3M1522 Study of the unique flagellar apparatus of magnetotactic bacteria MO-1 by Electron cryotomography

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Recently, a new magnetotactic bacterium called magneto-ovoid strain MO-1 was isolated from the Mediterranean sea by Wu's group. The bacterium shows a unique flagellar architecture and swims at an unusually high velocity. Here, we studied this unique flagellar apparatus by electron cryotomography. We find that the MO-1 cell has two flagellar bundles and each bundle is composed of a sheath, filaments and fibrils. The sheath envelops filaments and fibrils, and it can be easily detached from the cell, resulting in the dispersion of the filaments and impaired cell motility. Three-dimensional image reconstructions of the flagellar bundle embedded in vitreous ice clearly visualized that the majority of bundles consists of seven filaments and 24 fibrils; six filaments have a hexagonal distribution with the seventh in the middle; the fibrils are also organized in a hexagonal array around each filament and each fibril is situated between two filaments. Occasionally, eight filaments were also found in one bundle with similar hexagonal arrangement. The 7 filaments and 24 fibrils wind around one another within the sheath, and they are attached individually to the cell with a pit-like structure at the base. The 3D image reconstruction also revealed that the sheath has a left-handed helical structure formed by subunits that appear to be different from the cell surface structure. Therefore, unlike the single-flagellum sheath, that of MO-1 flagellar bundle does not seem to be the extension of the cellular wall.

3M1534 べん毛蛋白質輸送における FlgN シャペロンの役割**Role of a bacterial flagellar chaperone FlgN in flagellar type III protein export**

Akira Hida¹, Tohru Minamino^{1,2}, Keiichi Namba¹ (¹Graduate School of Frontier Biosciences, Osaka University, ²PRESTO, JST)

The bacterial flagellum is a rotary motor for motility. It consists of the basal body, the hook and the filament. Most of component proteins are transported to the growing end of the flagellum by the flagellar type III protein export apparatus, consisting of integral membrane proteins (FlhA, FlhB, FlhO, FlhP, FlhQ, FlhR) and soluble proteins (FliH, FliI, FliJ). The FliH-FliI-FliJ complex escorts export substrates from the cytoplasm to the export gate complex made of the six integral membrane proteins. FlgN, FliA, FliS, and FliT act as substrate-specific chaperones to facilitate the export of their cognate substrates. FlgN binds to its cognate substrates, FlgK and FlgL, to prevent their premature aggregation. The FlgN-FlgK complex binds to the FliH-FliI complex. Recently, it has been also shown that FlgN interacts with the C-terminal cytoplasmic domain of FlhA to facilitate the export of FlgK and FlgL. Because FlgK and FlgL are exported after hook-basal body assembly is completed, it is thought that interactions of FlgN with these export apparatus proteins must be highly regulated during protein export. However, it remains unknown how.

To investigate the regulatory mechanism of FlgN, we carried out genetic analysis of a *Salmonella* mutant. A deletion of FlgN resulted in a significant decrease in the secretion levels of hook-type substrates. The extragenic suppressor mutations in FlhA and FliS improved the secretion activity of the $\Delta fliH-fliI fliB(P28T) \Delta flgN$ mutant. We will discuss the role of FlgN in protein export.

3M1546 部位特異的架橋法によるべん毛蛋白質 FliH の相互作用解析**Analysis of Interactions of the Flagellar Export Protein FliH by Site-Directed Crosslinking**

Noritaka Hara¹, Keiichi Namba¹, Tohru Minamino^{1,2} (¹Grad. Sch. of Frontier Biosci., Osaka Univ., ²PRESTO, JST)

Most of flagellar proteins are transported by the bacterial flagellar type III protein export apparatus translocates from the cytosol to the growing distal end of the flagellum where their self-assemble occurs. A flagellar ATPase FliI forms a heterotrimeric complex with a dimer of its regulator FliH in the cytoplasm. The FliH2-FliI complex delivers export substrates to the export gate made of six integral membrane proteins of the export apparatus through an interaction between FliH and a C ring protein FliN, and helps the initial entry of the substrates into the export gate. Trp-7 and Trp-10 of FliH are critical not only for the interaction with FliN but also for efficient docking of the FliH2-FliI complex

to the export gate. However, the molecular details of the interactions of FliH with FliN and the export gate remain to be elusive. Here, to clarify these interactions in more detail, we carried out site-directed crosslinking and genetic analysis.

Disulfide crosslinking between FliH and FliN shows that Trp-7 and Trp-10 of FliH directly interact with the hydrophobic patch of FliN. Co-expression of FliI with FliH increased the efficiency of formation of the crosslinking product in FliH-7C/FliN-111C pair, indicating that FliI increases the binding affinity of FliH for FliN. Site-directed photo-crosslinking with p-benzoyl-phenylalanine revealed that Trp-7 and Trp-10 of FliH interact with the export gate component FlhA. We are currently attempting in vivo cross-linking to dissect the export process.

9:00~12:00 N会場 / Room N

蛋白質_蛋白質工学/進化学、生命の起源・進化

Protein: Measurement & Analysis, Origin of life & Evolution

3N0912 Experimental and computational studies on mutational effects on nylon-6 byproducts-degrading enzyme

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For material synthesis and degradation processes, usages of proteins or enzymes are now widely recognized as beneficial methods due to its high substrate preference and high reaction activity under mild conditions. Development of highly active nylon-6 byproducts-degrading enzyme leads to advanced environmental cleanup systems and is also useful for recycling raw materials from byproducts in wastes by the enzymes. For these purposes, our group has studied on this enzyme by combining with biochemical experiments, crystal structure analyses, and theoretical studies. According to these works, we found following points:

(1) There are two kinds of the enzymes (*Arthrobacter* sp. and *Pseudomonas* sp.), where the difference between these enzymes is that the value of K_m of the later is lower than that of the former (our main target)

(2) From X-ray structure and molecular dynamics simulations, structural fluctuation of the carboxyl group of the substrate in aqueous solutions is found to be larger than that in crystal structure, where a glycerol, which is a cryoprotectant, stabilizes the carboxyl group in the crystal structure.

From these results, we focused on amino acid sequences around the carboxyl group of the substrate and prepared some mutants (G316I, F317Y, and Y370V) to tightly bind the substrate. We confirmed some mutant enzymes improve its degradation activity, determined the X-ray structures of the highest activity mutant, and proposed structure-function relationships of them from theoretical viewpoints.

3N0924 ファージディスプレイ法を用いたサルモネラ菌フック蛋白質結合ペプチドの探索**Selection of the flagella hook binding peptides using the Phage-display Technique**

Mai Nemoto, Tomoko Kanamaru, Hideyuki Yoshimura (*Sch. of Sci. and Tech., Meiji Univ.*)

The flagellar hooks of *Salmonella typhimurium* and *E. coli* exist between basal body and filament plays an important role to transmit the force of rotary motor to filament. However, compared with mechanical aspects of filament, that of hook has not studied enough because of the short length. In this presentation, we report selection of the peptides which have affinity against hook (polyhook) of *Salmonella* from peptide phage library. The hook binding peptides were fused into N-terminal of apoferritin in order to label the hook.

The peptide interacting with the hook was searched by the phage display with M13 phage. Polyhook produced by *Salmonella* SJW108 ($\Delta FliK$) was utilized for phage display and panning was performed 3 times. In consequence we succeed to get a peptide of 12 residues, GNLFASPQKMYR, as a candidate of the peptide interacting with polyhook. Then we constructed the expression vector in which the peptide sequence was fused into N-terminal of horse spleen L-apoferritin (fer8). We found that this fusion protein HK-fer8 was well-expressed in *E. coli* but it tended to have more aggregate than native fer8. HK-fer8 in soluble fraction was purified by chromatography and confirmed by SDS-PAGE. The shape of HK-fer8 was similar to that of native apoferritin. Now we are trying to analyze the interaction between the hook and HK-fer8.