DATE: Day 9 Month May Year 2025

SUMMARY of
FY2024 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		**Structure of Fra1 prolyl aminopeptidase from *Saccharomyces cerevisiae***
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Summary

E3 ligases N-recognins recognize the N-terminal degradation signal, N-degron in the proteins, and this proteolytic system is called 'N-degron pathway'. Gluconeogenesis is a metabolic pathway of generating glucose from non-carbohydrate substrates, and it is a ubiquitous metabolic process in almost all living organisms. In yeast, cells switch from gluconeogenesis to glycolysis to maintain the level of glucose. Gid ubiquitin ligase complex, composed of 9 subunits (Gid1~9), degrades gluconeogenic enzymes when cells return to the glucose-replete condition. The gluconeogenic enzymes Fbp1, Icl1, Mdh2, and Pck1 bearing N-terminal proline are degraded conditionally by the Gid4-dependent Pro/N-degron pathway. The Gid4 is the recognition component for the Pro/N-degron; however, these also recognize non-proline N-terminal residues. More intriguingly, this Pro/N-degron pathway is expanded by finding the coupled aminopeptidase P, such as Fra1 prolyl aminopeptidase from *Saccharomyces cerevisiae*. Before Pro/N-recognin binds to the Pro/N-degron, Fra1 trims the non-proline residue at the N-terminus to expose Pro at the N-terminus. Therefore, structural information on the Fra1 and N-recognins is indispensable for understanding the functional repertoire of the N-degron pathway. Furthermore, the exact enzymatic mechanism of the peptide bond cleavage right before the proline residue remains elusive, and thus, we aim to determine the structures of Fra1 aminopeptidase in complex with prolyl or non-prolyl peptides to understand its enzymatic mechanism. Previously, we obtained the low-resolution cryo-EM map and, in this study, determined the atomic resolution structure of Fra1 prolyl aminopeptidase. The electron density map obtained from X-ray crystallography was superior to the partial structure of yeast Fra1 with cryo-EM data. Based on the current structure, we plan to mutate critical residues at the dimeric interface, which is known to control the proteolysis activity and the active site of Fra1, including metal-coordinating residues, and then check the *in vitro* enzymatic activity of Fra1 prolyl-aminopeptidase.

***Deadline: May 9, 2025**
***Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.**
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