Title of Project : Structural study of bacterial flagellar motor and protein export apparatus by electron cryomicroscopy

Keiichi Namba
(Osaka University, Graduate School of Frontier Biosciences, Professor)

Research Area : Biophysics
Keyword : Macromolecular assembly, Structural analysis, Electron cryomicroscopy, X-ray crystallography, Bacterial flagellum

【Purpose and Background of the Research】

The bacterial flagellum is a rotary nanomachine composed of the basal body as a rotary motor, the filament as a helical propeller, and the hook as a universal joint connecting these two parts. The flagellum is a large macromolecular assembly composed of about 30 different proteins with copy numbers from a few to a few tens of thousands, and its assembly relies on protein export through the central channel of the growing flagellum to the distal end where subunits self-assemble. The mechanisms of torque generation and protein export remain unknown due to the lack of structural information of the functional flagellar basal body. The core part of the basal body can be isolated from the cell membranes, but many components such as the stator and the protein export apparatus are lost during the purification procedure by the use of detergents to solubilize the membranes. The purpose of this project is to stabilize functional basal body complexes and solve the structures by electron cryomicroscopy (cryoEM) to provide the basis for our understanding of the mechanisms of torque generation and protein export.

【Research Methods】

One way to stabilize functional flagellar basal body complexes is *in vitro* reconstitution by adding lost component proteins that are purified from overexpression systems to the basal body that is isolated by the conventional method. We can also search for mutations by which functional complexes are stabilized. CryoEM and single particle image analysis will be used to obtain 3D structures of reconstituted or stabilized basal body complexes, and atomic models of component proteins obtained by X-ray crystallography will be docked into the 3D maps of the functional complexes to closely look into the intermolecular interactions for detailed analysis of possible conformational changes for various functions and mechanisms.

【Expected Research Achievements and Scientific Significance】

Structural information of the flagellar basal body complex representing various functional stages will provide crucial clues to our detailed understanding of the mechanisms. It will also provide crucial information to understand how biological nanomachines can work at an extremely high energy efficiency, which is also one of the central questions in biophysics. This approach will also be generally applicable to many different macromolecular assemblies for deducing their mechanisms. CryoEM and image analysis is becoming increasingly powerful in visualizing high-resolution structures of biological macromolecular assemblies without crystallization and will be an essential tool to achieve such difficult tasks as visualizing biological structures at various functional stages that are not stable.

【Publications Relevant to the Project】

Minamino, T., Imada, K. & Namba, K. (2008)
Mechanisms of type III protein export for bacterial flagellar assembly. *Mol. BioSyst.* **4**, 1105-1115.

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Molecular motors of the bacterial flagella. *Curr. Opin. Struct. Biol.* **18**, 693-701.

Kojima, S., Furukawa, Y., Matsunami, H., Minamino, T. & Namba, K. (2008)
Characterization of the Periplasmic Domain of MotB and Implications for Its Role in the Stator Assembly of the Bacterial Flagellar Motor. *J. Bacteriol.* **190**, 3314-3322.

González-Pedrajo, B., Minamino, T., Kihara, M. & Namba, K. (2006)
Interactions between C ring proteins and export apparatus components: a possible promotion mechanism for facilitating type III protein export. *Mol. Microbiol.* **60**, 984-998.

【Term of Project】 FY2009-2013

【Budget Allocation】 157,600 Thousand yen

【 Homepage Address and Other Contact Information】

http://www.fbs.osaka-u.ac.jp/eng/labo/02/