Universal Joint Mechanism of the Bacterial Flagellar Hook

Bacteria swim by rotating helical flagellar filaments, which are about 15 μm long and 120-250 Å in diameter. A membrane-embedded rotary motor at the base of each filament drives the helical propeller at hundreds of revolutions per second. To be able to rotate the filament off axis of the motor, the filament is connected to the motor by a short filament called the hook, which works as a universal joint. It has a relatively well-defined length of 55 nm and is made by polymerization of about 130 molecules of a 41 kDa protein, FlgE. To understand the universal joint mechanism we solved its high-resolution structure. Because FlgE polymerizes we over-expressed, purified and crystallized a 31.2 kDa fragment, FlgE31, made of segment G71-S363 [1] and solved the crystal structure at 1.8 Å resolution [2]. FlgE31 contains two domains, D1 and D2 (Fig. 1). D1 is made of an N-terminal segment, Gly71 - Ala144, and a C-terminal segment, Pro285 - Ser363. The fold can be described as randomly oriented β-strands. D2 is made of a central segment, Ala145 - Lys284, and can be described as an irregular β-barrel.

An atomic model of the straight hook was then built by docking the crystal structure of FlgE31 into the density map of the straight hook obtained by electron cryomicroscopy. Because domain D0 of FlgE missing in the crystal structure is known to form the inner core portion of the hook, domains D1 and D2 were docked to the middle and external parts of the hook, respectively (Fig. 2). Each domain was separately docked, manually connected and then refined by a real-space refinement program [3]. The model shows strong interactions between D2 domains along the 6-start helix on the surface, but the axial interactions along the 11-start helix are between domain D2 of the lower subunit and a triangular loop (Thr 116 - Pro 135) of domain D1 of the upper subunit [2]. This mesh structure of the tube wall explains why the hook is rigid against twisting.

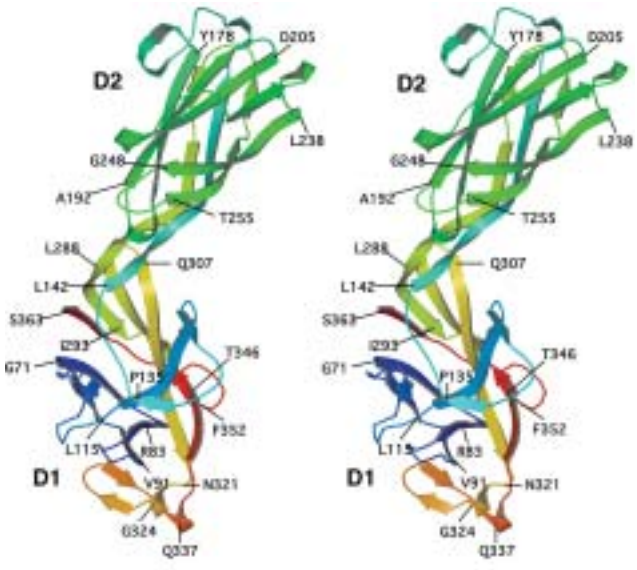

Fig. 1. Stereo view of the Cα backbone trace of FlgE31. The chain is color-coded from blue to red, going through the rainbow color from the N- to C-terminus.

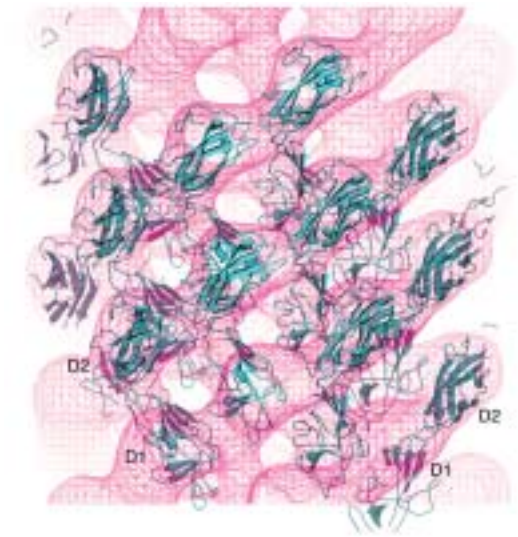

Fig. 2. Docking of the FlgE31 atomic model into two outer domains of hook. Side view.

The hook at work is highly curved and it is part of a supercoil. This implies that the repeat distance of protofilaments on the inner side of the curve must be shorter than those on the outer side. We built an atomic model of a supercoiled hook by continuously deforming the helical lattice of the straight hook so that the hook axis follows a right-handed helix with a pitch of 950 Å and a diameter of 350 Å as observed by electron microscopy (Fig. 3(a)). Subunits located on the inner side are axially more packed than those located on the outer surface (Figs. 3(c), 3(d)). The

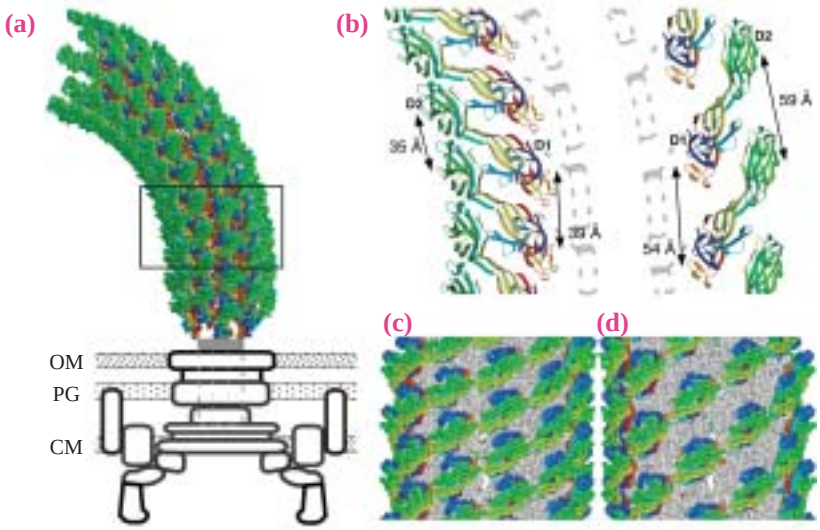

Fig. 3. Atomic model of a supercoiled hook. **(a)** Atomic model of a coiled hook with schematic diagram of the basal body. **(b)** Magnified image of the coiled hook with the innermost and outermost protofilaments on the left and right, respectively. The inner core domains forming the central channel are represented by dotted grey lines. **(c)**, **(d)** Intermolecular packing arrangements of D2 on the inner side **(c)** and outer side **(d)** of the coiled hook. Only D2 is color-coded as in Fig. 1, while D1 is colored light grey.

difference in repeat distance is almost 1.7 times (Fig. 3(b)). During the rotation of the hook around its axis, the protofilaments will successively be compressed and extended a few hundreds times per second. To understand how this dynamic change is achieved we performed a molecular dynamic simulation of the extension and compression of a protofilament model made of three subunits with surrounding water molecules (Fig. 4). The bonding interactions between residues of the triangular loop of D1 and the inner face of D2 show multiple steps of exchange in bonding partners, resulting in a large slippage at this D1-D2 interface. The bending flexibility of the hook, which is essential for its universal joint function, is probably due to this stepwise axial sliding along with the flexibility in relative domain orientation (see Ref. [2]).

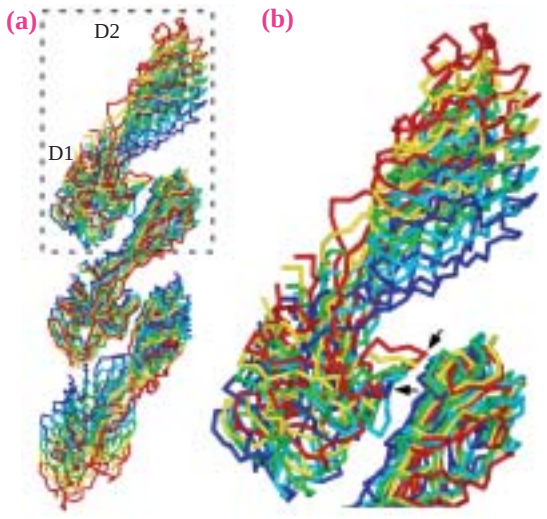

Fig. 4. Simulated extension and compression of a hook protofilament. Protofilament models at five different stages of simulated extension at 5 Å intervals are superimposed with different colors: dark blue, light blue, green, yellow, and red, from the most compressed to the most extended state. **(a)** Whole three subunits. **(b)** Magnified view of upper half. D2 at the top and D1 at the bottom have equal intervals (2.5 Å) along the vertical axis. The triangular loop of D1 and the surface of D2 have distinct side chain bonding partners depending on the state of extension or compression.

Fadel A. Samatey[a], Hideyuki Matsunami[a] and Keiichi Namba[a,b,*]

[a] Dynamic NanoMachine Project, ICORP, JST
[b] Graduate School of Frontier Bioscience, Osaka University

*E-mail: keiichi@fbs.osaka-u.ac.jp

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