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Current Opinion in Microbiology

# Dynamism and regulation of the stator, the energy conversion complex of the bacterial flagellar motor

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Many motile bacteria swim by rotating their motility organ, the flagellum. Rotation of the flagellum is driven by a motor at its base, and torque is generated by the rotor–stator interaction coupled with the specific ion flow through the channel in the stator. Because the stator works as an energy-conversion complex in the motor, understanding the functional mechanism of the stator is critically important. But its characterization has been hampered due to the difficulty in isolating the functional stator complex from the membrane. Recently, successful new approaches for studying the stator have been reported that reveal its novel properties. Two of those, visualization of the *in vivo* behavior of stator units using fluorescently tagged proteins and structure-guided functional analyses of the soluble region in the stator, are summarized in this short review.

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**Current Opinion in Microbiology** 2015, **28**:66–71

This review comes from a themed issue on **Growth and development: eukaryotes and prokaryotes**

Edited by **Sophie G Martin** and **Dan B Kearns**

For a complete overview see the Issue and the Editorial

Available online 23rd October 2015

http://dx.doi.org/10.1016/j.mib.2015.07.015



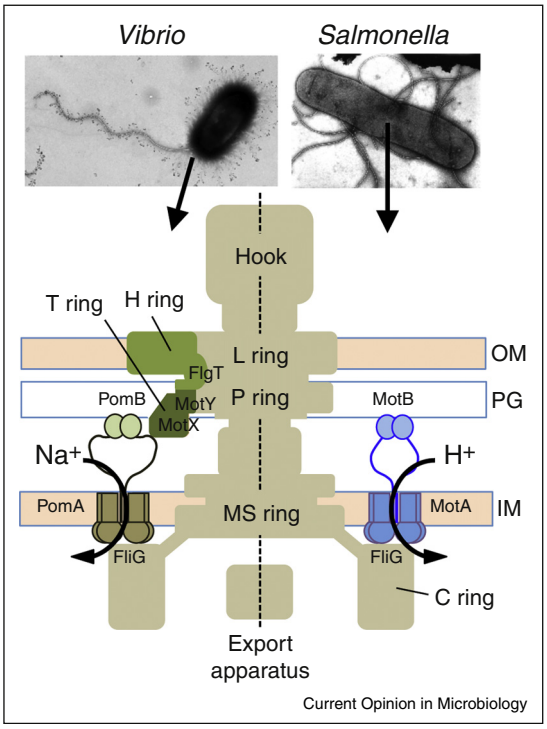
## Introduction

Motility is one of the basic functions bacteria use to survive in response to environmental changes. Most motile bacteria swim in a liquid or swarm on a solid surface using their motility organ, the flagellum [1,2]. The bacterial flagellum is structurally and functionally different from its eukaryotic counterpart. It is a helical propeller that extends from the cell body, and its rotary motion thrusts the cell body to move. Rotation of the flagellum is driven by the reversible rotary motor, which is embedded at its base in the cell envelope. The energy source of the flagellar motor is the electrochemical gradient of the coupling ions across the membrane, $H^+$ for *Escherichia coli*, *Salmonella* and most neutrophiles, and $Na^+$ for *Vibrio*, *Shewanella* and alkalophilic *Bacillus* (Figure 1) [3–5]. The motor consists of a rotary part (the rotor) and up to a dozen stator units that surround each rotor [6]. Motor torque is generated by the rotor–stator interaction that couples with the ion flow through the channel in the stator [7]. Therefore, the stator converts electrochemical energy into mechanical energy. Extensive studies have unraveled many aspects of the flagellum: its protein components and overall structure [8], the assembly process that couples with the flagellar gene regulation [9] and the biophysical properties of the motor [10]. However, the mechanism of torque generation, especially the energy conversion steps in the stator, is still mostly unknown because the membrane-embedded feature of the stator hampers its biochemical, biophysical and structural characterization. Therefore, characterization of the stator has proceeded in ways without isolating the whole stator complex. Single molecule technology using GFP-fusion proteins have contributed to the visualization of individual stator units in living cells, and that approach has revealed the unexpected dynamic properties of the stator [11]. Structural analyses of the stator have been performed for its soluble parts essential for function, and structure-guided functional analyses have provided insightful results that suggest the assembly-coupled activation mechanism of stator units [12,13••]. Those two advances in dynamics and structure of the stator are highlighted in this short review.

## Architecture of the bacterial flagellar basal body

To help understand the stator properties, current knowledge of the flagellar basal body, where the stator is assembled for function, is briefly summarized. Electron microscopic observations of purified flagella from *Salmonella* have contributed to unraveling its detailed ultrastructure [14•,15]. Recent progress in visualizing the intact flagellar motor in cells at molecular resolution using electron cryo-tomography can be found in Refs. [16,17]. Each flagellum is composed of three parts, the filament, the hook and the basal body that works as a reversible rotary motor at its base. The basic structure of the basal body is well conserved among bacterial species, and is composed of several rings mounted on an axial rod connected to the hook (Figure 1). Each ring structure is associated with parts of the cell envelope: the MS-ring is embedded in the inner membrane (IM), the P-ring is associated with the peptidoglycan (PG) layer, and the L-ring is attached to the outer membrane (OM) [2]. In the cytoplasmic side, there is another ring (the C ring) essential for motor rotation and flagellar assembly [18], whose component FliG most

**Figure 1**




Schematic representation of the bacterial flagellar motor. The left side shows the sodium-driven polar flagellar motor of *Vibrio alginolyticus*, and the right side shows the proton-driven flagellar motor of *Salmonella enterica*. The *Vibrio* motor contains additional ring structures, the T ring (dark green) and the H ring (green). The stator complex is composed of two integral membrane proteins, PomA and PomB for the *Vibrio* motor, MotA and MotB for the *Salmonella* motor. The stator is anchored at the PG layer around the rotor via the periplasmic PGB domain of the B subunit. In the *Vibrio* motor, the T ring is required for stator incorporation into the motor, and the N-terminal disordered region of $PomB_C$ (see the text) is postulated to interact with the T ring. The most likely locations for MotX, MotY, FlgT and FliG are shown in the scheme. OM, outer membrane; IM, inner membrane.

closely participates in torque generation in the rotor side through the interaction with the stator [19–21].

The basal body of the $Na^+$-driven polar flagellum of *Vibrio* species contains two additional ring structures, the T and H rings (Figure 1) [22,23]. These extra rings are thought to reinforce the motor to resist its high-speed rotation, up to 1700 Hz (for $H^+$-driven *Salmonella* and *E. coli* motor, up to 300 Hz). The T ring appears as an appendage beneath the P ring, and is composed of MotX and MotY that are essential for incorporating the stator unit into the motor and stabilizing it, and thereby are crucial for rotation [22]. Determination of the crystal structure and subsequent functional analyses of MotY revealed that it consists of two distinct domains: an N-terminal domain that directly interacts with MotX and the basal body, and a C-terminal domain that shows remarkable similarity to the OmpA-like domain known to bind the PG layer [24]. MotX is located at the outer rim of the T ring [22] and was suggested to interact with PomB [25]. The interface of MotX-PomB has not been identified, but a plausible model for this interaction is discussed later in this review. The H ring surrounds the L and P rings, and is also required for proper assembly of the stator units around the rotor [23]. FlgT, a soluble periplasmic protein, is needed to form both the T ring and the H ring [23]. FlgT is a part of the H ring, associates with the basal body and interacts with MotY [26•]. FlgO and FlgP might be the main components for the H ring because they are OM proteins known to be important for motility and flagellar stability in *Vibrio cholerae* [27]. Details of the crystal structures of MotY and FlgT, and the assembly process of the H and T rings, are reviewed elsewhere [8].

## The stator and its dynamic properties

The stator is a non-rotating part of the motor and is responsible for the energy conversion in the motor. Up to a dozen stator units surround each rotor and each can work as a functionally independent torque generating units [6,28,29]. The stator complex is composed of two membrane proteins: MotA and MotB for the $H^+$-driven motor of *E. coli* and *Salmonella*, and PomA and PomB for the $Na^+$-driven polar flagellar motor of *Vibrio* (Figure 1) [30–32]. They form an ion-conducting complex with an $A_4B_2$ stoichiometry [33–36], which is anchored to the PG layer through the OmpA-like domain within the C-terminal periplasmic region of MotB or PomB, when it is assembled into the motor [37,38].

It has been long believed that stator units are static and once incorporated into the motor, they are stably anchored around the rotor to smoothly generate torque. However, *in vivo* observation of GFP-fused MotB in the functioning *E. coli* motor at the single molecule level revealed the dynamic property of the stator unit, showing a rapid turnover between the motor and membrane pool (the dwell time of a given stator in the motor is only ~0.5 min) [11]. The dynamic property is also observed in the $Na^+$-driven PomA/PomB stator in *Vibrio* and *Shewanella* [39,40]. In those cases, the incorporation of stator units around the rotor appears to be dependent on the $Na^+$-motive force: stator units dissociate from or associated with the motor in response to decreases or increases in $Na^+$ concentration in the medium (Figure 2a,b). These observations change the static image of the stator and indicate that incorporated stator units are exchangeable. Recently, stator incorporation or dissociation in response to the external load was reported [41••,42••,43••], suggesting that the stator can work as a mechanosensor. Therefore, it is plausible that cells can control their motility by regulating the stator number of a single motor in response to sudden environmental changes. In this point of view, the dynamic property of the stator is biologically relevant.