学位論文抄録

Deviation of the typical AAA substrate-threading pore prevents fatal protein degradation in yeast Cdc48

（酵母 Cdc48 における基質糸通しに係る典型的な AAA ポアからの逸脱は致死的タンパク質分解を抑制する）

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Abstract of Thesis

Background and Purpose: Cdc48 is a highly conserved AAA-type ATPase found in all eukaryotes, which plays essential roles in protein homeostasis. Typically, AAA ATPases form a hexameric ring-shaped structure with a central pore, and facilitate unfolding of substrate proteins by threading them through the pore. The threading mechanism relies on an ATP-dependent movement of a loop structure protruding into the pore (pore loop) with a conserved ΦXG (an aromatic amino acid, any residue, and a glycine residue) motif sequence. Conversely, Cdc48 lacks the ΦXG motif in one of the two AAA domains, thereby much less is known about how substrate proteins are processed by Cdc48.

Methods: We used the budding yeast *Saccharomyces cerevisiae* as a model organism because of convenient genetic manipulations. Strains with disruption of the genomic *CDC48* gene expressing a mutant Cdc48 under the control of the authentic promoter from a single-copy plasmid were constructed.

Results: Methionine or leucine residue occupies the first position of the corresponding ΦXG motif sequence in the first AAA domain of all Cdc48 homologs. Replacements of the methionine residue of yeast Cdc48 with any of three aromatic residues were found to exert lethal phenotype for the cell. The lethality depended on interaction with the 20S proteasome. Because the mutations were suggested to endow a hyperactive unfolding activity, we hypothesized that the hyperactive Cdc48 complexed with the 20S proteasome may degrade some essential proteins.

To explore substrate proteins of the Cdc48-20S proteasome, we performed proteomic analyses, which showed instability of about 200 intracellular substrate proteins in the hyperactive Cdc48 mutant strain. Among them, Sod1, a cytosolic copper-zinc superoxide dismutase, has been identified as the first endogenous substrate of the Cdc48-20S proteasome.

Conclusion: The hyperactive threading mutant of Cdc48 led to degradation of otherwise stable Sod1. So, we claim that the widely-applicable threading mechanism for substrate proteins is less effective in wild type Cdc48, and instead Cdc48 would function mainly through an as-yet-undetermined mechanism.