Review

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The bacterial flagellar motor and its structural diversity

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The bacterial flagellum is a reversible rotary motor powered by an electrochemical-potential difference of specific ions across the cytoplasmic membrane. The H^+-driven motor of *Salmonella* spins at ~300 Hz, whereas the Na^+-driven motor of marine *Vibrio spp.* can rotate much faster, up to 1700 Hz. A highly conserved motor structure consists of the MS ring, C ring, rod, and export apparatus. The C ring and the export apparatus show dynamic properties for exerting their functional activities. Various additional structures surrounding the conserved motor structure are observed in different bacterial species. In this review we summarize our current understanding of the structure, function, and assembly of the flagellar motor in *Salmonella* and marine *Vibrio*.

Dynamic structural properties of the bacterial flagellar motor

The bacterial flagellum, which propels the bacterial cell body in liquid environments, is a supramolecular complex made of at least three parts: the basal body (reversible motor), the hook (universal joint), and the filament (helical propeller). *Escherichia coli* and *Salmonella enterica* are model organisms that have provided detailed insights into the structure, assembly, and function of the flagellum. The flagellar basal body (FBB) of *S. enterica* consists of the C ('cytoplasmic') ring, MS ('membrane/supramembrane') ring, P ('peptidoglycan') ring, L ('lipopolysaccharide') ring, and the rod. The C, MS, P, and L rings are located in cytoplasm, cytoplasmic membrane, peptidoglycan (PG) layer, and outer membrane, respectively. The rod is a drive shaft composed of three proximal rod proteins (FlgB, FlgC, and FlgF) and a distal rod protein (FlgG), and traverses the periplasmic space [1–3]. The MS–C ring complex is a reversible rotor. FliG, FliM, and FliN form the C ring on the cytoplasmic face of the MS ring, which is formed by a single flagellar protein, FliF [4–6]. FlgI and FlgH assemble around the rod in the PG layer and outer membrane, respectively, forming the LP ring complex that functions as a molecular bushing (Figure 1) [7].

The flagellar motor can operate in both the counterclockwise (CCW; viewed from the filament to the FBB) and clockwise (CW) directions. FliG, FliM, and FliN are responsible for switching the direction of motor rotation. The binding of a chemotactic signaling protein, phospho-CheY, to FliM and FliN induces a co-operative conformational change in the FliG ring that allows the motor to spin CW [8,9]. FliM and FliN labeled with fluorescent proteins such as green fluorescent protein (GFP) and yellow fluorescent protein (YFP) alternate rapidly between localized and freely diffusing forms while the motor is rotating. This behavior indicates that the C ring is a highly dynamic structure even when the motor is in action [10–14]. This dynamic nature is responsible for the ultrasensitivity of the motor to chemotactic signals [15].

The *E. coli* and *S. enterica* flagellar motors contain about a dozen stators distributed around the rotor [16]. Motor torque is produced by sequential rotor–stator interactions coupled with downhill ion (H^+ or Na^+) translocation along the electrochemical gradient of the coupling ion across the cytoplasmic membrane [17,18]. Using electron cryo-tomography (ECT) and subtomogram averaging, the stators have been shown to be assembled around the MS-C ring [19]. The stator comprises an ion-channel that is attached to the PG layer and converts the ion flow through the channel into the mechanical work required for flagellar motor rotation [20–22]. In *E. coli* and *S. enterica*, MotA and MotB form a proton channel with four copies of MotA and two copies of MotB (Figure 1, left) [23]. In *Vibrio alginolyticus*, PomA and PomB constitute a sodium ion channel similar to the MotAB proton channel (Figure 1, right) [24]. The stator is anchored to the PG layer through a typical OmpA-like structure within the C-terminal periplasmic domain of MotB ($MotB_C$) or PomB ($PomB_C$) when it is assembled into the motor [25–27]. GFP–MotB shows rapid turnover between the FBB and the membrane pool while the motor is rotating [28]. Furthermore, each stator associates with, and dissociates from, the motor in response to changes in the ion motive force or the external load on the flagellum [29–32]. These observations indicate that the stators are not permanently fixed in place around the motor. Consistently, no clearly defined stator structure is observed in *E. coli*, *S. enterica*, or *Vibrio cholerae* by ECT [33,34]

The FBB contains a specific protein-export apparatus that is required for construction of the flagellum. The export

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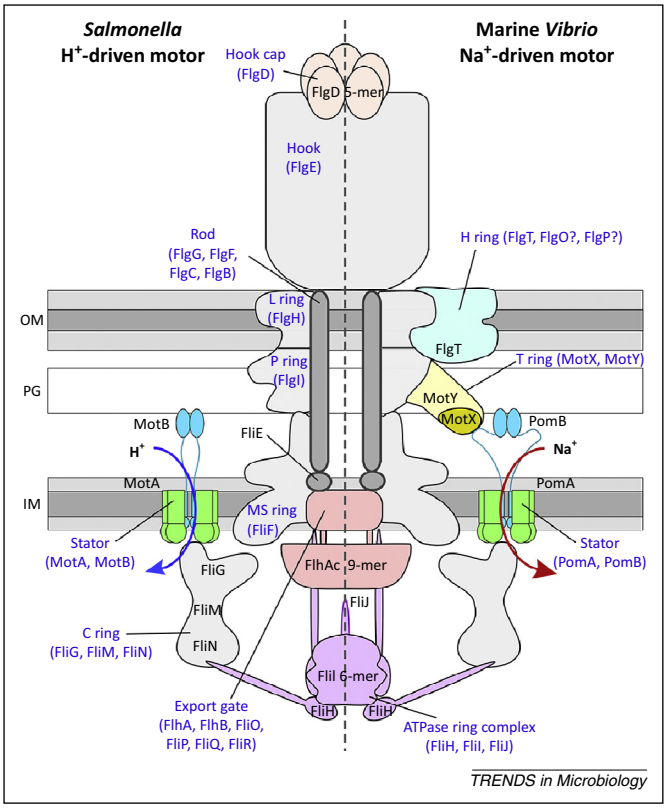

Figure 1. Schematic drawing of the bacterial hook basal-body architecture. The left side is the hook basal-body complex of the *Salmonella* H^+-driven motor and the right side is that of the *Vibrio* Na^+-driven motor. The schematic model is drawn based on the electron microscopy (EM) analyses of the *Salmonella* and *Vibrio* motors. Abbreviations: IM, inner membrane; OM, outer membrane; PG, peptidoglycan layer.

apparatus consists of an export gate that is a transmembrane complex containing FlhA, FlhB, FliO, FliP, FliQ, and FliR, as well as a cytoplasmic ATPase complex composed of FliH, FliI, and FliJ (Figure 1) [35,36]. The export gate of *S. enterica* is located within the MS ring and is fueled by the proton motive force (PMF) across the cytoplasmic membrane [37,38]. A nonameric ring structure consisting of the C-terminal cytoplasmic domain of FlhA projects from the export gate into the large central cavity of the C ring (Figure 1) [39–41]. FlhA–YFP turns over between the FBB and the freely diffusing membrane pool during flagellar assembly. This property suggests that the $FlhA_9$ ring is also a dynamic structure [41,42].

The FliI ATPase forms a homo-hexamer to facilitate flagellar protein export [43,44]. The $FliI_6$ ring is located on the cytoplasmic face of the FBB and is secured through interactions of FliH with FlhA and FliN (Figure 1) [33,45–47]. FliJ binds to the center of the $FliI_6$ ring to form the $FliI_6FliJ$ ring complex [48]. An interaction between FliJ and FlhA allows the export gate to efficiently utilize the PMF to drive protein export [49,50]. FliI also forms a $FliH_2FliI$ complex with a homo-dimer of FliH in the cytoplasm [51]. More than six FliI–YFP molecules are associated with the FBB, suggesting that not only the $FliI_6$ ring but also several $FliH_2FliI$ complexes associate with the FBB [47]. FliI–YFP exchanges rapidly between the FBB-localized and free-diffusing forms in an ATP-independent manner. This indicates that ATP hydrolysis by FliI does not drive the assembly–disassembly cycle of FliI during flagellar assembly [47]. Because export substrates and chaperone–substrate complexes bind to the $FliH_2FliI$ complex [52–54], it is proposed to be a dynamic carrier that delivers export substrates and chaperone–substrate complexes from the cytoplasm to the PMF-driven export gate [47].

Structural diversity of the FBB

Intact flagellar motor structures derived from different bacteria species such as *E. coli, S. enterica*, *Campilobacter jejuni*, *V. cholerae*, *Borrelia burgdorferi*, *Leptospira interrogans*, and *Treponema primitia* have been visualized by ECT (Table 1). The flagella have been purified from many bacteria such as *S. enterica*, *V. alginolyticus, Rhodobacter sphaeroides*, and *Bacillus subtilis* and observed by electron microscopy (EM) (Table 1). The MS ring, C ring, rod, and export apparatus of these bacteria show similar architectures to those in the FBB of *S. enterica*, suggesting that most components of the core structure of the FBB and their organization are well conserved, although their stoichiometry varies considerably between species [33,34]. Interestingly, novel and divergent structures with different symmetries surround the conserved core structure in different species (Table 1) [33,34]. For example, the FBB of marine *Vibrio* spp. contains two additional ring structures, the T and H rings (Figure 1, right) [55,56]. MotX and MotY form the T ring, which is located beneath the P ring, and this presumably facilitates efficient assembly and stable anchoring of the PomAB stator complex around the rotor [55]. The H ring surrounds the LP ring complex [56]. FlgT is directly involved in the construction of the H and T rings because a *flgT* null mutant produces an FBB lacking the H and T rings [56,57]. The flagellum produced by the *flgT* mutant of *V. cholerae* is released into the culture supernatant from the cell upon completion of flagellar assembly, suggesting that the H and T rings are responsible for stable anchoring of the flagellum to the cell [58]. Because the Na^+-driven motor of *V. alginolyticus* can rotate at up to 1700 Hz [59], whereas the H^+-driven *S. eneterica* motor rotates at a maximum of 300 Hz [60], these two ring structures may reinforce the motor to resist the high-speed rotation.

Assembly of the conserved core structure of the FBB

Flagellar assembly proceeds from more-proximal structures to more-distal ones in a temporally and spatially regulated fashion [1,2]. The MS ring, which is made of 26 copies of FliF in *E. coli* and *S. enterica* [5], is the base for flagellar structure, assembly, and function. In addition to being the mounting platform for the C ring, the MS ring serves as a housing for the export apparatus [3]. FliF can self-assemble into the MS ring structure in the cytoplasmic membrane when overexpressed in *E. coli* [61], and FliF–YFP shows punctate patterns of localization in *E. coli* and *S. enterica* [41,42]. Similar punctate patterns of localization are observed in *Salmonella* Δ*flhA*, Δ*flhB*, Δ*fliH–fliI–fliJ*, Δ*fliM*, Δ*fliN*, Δ*fliO*, Δ*fliP*, Δ*fliQ*, and Δ*fliR* mutants, but not in the Δ*fliG* mutant. Thus, FliF requires FliG for efficient ring formation [41]. Because FliG directly associates with the cytoplasmic face of the MS ring with a 1 FliG to 1 FliF stoichiometry [62], FliF and FliG apparently associate cooperatively to form the MS ring. FliG consists