1P-185 MotB ペリプラズム側断片を用いた細菌べん毛モーター固定子複合体の自己集合の解析

Analysis of the stator assembly by using periplasmic fragment of MotB, a stator component of the bacterial flagellar motor

Yoshio Yuasa (1), Seiji Kojima (1) and Michio Homma (1). (1: Div Biological Science, Graduate School of Science, Nagoya Univ)

In the proton driven flagellar motor, membrane proteins MotA and MotB are required for flagellar rotation. MotA and MotB form a stator complex and function as a proton channel that couples proton flow with torque generation. MotB has a single transmembrane segment and a peptidoglycan-binding (PGB) motif in the C-terminal periplasmic domain. The PGB motif of MotB is believed to associate with the peptidoglycan layer to anchor the MotA/MotB stator complex around the rotor. However, it remains still unknown how the stator complex is targeted and anchored near the rotor. To investigate the stator assembly more precisely, we used an N-terminally truncated MotB fragment from *Salmonella* (MotBC). Previously we showed that MotBC significantly inhibited motility of wild-type cells when exported into the periplasm, and it forms a stable dimer. Here, we used the *E. coli* strain that expresses GFP-MotB, which is localized around the motor as judged by a fluorescent dot on the cell, to investigate how MotBC affects GFP-MotB localization. When MotBC was overexpressed in the cytoplasm of GFP-MotB background, we could observe a number of fluorescent dots, whereas they were significantly reduced when MotBC was overexpressed in the periplasm. Flagellar rotation was examined by the tethered cell assay for these strains, and none of the motor rotates when MotBC was overexpressed in the periplasm of GFP-MotB cells. These results suggest that MotBC in the periplasm inhibited the functional stator assembly so that impaired motility.

1P-186 細菌べん毛モーター固定子蛋白質 MotB のペリプラズムドメインの結晶構造

Crystal structure of the periplasmic domain of MotB, a stator protein essential for torque generation of the bacterial flagellar motor

Seiji Kojima (1), Mayuko Sakuma (1), Yuki Sudo (1), Chojiro Kojima (2), Tohru Minamino (3), Keiichi Namba (3), Michio Homma (1) and Katsumi Imada (3). (1: Division of Biological Science, Graduate School of Science, Nagoya Univ.; 2: Graduate School of Biological Sciences, Nara Institute of Science and Technology; 3: Graduate School of Frontier Biosciences, Osaka Univ.

MotA/MotB stator complex functions as a proton channel and couples proton flow with torque generation in the bacterial flagellar motor. The stator must be anchored to an appropriate place of the motor, which is believed to occur through a putative peptidoglycan-binding (PGB) motif within the C-terminal periplasmic domain of MotB. In this study, we constructed, characterized and determined the crystal structure of a periplasmic fragment of *Salmonella* MotB consisting of residues 88 through 291 (MotB_{C6}). MotB_{C6} fused with a leader sequence at its N-terminus was exported to the periplasm and significantly inhibited motility of wild-type cells. Purified His₆-tagged MotB_{C6} (MotB_{C6}-His₆) formed dimer as judged by the size exclusion chromatography and analytical ultracentrifugation. Native and selenoMet-derivative crystals of MotB_{C6}-His₆ with the space group of P3₂2₁ were obtained by sitting-drop vapor-diffusion method. The structure was determined at 2.0 Å resolution using diffraction data collected at SPring-8 beamline BL41XU. It revealed the MotB_{C6} dimer structure with a single domain. Its core structure including PGB motif shows remarkable structural similarities to peptidoglycan binding proteins such as Pal, RmpM and MotY. The N-terminus of PGB core is connected to an extra α -helix and β -sheet sandwich structure, which contains a long N-terminal α -helix protruding from the domain. Mutational analyses in this domain based on structural insights are currently ongoing. Possible mechanisms of stator assembly and function will be discussed.

1P-187 ネクロシスの蛍光イメージング ; MPT の関与

Fluorescence imaging of Necrotic Cell Death; Involvement of MPT

Chisako Fujita (1), Xiaolei Shi (1), Yoshihiro Matunomoto (1), Yoshihiro Ohta (1). (1: Div of Biotech & Life Science, Tokyo Univ of Agri and Tech)

The aim of the present study is to investigate whether or not the increase in the permeability of inner mitochondrial membrane (MPT) is responsible for necrotic cell death with imaging techniques. Although a lot of intracellular mechanisms of necrosis remain unclear, there is a hypothesis that MPT triggers necrosis when oxidative stress is given to cells. This hypothesis is based on the fact that the mitochondrial membrane potential is collapsed upon necrosis and that MPT is observed in cells which have undergone necrosis. The collapse of the mitochondrial membrane potential, however, does not always indicate MPT. Further, MPT can occur even after necrotic cell death. Here we examined whether MPT is the cause of necrosis or not by observing the release of hydrophilic fluorescent probe from mitochondria with fluorescence microscopy. We used calcein as a fluorescent probe entrapped in mitochondria, t-BuOOH as pro-oxidant and C6 glioma cells. Tert-BuOOH first induced the shrinkage of cells followed by the rupture of the plasma membrane. When cells begun to

shrink, mitochondria still maintained the membrane potential and dissipated it just before the rupture of plasma membrane at the end of necrotic cell death. These results imply that MPT is not the trigger of t-BuOOH induced cell death. To examine whether MPT takes place in the end of necrosis, we observed the release of calcein previously entrapped in the mitochondria. The results will be discussed at the conference.

1P-188 低出力超音波刺激による初代培養グリア細胞の機能変化

Effects of low-intensity pulsed ultrasound stimulation on the functions of primary cultured astrocytes

Sadahiro Iwabuchi and Koichi Kawahara. Laboratory of Cellular Cybernetics, Graduate School of Information Science and Technology, Hokkaido University.

Astrocytes, which represent the most abundant cell population in the brain, guide axonal growth during development. Recently, high intensity (1 W/cm²) ultrasound promotes the nerve conduction velocity and also, therapeutic ultrasound (0.4 W/cm²) enhances nerve regenerations in the *in vivo* neuropathy models, revealing that ultrasound stimulations might be responsible for regenerating neurons and glial cells. However, the detail of the effects of ultrasound on the nerve tissues is still unknown. This study aims at elucidating whether non-thermal therapy of low-intensity pulsed ultrasound (LIPUS; 0.03 W/cm²) stimulation has a potential to affect astrocytic proliferation and migration. Primary rat astrocytic cultures were obtained from the cortical hemispheres. LIPUS stimulations were applied for 20 min every day during culture periods. LIPUS apparatus, which is widely used for healing a bone fracture, was obtained from Smith & Nephew. An analysis by MTT-assay demonstrated that LIPUS stimulations did not affect the proliferation activity of astrocytes. Next, we carefully scratched the cell cultures in the non LIPUS-treated groups to make a pore space, and 1 day later, we calculated the healing (migration) rate in the culture groups with or without a single LIPUS stimulation. The migration rate in the culture group without and with LIPUS-treatment was 52.3±1.7 and 64.0±1.6% (mean±S.E.), and significantly differed. These results indicated that LIPUS stimulations are effective for astrocytic migrations.

1P-189 Spiroplasma バクテリア運動の流体力学

Hydrodynamics of Spiroplasma bacterial motility

Hirofumi Wada(1), Roland R. Netz(2). (1: Yukawa Institute for Theoretical Physics, Kyoto University; 2: Physics Department, Technical University Munich)

Spiroplasma bacteria are tiny helical-shaped eubacteria with typically a few microns length. Since they lack a rigid cell wall, their helical cell shape is maintained by an internal ribbon-shaped cytoskeleton and the motility of Spiroplasma is provided by contractile motion of the cytoskeleton. Recent video-microscopy observations found that Spiroplasma meliferum swim through viscous media by generating kinks or domain walls between sections of opposite helicities and by sending them down their helical body. To reveal a underlying hydrodynamic mechanism for this directed propulsion of Spiroplasma, we formulate a simple elastic helical rod model with alternating helicities along the helical contour. Using hydrodynamic simulations and analytic arguments, we show that the kinks or domain walls propagation leads to a directed propulsion of the cell body opposite to the kink traveling direction. In particular we show that the helical pitch angle observed for Spiroplasma meliferum, 35°[deg], is optimized for maximal swimming speed and efficiency. Our analytic theory based on the slender-body hydrodynamics approximation agrees very well with our numerical data, giving the physical interpretation how the chirality switch propagating along the helical cell body is converted to a translational thrust for the cell body itself.

1P-190 カテコールアミン誘導性脂肪分解にともなう脂肪細胞内での脂肪滴の形態変化

Morphological changes of lipid droplets in adipocytes during catecholamine-induced lipolysis

Masafumi Nagayama (1), Kyoko Shimizu (2), Toshio Taira (2), Tsutomu Uchida (1) and Kazutoshi Gohara (1). (1: Division of Applied Physics, Graduate School of Engineering, Hokkaido Univ.; 2: Primary Cell Co., Ltd.)

Release of the energy as free fatty acid and glycerol via lipolysis is one of the fundamental functions in adipocytes. To date, many biochemical and molecular biological studies have identified receptors, kinases and lipases involved in the mobilization of lipid. In contrast, morphological changes of lipid droplets (LDs) during adipocyte lipolysis have not been clarified yet.

In the present study, we performed time-lapse observations of adipocyte lipolysis induced by stimulation with norepinephrine (NE). According to the time-lapse observations, large LDs shrank rapidly and finally disappeared from 2 to 8 h after the stimulation. However, not all of adipocytes shrank LDs in their cytoplasm. To examine the relation between translocation of endogenous lipases and morphological changes of LDs, we performed immunofluorescence observations of adipose triglyceride lipase (ATGL) and phospho-hormone sensitive lipase (phospho-HSL). ATGL showed a punctate distribution in the cytoplasm of all adipocytes, and the stimulation with NE did not alter the distribution of ATGL. On the other hand, phospho-HSL was localized on the surface of LDs when lipolysis was stimulated with NE. However, the number of phospho-HSL-positive adipocytes is inconsistent with that of adipocytes which shrank LDs. In summary, the translocation of ATGL and HSL alone cannot explain the shrinking mechanism of LDs during catecholamine-induced lipolysis.