学 位 論 文 内 容 の 要 旨

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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 Chapter I, the background about the functional switching of chaperones is introduced. In the 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 the protein folding, thereby preventing proteins from misfolding and/or promoting protein to be folded correctly. On the other hand, once 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 the 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 the dimerization accelerates the association rate of TF to the substrate proteins for enhanced holdase activity. Besides the dimerization, other strategies of TF for functional switching are 1) binding to the metal ion as observed for TF from *Thermus thermophilus* (*Tt*TF), and 2) the binding to the other chaperone as suggested in TF from *Escherichia coli* (*Ec*TF) in the 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. The mechanisms of the functional switching induced by Zn^{2+} and *Ec*ClpX are discussed in Chapter II and Chapters III-IV, respectively.

In Chapter II, to investigate how Zn^{2+} ions regulate the 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 follow the changes in the structure and oligomerization state of *Tt*TF by the addition of Zn^{2+}. The CD and NMR spectra showed that the zinc-binding induces the partial structural changes of *Tt*TF. SEC-MALS data showed that zinc-binding promotes the oligomerization of *Tt*TF. Given the previous report on *Ec*TF showing the relationship between the oligomerization and the activity modulation, the spectroscopic data suggest that the binding of Zn^{2+} to *Tt*TF induces the structural changes coupled with the oligomerization to form the assembled substrate-binding site, thereby effectively preventing proteins from misfolding. Thus, the mechanism of the functional switching in TF by the binding of a cofactor, Zn^{2+}, has been revealed.

In Chapter III, to investigate the functional switching of *Ec*TF from a holdase to an unfoldase by interactions with *Ec*ClpX, the interaction pattern between *Ec*TF and hexameric *Ec*ClpX was examined 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 nucleotide-

dependent binding of *Ec*TF to *Ec*ClpX^{AAA}: the tandem domain consisting of the proline peptidyl-prolyl *cis-trans* isomerase domain and substrate-binding domains of *Ec*TF (TF$^{PPD-SBD}$) selectively bind to the ADP-bound *Ec*ClpX subunits, whereas the ribosome-binding domain of *Ec*TF (TF^{RBD}) preferntially binds to the nucleotide-free *Ec*ClpX^{AAA} subunits (Fig. 1A). The nucleotide-dependence of interaction pattern implies the conformational changes of the *Ec*TF-*Ec*ClpX complex upon the ATP binding and hydrolysis: in the ATP-bound state, TF^{RBD} binds to the nucleotide-free *Ec*ClpX subunits, whereas *Ec*TF$^{PPD-SBD}$ binds to the ADP-bound *Ec*ClpX^{AAA} subunits when ATP is hydrolyzed (Figure 1B). The data suggests that the nucleotide-dependent conformational changes of ClpX are propagated to TF through the changing of the interaction sites, which explains the mechanism of the enhanced unfoldase activity of TF upon the binding to ClpX.

In Chapter IV, to obtain more detailed structural 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 *Ec*TF^{SBD} interacts with the zinc-binding domain of *Ec*ClpX (ClpX^{ZBD}) and both domains are responsible for the recognition of the substrate proteins, the interaction between TF^{SBD} and 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 that most of the substrate-binding sites on *Ec*TF^{SBD} are occupied by *Ec*ClpX^{ZBD}, whereas parts of the substrate-binding sites on *Ec*ClpX^{ZBD} are still exposed to the solvent even in the complex with *Ec*TF^{SBD} (Fig. 1B). Combined with the binding competition experiments, the substrate protein recognized by TF is found ro be transferred to ClpX through the interaction between *Ec*ClpX^{ZBD} and *Ec*TF^{SBD}, in which the substrate protein is released from *Ec*TF^{SBD} by the competitive binding of *Ec*ClpX^{ZBD} and, then, transferred to *Ec*ClpX^{ZBD} (Fig. 1B). This substrate transfer from *Ec*TF^{SBD} to *Ec*ClpX^{ZBD} would be essential to explain the efficient protein unfolding by the *Ec*TF-*Ec*ClpX complex. Associate with the efficient substrate transfer in the complex with ClpX, the unfolding activity of TF would be enhanced.

In Chapter V, the results and mechanisms of the functional switching in TF by cofactor (Zn^{2+}) and chaperone (ClpX) were summerized. The oligomerization mediated by Zn^{2+} ions is critical for the functional switching of *Tt*TF. The intricate interaction pattern, substrate transfer in the *Ec*TF-*Ec*ClpX complex can explain why *Ec*TF changes from a holdase to an unfoldase when it complexes with ClpX chaperone. Given the recent discoveries of the interactions and cooperative functions between chaperones, the mechanism of the functional switching investigated in this study will also provide new insights into the chaperone system in protein quality control in the cell.

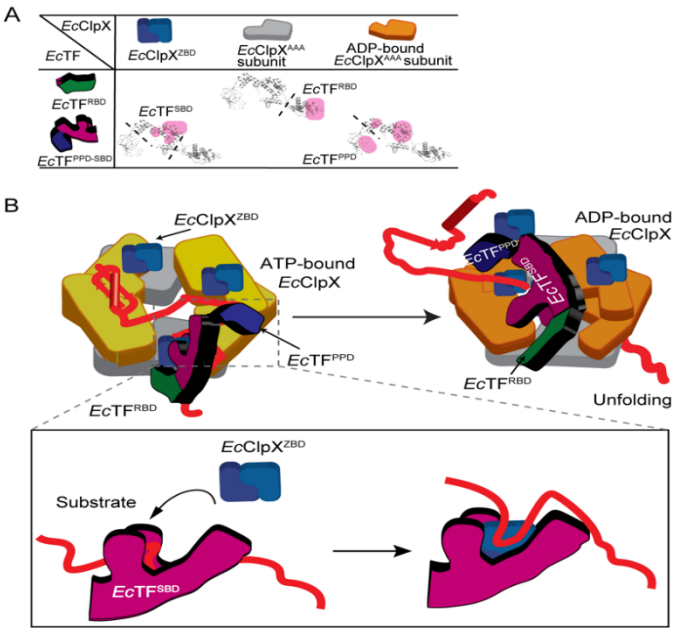

Fig. 1 The substrate unfolding by the *Ec*TF-*Ec*ClpX complex.