【Grant-in-Aid for Specially Promoted Research】

Biological Sciences

Title of Project : Structures and functions of macromolecular motors by electron cryomicroscopy

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Research Area : Biophysics
Keyword : Biophysics, Nanobio, Nanomachine, Molecular motor

【Purpose and Background of the Research】

Three-dimensional structural information of biological macromolecular machinery is essential for the understanding of mechanisms by which they function, and various methods for structural analyses have been developed to obtain structural information at highest possible resolution. The purpose of this project is to solve the structures of macromolecular complexes at atomic resolution to understand mechanisms of how force is generated and regulated by motor protein complexes.

【Research Methods】

The main method we will develop and use is electron cryomicroscopy (cryoEM) and image analysis of macromolecular complexes in combination with X-ray crystallography to visualize their 3D structures in detail. We will first introduce a direct electron-detection CMOS camera of which resolution is by far higher than the present CCD camera to further improve the achievable resolution and throughput of cryoEM image analysis. We will also automate image data collection by modifying the stage control and software development. The structures of various motor protein complexes including bacterial flagellar motor will be visualized at near atomic resolution in various functional states. Such structural analyses in combination with functional assays by genetics, biochemistry and single molecule nanophotometry at high temporal and spatial resolution should lead to clear understanding of how motor protein complexes generate force in well-regulated manner.

【Expected Research Achievements and Scientific Significance】

Motility and movement are essential properties of the function of life. Motility is driven by well-regulated motor protein complexes, such as actin-myosin, microtubule-kinesin, and microtubule-dynein, which are ATP-driven linier motors. The bacterial flagellar motor is a bi-directional rotary motor fueled by cation flow through an ion channel driven by electrochemical potential difference across the cell membrane. The level of minimum energy requirement is close to that of thermal noise and yet the energy conversion efficiency is close to 100%, which is in a sense far superior to man-made motors, but it remains unclear how they work. For our in-depth understanding of energy transduction mechanisms of such motor proteins, their high-resolution structures in many different functional states must be revealed. We will develop and apply substantially improved cryoEM image analysis techniques in combination with X-ray crystallography as well as advanced methods for functional assay to challenge these difficult problems. Our previous results suggest that a high-throughput, near atomic-resolution (3 - 4 Å) structural analysis would not be very far from actual achievement. If this challenge is proved to be successful, it will drastically change the fields of structural biology as well as many fields of biological sciences.

【Publications Relevant to the Project】

Ruan, J., Kato, T., Santini, C.-L., Miyata, T., Kawamoto, A., Zhang, W.-J., Bernadac, A., Wu, L.-F. & Namba, K. (2012)
Architecture of a flagellar apparatus in the fast-swimming magnetotactic bacterium MO-1. *Proc. Natl Acad. Sci. USA*, **109**, 20643-20648.

Gayathri, P., Fujii, T., Møller-Jensen, J., van den Ent, F., Namba, K., Löwe, J. (2012)
A bipolar spindle of antiparallel ParM filaments drives bacterial plasmid segregation. *Science* **338**, 1334-1337.

Fujii, T., Iwane, A. H., Yanagida, T. & Namba, K. (2010)
Direct visualization of secondary structures of F-actin by electron cryomicroscopy. *Nature* **467**, 724-729, 2010.

Yonekura, K., Maki-Yonekura, S. & Namba, K. (2003)
Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. *Nature* **424**, 623-650.

【Term of Project】 FY2013-2017

【Budget Allocation】 442,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.fbs.osaka-u.ac.jp/jpn/general/lab/02/