学位論文抄録

Functional characterization of UBXN-6, a C-terminal cofactor of CDC-48, in *C. elegans*
（CDC-48 の C 末端コファクターUBXN-6 の線虫における機能解析）

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Abstract of the Thesis

Background and Purpose: VCP, a human homolog of *C. elegans* CDC-48, is an abundant AAA-type molecular chaperon and is involved in many pathways maintaining cellular homeostasis including proteasome-dependent degradation of misfolded or damaged proteins, and endolysosomal sorting and autophagy. For the diverse cellular function, different cofactors cooperate VCP either by binding to N-terminal or C-terminal domain of VCP. However, UBXD1, a human homolog of *C. elegans* UBXN-6, is unique among the cofactors of VCP since it can interact with both terminals of VCP through a novel, bipartite binding mechanism. Disruption of the interaction between VCP and UBXD1 leads to the pathogenesis of neurodegenerative diseases like IBMPFD (Inclusion Body Myopathy with Paget disease and Frontotemporal Dimentia). For the study of disease-pathophysiology, it is necessary to characterize the physiological role of UBXD1 in a suitable animal model. Here we showed that expression of UBXN-6 is induced upon starvation for proper functioning of lysosomal clearance in *C. elegans*.

Methods: Synchronized young adults were used throughout the study. Life span measurement was analyzed. Western blotting analyses were employed to detect UBXN-6 and GFP-tagged protein using anti-UBXN-6 and anti-GFP antibodies, respectively. Confocal microscopy was used to monitor GFP-tagged proteins in *C. elegans*.

Results: We created the UBXN-6 deletion strain (*Δubxn-6*) using CRISPR-Cas9 genome editing tools. The *Δubxn-6* mutant showed reduced life span comparing with wild-type (WT) strain. The expression of UBXN-6 was induced upon starvation. Starvation-induced expression is unique characteristic for UBXN-6 among C-terminal cofactors of CDC-48, since other C-terminal cofactors, UFD-2 and UFD-3, were not induced upon starvation. In contrast, UBXN-6 was not induced upon other stresses such as tunicamycin (ERAD stressor) and rapamycin (autophagy inducer). During starvation, lysosomal activity is triggered for rapid clearance of cellular materials (proteins, lipids etc.). We observed the lysosomal activity by monitoring GLO-1::GFP, a marker for lysosome related organelles. Western blotting analysis showed that the level of GLO-1::GFP was decreased after 12 hrs of starvation in both WT and *Δubxn-6* mutant, but accumulation of GLO-1::GFP puncta was observed more in *Δubxn-6* mutant by microscopic analysis. Together, the results indicate the involvement of UBXN-6 in clearance of cellular materials upon starvation in *C. elegans* system.

Conclusions: Among the C-terminal cofactors of CDC-48, the UBXN-6 is unique that it is induced upon starvation for maintaining proper cellular homeostasis in *C. elegans*.