Structural basis of type IVb pilus-mediated bacterial adhesion to the intestinal epithelium

Initial attachment and subsequent colonization of the host cell surface are critical events that allow bacterial pathogens to survive in the host environment and exert their pathogenic effects. For efficient attachment and colonization, enteric pathogens have evolved numerous surface organelles, with filamentous protein polymers termed pili or fimbriae being the most prominent [1]. Enterotoxigenic *Escherichia coli* (ETEC) (Fig. 1(a)), a major pathogen causing diarrhea in travelers and children in developing countries, expresses a long, rod-like proteinaceous fiber called colonization factor antigen/III (CFA/III), which is an operon-encoded type IVb pilus (T4bP) synthesized through the assembly of its major and minor type IV pilin subunits, CofA and CofB, respectively.

Despite its known importance in bacterial pathogenesis, little is known about the structure and adhesion mechanisms of CFA/III. Therefore, we first determined the crystal structure of the CFA/III major pilin subunit, CofA, at a resolution of 0.90 Å by single-wavelength anomalous dispersion (SAD) phasing using five sulfurs in the protein. Given the counterbalance between anomalous signal strength and the undesired X-ray absorption of the solvent, we collected diffraction data selectively at a wavelength of 1.5 Å on SPring-8 **BL38B1**, which were sufficient to perform successful sulfur SAD phasing [2]. The determined CofA structure adopts an α/β-roll fold typical of type IVb pilin protein (Fig. 1(b)) and shows high similarity to that of another type IVb pilin, TcpA, which is the major pilin subunit of the toxin-coregulated pilus from *Vibrio cholerae*, including the spatial distribution of key residues critical for pilus assembly. Although this allows us to build a pilus-filament model of CofA via computational docking using a previously proposed filament model of TcpA as a structural template, the lack of structural information regarding the minor pilin subunit, CofB, hampers the acquisition of comprehensive data on CFA/III pilus. Therefore, the crystal structure of CofB was determined at a resolution of 1.88 Å by the SAD method using SeMet-derivatized crystals of CofB. The diffraction quality of CofB crystals was initially very poor. However, several post-crystallization protocols including dehydration of the crystals substantially improved the resolution limit of these crystals from ~4.0 Å to ~2.0 Å when measured at BL38B1 [3]. The determined CofB structure shows novel three-domain architecture, where each domain is connected by a short linker loop (Fig. 1(c)). In addition to the N-terminal type IVb pilin-like domain, the C-terminal

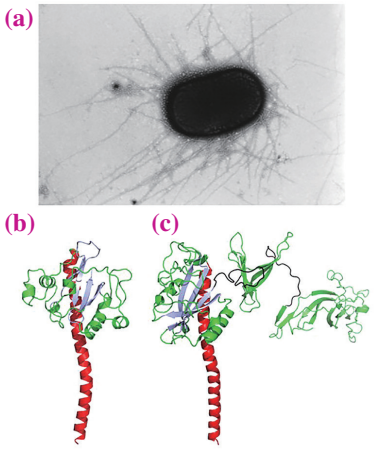

Fig. 1. Type IVb pili of enterotoxigenic *Escherichia coli* (ETEC). **(a)** Transmission electron micrograph of wild-type ETEC 31-10 strain. **(b)** Full-length structure of CFA/III major pilin CofA. **(c)** Full-length structure of CFA/III minor pilin CofB. Because the conserved N-terminal hydrophobic segment (1-28) of each pilin was truncated to solubilize the protein, the corresponding N-terminal segment was modeled using the full-length PAK pilin structure of type IVa pilin (PDB entry: 1OQW) as a template.

region of CofB is composed of two characteristic β-strand-rich domains that were subsequently revealed to homotrimerize in solution by domain-swapping to form a pilus assembly initiator complex. This initiator complex efficiently promotes T4bP assembly (Fig. 2) [4]. We also noted that at the distal pilus end, the CofB C-terminal β-strand-rich domain adopts an H-type lectin fold that bears substantial structural similarity with that of trimeric discoidin I from *Dictyostelium discoideum* (Fig. 2).

As H-type lectins generally bind N-acetylgalactosamine (GalNAc) molecules at the conserved binding pocket of their trimeric interfaces, it was initially thought that CofB may function as a lectin, targeting the small intestine mucosal glycome. However, we unexpectedly found that, in order to attach to the target cell surface, CFA/III pilus requires additional interaction with a protein named CofJ, a secreted protein of unknown function encoded by the CFA/III operon, at the expected binding interfaces of the CofB trimer located at its pilus-tip [5]. Interaction analyses indicated that the N-terminal flexible 24 residues of CofJ were responsible for the CofB-CofJ interaction. We subsequently elucidated the crystal structure of the corresponding N-terminal region (namely CofJ (1-24) peptide) in complex with CofB at a resolution of 3.52 Å

on SPring-8 **BL26B1** (Fig. 3(a)). In each CofB trimeric interface, the complex structure clearly revealed that the CofJ (1-24) peptide fragment from Ser5 to Pro15 was bound to the groove, initially considered as a sugar binding pocket, sandwiched between 2 CofB C-terminal H-type lectin domains. This intriguing binding feature reasonably explains the fact that it precludes GalNAc binding and suggests functional "repurposing" of the H-type lectin domain of CofB for efficient attachment of ETEC to the target cell surface.

Based on the data from analytical ultracentrifugation which demonstrated that the CofJ-CofB complex in solution exists as a heterotetramer with one intact CofJ molecule and three CofB molecules, we generated a structural model of the CofJ-CFA/III pilus, in which a secreted protein CofJ was situated further above the minor pilin CofB at the tip of T4bP (Fig. 3(b)). Cell adherence assay with the CFA/III-positive HB101 strain carrying the recombinant plasmid which harbored all CFA/III operon genes except *cofJ* (*ΔcofJ* strain) indicated a marked reduction in bacterial adherence to Caco2 cells, where the observed reduction recovered noticeably when recombinant CofJ was added (Fig. 3(c)). These results strongly support the notion that the secreted protein CofJ serves as an anchor, bridging the host cell surface and the pilus-tip of T4bP during the process of ETEC adherence [5]. Initial attachment is a critical step common to all enteric pathogens. Thus, the interplay between secreted protein and T4bP of ETEC shown in this study may constitute an attractive therapeutic target for vaccination and/or antiadhesive treatment against ETEC infection.

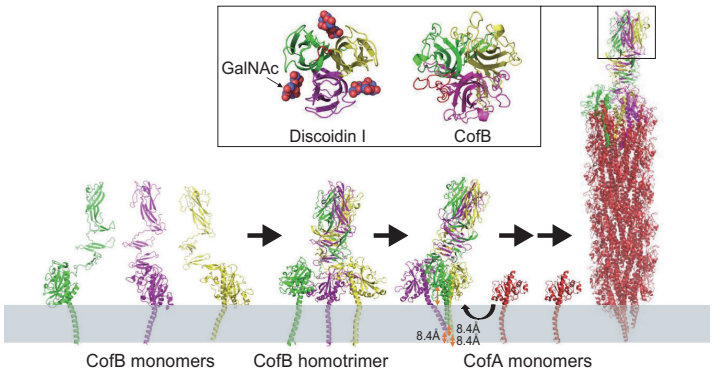

Fig. 2. Proposed mechanism for initiation of CFA/III assembly by CofB homotrimer. Three CofB monomers with their N-terminal α-helices initially embedded deeply in the inner membrane form a homotrimeric complex by association of C-terminal two β-strand-rich domains. Three cycles of extraction (~8.4 Å) of the N-terminal α-helix, possibly occurring in collaboration with inner membrane proteins, bring the α-helices into close proximity. This structural "tip" complex allows for easy docking of the major pilin CofA by shape and charge complementarity. Of note, the C-terminal domain of CofB has substantial structural similarity with H-type lectin domain of discoidin I from *Dictyostelium discoideum* (PDB entry: 2WN3).

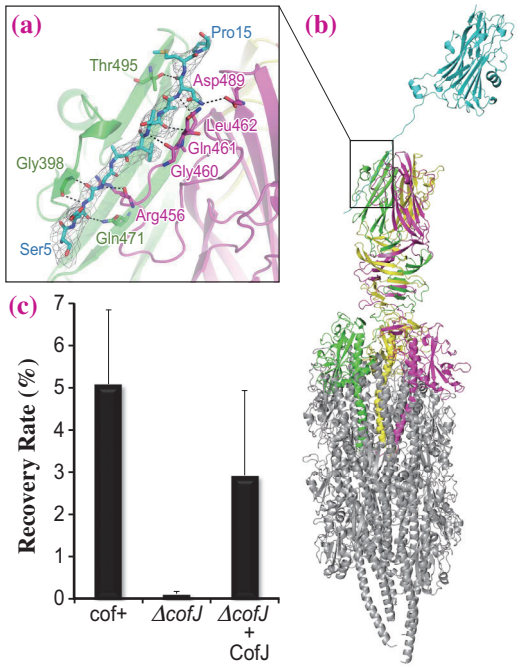

Fig. 3. Interplay of a secreted protein CofJ with CFA/III for bacterial adhesion to target cell surface. **(a)** Binding interface of CofJ(1-24)-CofB complex. A 2mFo-DFc omit map contoured at 1.0 σ corresponding to the region of the peptide binding groove is shown. **(b)** Structural model of CofJ-CFA/III pilus complex, with the CofJ monomer situated further above the minor pilin CofB homotrimer at the pilus tip. The model was built by superposing the CofJ(1-24)-CofB crystal structure onto the CFA/III pilus model. The CofJ(1-24) segment was then modeled and connected with the CofJ globular domain, the crystal structure of which was previously solved using its Dy derivative crystal at SPring-8 BL38B1. **(c)** CFA/III-mediated *E. coli* adherence to Caco-2 cells. Adherence values correspond to the recovery rate after incubation for 3 h. cof+ is an *E. coli* strain HB101 harboring all CFA/III operon genes on the plasmid pTT240 and *ΔcofJ* is a *cofJ* deletion mutant.

Kazuki Kawahara[a,*], Hiroya Oki[a] and Shota Nakamura[b]

[a] Graduate School of Pharmaceutical Sciences, Osaka University
[b] Research Institute for Microbial Diseases, Osaka University

*Email: kkkazuki@phs.osaka-u.ac.jp

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