The 88th iCeMS

SEMINAR

CeMI Seminar Series 25

Mon 29 Aug 2011
10:00-11:00

Lecturer: **Osamu Nureki, Ph.D.**
Department of Biophysics and Biochemistry
Graduate School of Science
The University of Tokyo

Structural Basis for Dynamic Protein Translocation across Plasma Membrane by Sec Machinery

Venue: 2nd floor Seminar Room (#A207)
Main Building, iCeMS Complex 1
Kyoto University

Contact: Harada Group at harada-g@icems.kyoto-u.ac.jp
Hosted by: iCeMS (Institute for Integrated Cell-Material Sciences), Kyoto University
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Abstract of the seminar by Prof. Nureki on Aug. 29

About one-third of newly synthesized proteins are secreted across or integrated into membranes. The evolutionarily conserved Sec translocon (SecYEG in prokaryotes and Sec61$\alpha\gamma\beta$ in eukaryotes) functions as a protein-conducting channel to facilitate these processes that take place either co-translationally or post-translationally. In bacteria, SecA ATPase drives the post-translational translocation. We first present the 3.2 Å-resolution crystal structure of the SecYE translocon (ttSecYE) complexed with its specific Fab fragment, from a SecA-containing organism, *Thermus thermophiles* (1). The structure reveals a "pre-open" state, in which several SecY transmembrane helices are moved to create a hydrophobic crevasse open to the cytoplasm. Fab and SecA proved to competitively bind to a common site at the tip of the cytoplasmic domain of SecY. Molecular dynamics and disulfide mapping analyses suggested that the pre-open state represents a SecYE conformational transition that is inducible by SecA binding. Moereover, we identified SecY-contacting residues of SecA, which are buried in the isolated SecA molecule, suggesting that SecA also undergoes a conformational change upon binding to the translocon. These results suggest that the channel and the motor components of the Sec machinery undergo cooperative conformational changes to achieve dynamic interplay for transmembrane protein translocation.

In addition to the coupling with the ATP hydrolysis cycles of SecA, efficient export of proteins emerging from the translocon requires the proton-motive force (PMF) generated across the plasma membrane. A membrane-integrated Sec component, SecDF, associates with SecYEG and facilitates the completion of protein export, but its structure and exact function have remained elusive. Second, we show the 3.3 Å-resolution crystal structure of SecDF from *Thermus thermophilus* and higher resolution structures of its periplasmic domains. SecDF consists of a pseudo-symmetrical 12-helix transmembrane (TM) domain and two protruding periplasmic domains, suggesting that the TM region acts as a proton transporter and the first periplasmic region converts its architectures with a hinge motion during the protein translocation (2). Patch-clamp approach revealed the ion-channel activity of SecDF in the presence of PMF and a unfolded protein, which is also supported by *in vivo* functional analysis of Vibrio SecDF-1. Structural-based functional analysis of SecDF identified that several conserved charged residues at the transmembrane SecD/SecF-interface that are assumed to be relate to proton-transport and the flexible periplasmic region are crucial for the SecDF function. Moreover, *in vitro* translocation assays showed that the completion of protein translocation requires both SecDF and PMF and also detected interactions of periplasm domain with translocating polypeptides. We propose that SecDF functions as a dynamic, membrane-integrated chaperone, which is powered by PMF to actively complete SecYEG-mediated protein translocation.

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