Extensive Structural Similarity between the Flagellar Type III ATPase FliI and F_1-ATPase Subunits

In response to environmental stimuli, bacteria swim by rotating a filamentous organelle called the flagellum. It is a molecular complex composed of more than 25 kinds of proteins, each in multiple copies from several to several tens of thousands. The flagellar axial proteins and other proteins involved in their assembly and regulation are synthesized in the cytoplasm. They are selectively exported into the central channel of the growing flagellar structure by the flagellar type III export apparatus, and travel through the narrow channel to the growing end for their self-assembly [1]. The flagellar type III export apparatus, which is composed of six integral membrane proteins and three soluble proteins [2] (Fig. 1), is homologous in the quaternary as well as the primary structure level to the virulence type III protein secretion system, which bacterial pathogens use to export its axial component proteins for self-assembly and to secrete effecter proteins into their host cells for invasion. Characterization of these type III protein export systems has been intensively carried out by many groups, and many intriguing features have been revealed, such as export specificity switching for controlling assembly size and selective secretion, involvement of cytoplasmic chaperones, and unfolding of export substrate proteins for export through the channel of about 2 nm in diameter. However, the mechanisms of these functions are still obscure due to lack of structural information.

FliI is a soluble component subunit of the flagellar type III export apparatus and an ATPase that is thought to facilitate the flagellar protein export process. To elucidate the mechanism of type III protein export, we determined the crystal structure of monomeric FliI in complex with ADP at 2.4 Å resolution by using X-ray diffraction data collected at beamline **BL41XU**. Because of the low solubility of native FliI, we prepared a highly soluble variant lacking the first 18 residues, FliI(Δ1-18), crystallized it and solved its structure .

FliI consists of three distinct domains; the N-terminal domain, the ATPase domain and the C-terminal domain (Fig. 2). The central ATPase domain of FliI has a significant similarity in the primary sequence to the α and β subunits of F_oF_1-ATPsynthase [3], but the overall sequence similarity is rather low, and the functions of these two systems are quite different from each other. FliI is involved in protein export and is therefore thought to be a linear motor that unfolds and threads the export substrate proteins through its central channel, while F_oF_1-ATPsynthase is a rotary motor. However, we have

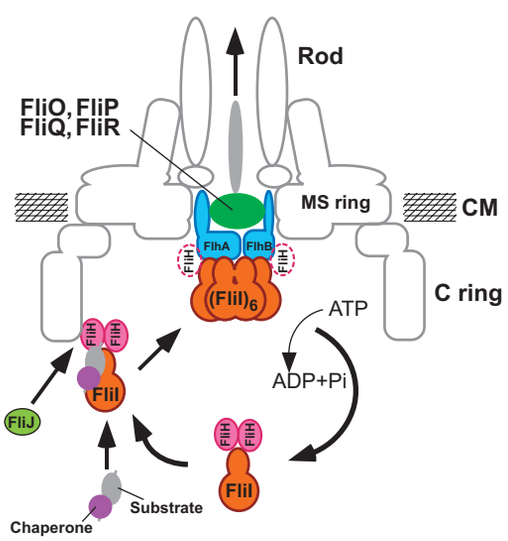

Fig. 1. Schematic diagram of the bacterial flagellar type III protein export apparatus. FlhA, FlhB, FliO, FliP, FliQ and FliR are integral membrane components and FliH, FliI and FliJ are cytoplasmic components. FliI forms a hetero-trimer with the FliH dimer in the cytoplasm and assembles into hexamer upon docking to the cytoplasmic domains of FlhA and FlhB. An export general chaperon FliJ interacts with the FliH/I complex in the cytoplasm.

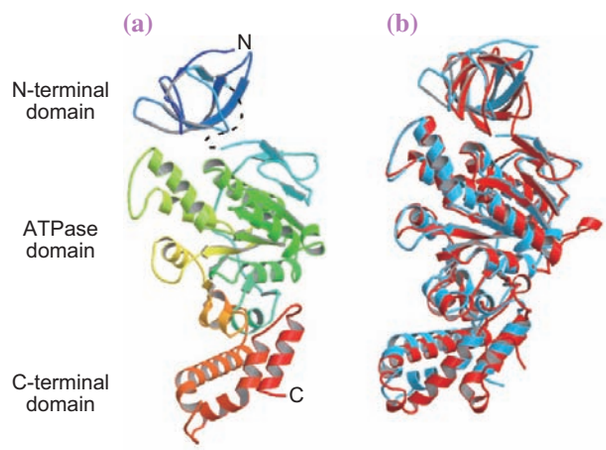

Fig. 2. **(a)** Cα ribbon representation of FliI(Δ1-18). **(b)** FliI(Δ1-18) (cyan) is superimposed to the β_{ADP+Pi} subunit (red) in the ADP-AlF_4 bound form of the F_1-ATPase (PDB code: 1H8E).

found that the entire structure of FliI has a striking similarity to the α and β subunits of F_oF_1-ATPsynthase, both in the conformation of the whole molecule and the ATP binding site. The structure of FliI with ADP exhibited a half closed conformation, which is very similar to the β_{ADP+Pi} subunit of an inhibited form of F_1-ATPase obtained in the presence of aluminium fluoride and Mg-ADP (Fig. 2). Amino acid residues of the F_1-α/β subunits known to be involved in ATP hydrolysis are highly conserved in FliI (Fig. 3). These structural similarities strongly suggest that FliI and F_1-ATPase share a similar catalytic pathway for ATP hydrolysis.

A FliI hexamer model we built by superimposing the atomic model of FliI monomer to each subunit of the $\alpha_3\beta_3$ hexameric ring structure shows that all the differences are found on the outer surface of the ring, while the intersubunit interface and the region surrounding the central channel of the ring are structurally very well conserved (Fig. 4). These results suggest that these two complex molecular machines have been evolved from a common ancestral system and share a common working mechanism albeit the present functions appear to be quite different from each other.

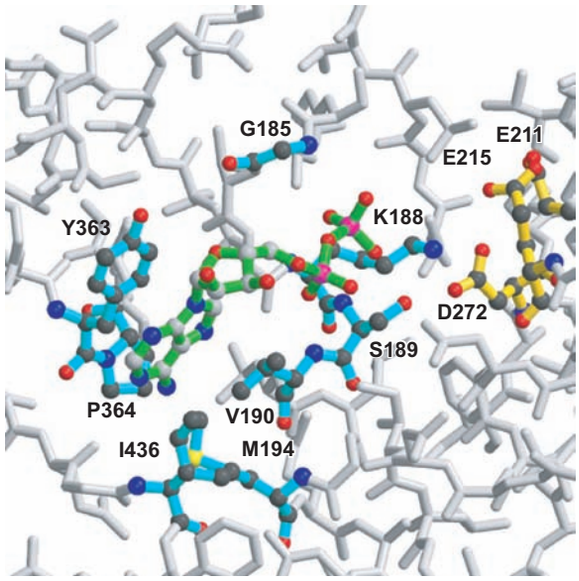

Fig. 3. Close up view of the nucleotide-binding site. The bound ADP is colored green. Residues interacting with ADP are shown in cyan and conserved residues involved in catalysis are colored yellow.

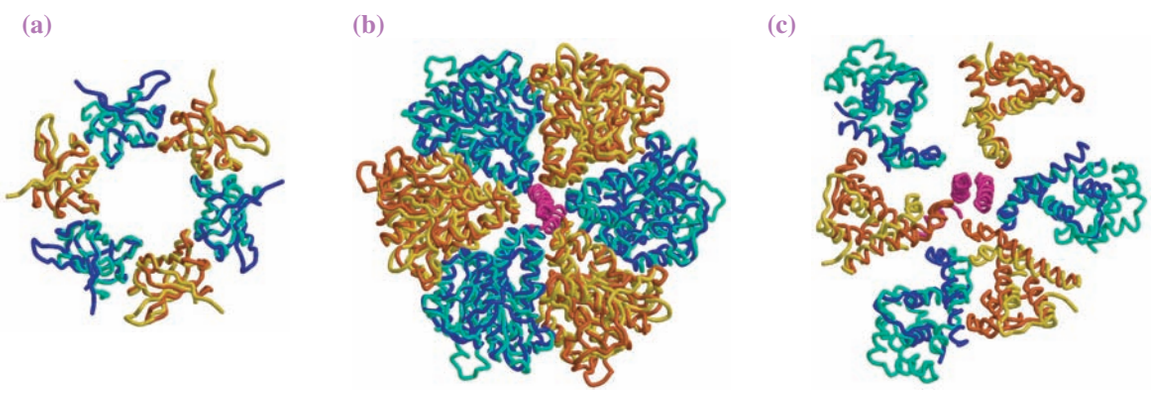

Fig. 4. Superposition of FliI (blue and yellow) to the α (blue green) and β (orange) subunits of F_1-ATPase. **(a)** The N-terminal domain, **(b)** the ATPase domain, and **(c)** the C-terminal domain. The γ subunit of F_1-ATPase at the center is shown in purple.

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