DATE: Day 13 Month 04 Year 2021

SUMMARY of
2020 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Structural study of dUMP hydroxymethylases from bacteriophage**
Applicant	**Name**	**Hyun Kyu Song**
	Affiliation	**Korea University**
	Present Title	**Crystal structure of UBR box of PRT6 from *Arabidopsis thaliana***
Research Collaborator (Host PI)		**Atsushi Nakagawa (Professor)**

Summary

The amount of proteins in the cells is elaborately regulated to maintain cellular homeostasis. A mechanism for this precise regulation in eukaryotes is the ubiquitin/proteasome system. One of the well-characterized degradation mechanisms is the N-degron pathway, which is a process that determines the half-life of proteins based on the N-terminal residue, called the 'N-degron'. The N-degrons are recognized by adaptor proteins (N-recognin), which deliver them to proteases for degradation. In contrast to the wealth of structural information on UBR box from fungi and mammals, the structural information from plants is very limited. Furthermore, the recognition of oxidized cysteine at the second residue of N-degron might be very critical in plant because one of the transcription factor, ERF-VII degrades continuously under normal oxygen condition by recognition of UBR box in PRT6, N-recognin. Therefore, it is of interest to understand how UBR box from plants can recognizes the sulfonylcysteine residue at the second position of N-degron. The crystal structure of UBR box of AtPRT6 was solved by molecular replacement and the coordinates of yeast UBR box (PDB ID: 3NIT) were used as a search model. The bound N-degron was quite evident in the initial electron density map calculated by the MR phases. By taking advantage of LC3B-fusion method, we determined the crystal structures of RLGS–AtPRT6 UBR box and thus, the structure was solved in the N-degron complexed state. The PRT6 UBR box from *Arabidopsis* also showed a heart-shaped domain with three zinc-coordinated sites. In addition, as compared with the structure of the complex formed by the yeast Ubr1 UBR box and RLGES peptide, both the yUbr1 UBR box and the AtPRT6 box recognized Arg-1* and Leu-2* residues in a similar manner (where the asterisk indicates the N-degron sequence attached to the N terminus). We initiate the collaboration with Dr. James Meng-Chiao Ho at Institute of Biological Chemistry, Academia Sinica, Taiwan. His group obtained the structure the same protein from *Oryza Sativa* (rice). We exchanged the refined coordinates for structural comparison. The overall fold of both UBR boxes is very similar, however, the arginine residue, one of the key arginine residue near N-degron binding site shows different conformation, which might be a key determinant for the second residue specificity. These results provide a framework for understanding the substrate specificity of the N-degron pathway in higher eukaryote plant.

***Deadline: May 14, 2021**
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