1P185

Interactions between *Vibrio* phage KVP40 gp5 and gene product of ORF334, T4 gp27 homologue

Mai Nemoto, ○Shuji Kanamaru, Fumio Arisaka

Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology

Recently, the whole genome sequences of many bacteriophages were determined. In T4-related phages, there are four subgroups based on the homology of major capsid and sheath proteins of T4 phage with that of other phages; namely T-even, Pseudo T-even, Schizo T-even and Exo T-even.

In some phages belonged to T-even or Pseudo T-even, the amino acid sequence of gp5 is well conserved. However, gp5 of *Vibrio* phage KVP40, which belongs to Schizo T-even, has high homology in N- and C-terminal domain, but lacks the lysozyme domain including the five essential residues for the lysozyme activity. We have expressed and isolated gp5 from KVP40 phage, and demonstrated that it forms a trimeric β -helix like that of T4 phage by analytical ultracentrifugation (AUC) and the CD spectrum. The lysozyme activity was examined by a spot test against chloroform treated *E. coli* and *Vibrio cholerae*.

On the other hand, PSI-BLAST search cannot detect any ORF in the KVP40 genome that corresponds to gp27 of T4. Nevertheless, believing that there must be a T4 gp27 homologue, we picked up the ORF334 as the candidate based on the size and the location in the genome. ORF334 was then cloned and successfully co-expressed with gp5. It was subsequently found that the gene product of ORF334 strongly interacts with gp5. Purified complex was analyzed by AUC and it forms a 3:3 complex.

1P187

Role of the N-terminal disordered region in *Salmonella* hook protein FlgE in its export and self-assembly

○Nao Moriya^{1,2}, Tohru Minamino^{1,3}, Hedda U. Ferris⁴, May Macnab⁴, Keiichi Namba^{1,3}

¹Graduate School of Frontier Biosciences, Osaka University, ²JSPS Research Fellow DC, ³Dynamic NanoMachine Project – ICORP – JST, ⁴Department of Biophysics and Biochemistry, Yale University

The bacterial flagellum is a motile organelle, which consists of three parts: the basal body, the hook, and the filament. The hook is a short, highly curved tubular structure made of the hook protein FlgE, connects the filament and the basal body, and functions as a universal joint to smoothly transmit torque produced by the flagellar motor to the filament. Samatey *et al.* (2004) revealed the structure of FlgE missing the disordered regions of both termini by X-ray crystallography and proposed a model of the hook structure and the switching mechanism for a universal joint function. However, without these terminal regions, FlgE cannot properly polymerize into the hook either *in vivo* or *in vitro*. Thus, it still remains unknown at atomic level how FlgE self-assembles stably into the hook structure to act as a universal joint. Also, the disordered N-terminal region is found to be essential for recognition by the flagellar type III protein export apparatus. However, the signal recognized by the export apparatus is not known. In this study, to investigate how the N-terminal disordered region of FlgE contributes to its recognition by export apparatus, to FlgE assembly and to the hook polymorphism in more detail, we constructed scanning N-terminal deletion variants of FlgE and tested their complementation ability. We examined their secretion ability, swimming behavior and hook morphology. From the results obtained, the role of the N-terminal region of FlgE in the export and self-assembly will be discussed.

1P186

Role of the T3S4 Domain of the Hook-Length Control Protein FliK in Export Specificity Switching of the Flagellar Type III Export Apparatus

○Tohru Minamino^{1,2}, Hedda F. Ferris³, Nao Moriya^{1,4}, May Kihara³, Keiichi Namba^{1,2}

¹Grad. Sch. of Fron. Biosci., Osaka Univ., ²Dynamic Nano. Proj., ICORP, JST,

³Dept. of MB & B, Yale Univ., ⁴JSPS Research Fellow DC

The bacterial flagellum functions as a rotary nanomachine, consisting of the basal body, the hook and the filament. Most of the flagellar components are translocated through the central channel of the growing structure by the type III flagellar protein export apparatus.

Export specificity switching of the type III flagellar protein export apparatus is believed to occur upon completion of hook assembly by way of an interaction of the T3S4 domain (FliKT3S4) of the hook-length control protein FliK with an integral membrane export apparatus component, FlhB. FliKT3S4 consisting of amino-acid residues 265-405 has an unstable and flexible conformation in its last 35 residues (FliKCT). FliK-driven switching is highly regulated and occurs only in the presence of the hook, and the conformational flexibility of FliKCT appears to play a role in preventing the interaction of FliKT3S4 with FlhBC at inappropriate stages of flagellar assembly.

To investigate the role of FliKT3S4 in substrate specificity switching, we constructed and characterized sequential 10-amino-acid in-frame deletion and point-mutation variants of FliK and also carried out a genetic analysis of FliKT3S4 by isolating suppressor mutants of the fliK(S319Y) mutant, which cannot change substrate specificity at the correct time in the flagellar assembly process. We show that residues 301-350 and the last five amino acids of FliK are essential for the switching. We will discuss the flagellar export specificity switching mechanism in terms of the FliK-FlhB interactions.

1P188

Reconstitution of V_1 complex of *Thermus thermophilus* V-ATPase revealed that ATP binding to the A subunit is crucial for V_1 formation

○Hiromi Imamura¹, Sacko Funamoto², Masasuke Yoshida^{2,3}, Ken Yokoyama²

¹Inst. Sci. Indust. Res., Osaka Univ., ²ATP System Project, JST, ³Chem. Res. Lab., Tokyo Inst. Tech.

Vacuolar-type H^+ -ATPase (V-ATPase) is a multi-subunit complex comprised of a water-soluble V_1 complex, responsible for ATP hydrolysis, and a membrane-embedded V_o complex, responsible for proton translocation. Although efforts have been made to elucidate assembly pathway of V-ATPase subunits, assembly of V_1 subunits into the complex is still ambiguous. In this study, we investigated the assembly pathway into V_1 complex by reconstituting the *Thermus thermophilus* V_1 complex from the monomeric A and B subunits and DF subcomplex. The V_1 complex of *T. thermophilus* V-ATPase is with a subunit composition of A_3B_3DF , in which the A and B subunits form a hexameric ring structure and the central stalk composed of the D and F subunits is penetrated inside the ring. Assembly of these components into the V_1 complex required binding of ATP to the A subunit, although hydrolysis of ATP is not necessary. In the absence of DF subcomplex, the A and B monomers assembled into A_2B_1 and A_3B_3 subcomplexes in an ATP binding-dependent manner, suggesting that ATP binding-dependent interaction between the A and B subunits is a crucial step of assembly into V_1 complex. Kinetic analysis of assembly of the A and B monomers into A_2B_1 heterodimer using fluorescence resonance energy transfer (FRET) revealed that the A subunit binds ATP very rapidly and prior to binding the B subunit. Kinetics of binding of a fluorescent ADP analog, N-methylanthraniloyl ADP (mant-ADP), to the monomeric A subunit also supported the rapid nucleotide binding to the A subunit.