title	Structural basis of ribosome associated quality control	
Principal Investigator	Roland Beckmann (University Munich LMU・Professor)	
Project Member(s)	**IMSUT Host Researcher**	Toshifumi Inada (The Institute of Medical Science, The University of Tokyo・Professor)
	Project Member (s)	Roland Beckmann (University Munich LMU・Professor, Chair of Biochemistry)
	Project Member (s)	Thomas Becker (LMU・Staff scientist)
	Project Member (s)	Timo Denk (University of Munich LMU・PhD student)

(Form 2)

Principal Investigator
Roland Beckmann

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

Report on Proposal Number K23-1050

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Target period: April 1, 2025 to March 31, 2026

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, the cryo-EM studies did not yield clear 3D information on the hRQT-ribosome complex, therefore leading us to invest in further optimization of these complexes.

The second step of RQC, the proteasomal-degradation of the aberrant polypeptide on 60S subunit, involves first the recognition of the 60S-polypeptide complex by Rqc2 (NEMF in humans) and Ltn1 (LTN1 or Listerin in humans). 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 been published (Tesina et al., 2023). Now, the Inada lab has developed a 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 the affinity 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 how 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 have been prepared for publication and will be submitted soon.

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 have prepared a manuscript which combines the functional data by the Inada lab and our cryo-EM structural results to be submitted soon.

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 (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).

A major focus has been on the factors contributing to 18S NRD (Non-functional ribosome decay), the degradation of entire ribosomal subunits, here the 40S small subunit from yeast. The Inada lab (and the Beckmann lab) have discovered new factors which are essential in this process downstream of the ubiquitylation state (by Hel2 or Mag2/Fap1). We thus understand which factors recognize the uS3-uS5 modification by ubiquitin and which factors are required for downstream reactions to initialize destruction of the ribosomal subunit. For three of these factors the Beckmann lab has determined cryo-EM structures when in complex with the (partially degraded) ribosomal subunit and are currently combine these data with the biochemical and molecular biology analysis of the Inada lab.

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