学 位 論 文 の 要 約

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学 位 論 文 題 名

Unraveling the Mechanism of Functional Switching of Trigger Factor Chaperone
（トリガーファクターシャペロンにおける機能変換機構の解明）

In a crowded cellular environment, proteins are at risk of misfolding and aggregation. Molecular chaperones play multiple roles in the regulation of protein quality. Chaperones exert their holdase activity to keep the substrates in the unfolded state for protein translocation or exert their foldase activity to promote protein folding, thereby preventing proteins from misfolding and/or promoting protein to be folded correctly. On the other hand, once a malfunctional protein appears, molecular chaperones work as an unfoldase to unfold such proteins for degradation or refolding, thereby removing these harmful proteins. Trigger factor (TF) chaperone from prokaryotes is a multifunctional chaperone and has holdase, foldase, and unfoldase activities to control the protein quality. Despite its importance in maintaining protein homeostasis in the cell, the mechanism of how TF switches its functions is poorly understood. TF takes several strategies to change its functions. One of the examples is that the dimerization of TF can switch/regulate the holdase and foldase activity, in which dimerization accelerates the association rate of TF to the substrate proteins for stronger holdase activity. Besides the dimerization, other strategies of TF for functional switching are 1) binding to the metal ion as seen in TF from *Thermus thermophilus* (*Tt*TF), and 2) binding to the other chaperone as seen in TF from *Escherichia coli* (*Ec*TF) in complex with *Ec*ClpX chaperone. The holdase activity of *Tt*TF is turned on upon the binding to Zn^{2+}, and *Ec*TF switches from holdase/foldase to unfoldase upon the binding to *Ec*ClpX. However, the mechanism of how the cofactor or other chaperone induces the functional switching of TF is still unclear. Thus, I aimed to explore the mechanism of functional switching of TF.

1) The mechanism of *Tt*TF functional switching induced by Zn^{2+} (Chapter II)

To investigate how Zn^{2+} ions turn on the holdase activity of *Tt*TF, a series of experiments including circular dichroism (CD), size exclusion chromatography-multi-angle light scattering (SEC-MALS), and nuclear magnetic resonance (NMR) were used to analyze the changes in the structure and oligomerization state of *Tt*TF in the presence and absence of Zn^{2+}. CD and NMR data showed that the zinc-binding induces partial structural changes of *Tt*TF (Fig.1 i). SEC-MALS data showed that zinc-binding promotes the oligomerization of *Tt*TF (Fig.1 ii). Given the previous report on *Ec*TF showing the relationship between the oligomerization and the activity modulation, the data suggest that *Tt*TF exploits zinc ion to induce the structural change coupled with the oligomerization to assemble the substrate-binding site (Fig.1 iii), thereby turning on its holdase activity and effectively preventing proteins from misfolding. Thus, the mechanism of TF functional switching in the presence of a cofactor (Zn^{2+}) has been revealed.

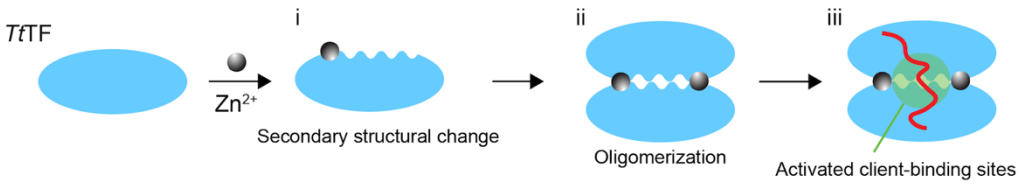

Fig.1 The possible mechanism for zinc-dependent activity modulation of *Th*TF.

2) The mechanism of *Ec*TF functional switching induced by *Ec*ClpX (Chapter III-IV)

To investigate how *Ec*TF changes from a holdase to an unfoldase when it interacts with *Ec*ClpX, the interaction pattern between *Ec*TF and *Ec*ClpX was observed by NMR titration experiments. Because *Ec*ClpX was known to undergo nucleotide-dependent conformational changes, the NMR titration experiments were performed in the absence and presence of the nucleotides. The NMR titration experiments showed *Ec*TF binding to *Ec*ClpX^{AAA} domain in a nucleotide-dependent manner: The NMR titration experiments also showed that the SBD domain of *Ec*TF (*Ec*TF^{SBD}) uses similar binding sites to interact with the ZBD domain of *Ec*ClpX (*Ec*ClpX^{ZBD}), showing *Ec*TF cannot bind to *Ec*ClpX^{ZBD} and *Ec*ClpX^{AAA} simultaneously. According to previous research, *Ec*TF$^{PPD\text{-}SBD}$ binds to ADP-bound *Ec*ClpX^{AAA} with higher affinity than the interaction between *Ec*TF^{SBD}-*Ec*ClpX^{ZBD}. Such results suggest that the nucleotide-dependent conformational changes of ClpX are propagated to TF-ClpX complex through the switching of the interaction sites.

To obtain more detailed information about the interaction between TF and ClpX, the structure of the *Ec*TF-*Ec*ClpX complex was investigated. Given the previous studies showing that the *Ec*TF^{SBD} interacts with the *Ec*ClpX^{ZBD} and both domains are responsible for the recognition of the substrate proteins, it is expected that the interaction between *Ec*TF^{SBD} and *Ec*ClpX^{ZBD} is a key to unveiling the mechanism of the handling of the substrate protein in the *Ec*TF-*Ec*ClpX complex. Thus, the structure of the *Ec*TF^{SBD}-*Ec*ClpX^{ZBD} complex was determined by NMR experiments. The structure showed how the substrate protein is released from *Ec*TF^{SBD} and transferred to *Ec*ClpX^{ZBD}. The substrate-handover between *Ec*TF^{SBD}-*Ec*ClpX^{ZBD} can be a key to explaining efficient protein unfolding by the *Ec*TF-*Ec*ClpX complex. Through the efficient substrate handover in complex with ClpX, TF achieves unfolding activity.

The oligomerization mediated by Zn^{2+} ions is a critical reason for the functional switching of *Tf*TF (Fig. 1). The intricate interaction pattern, substrate handover behavior can explain why *Ec*TF changes from a holdase to an unfoldase when it complexes with ClpX chaperone. Given the recent discoveries of the interaction and cooperativity between chaperones, the mechanism of the functional switching of TF chaperone investigated in this study will also provide new insights into the chaperone system in protein quality control in the cell.