Chapter 1

Salmonella Flagellum

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Abstract

Flagella-driven motility contributes to effective bacterial invasion. The bacterial flagellum of *Salmonella enterica* is a rotary motor powered by an electrochemical potential difference of protons across the cytoplasmic membrane. The flagellum is composed of several basal body rings and an axial structure consisting of the rod as a drive shaft, the hook as a universal joint and the filament as a helical propeller. The assembly of the axial structure begins with the rod, followed by the hook and finally the filament. A type III protein export apparatus is located at the flagellar base and transports flagellar axial proteins from the cytoplasm to the distal end of the growing flagellar structure where their assembly occurs. The protein export apparatus coordinates flagellar gene expression with assembly, allowing the hierarchy of flagellar gene expression to exactly parallel the flagellar assembly process. The basal body can accommodate a dozen stator complexes around a rotor ring complex in a load-dependent manner. Each stator unit conducts protons and pushes the rotor. In this book chapter, we will summarize our current understanding of the structure and function of the *Salmonella* flagellum.

Keywords: bacterial flagellum, motility, rotary motor, self-assembly, gene expression, torque generation, type III protein export

1. Introduction

Salmonella is well known as a zoonotic pathogen, which causes gastroenteritis. Motility of *Salmonella* assists in reaching an appropriate site for invasion and enhances the infectivity. The bacterial flagellum is a long filamentous organelle responsible for motility. *Salmonella* swims in liquid environments and moves on solid surfaces by rotating flagella. In addition, the flagella also facilitate bacterial adhesion and biofilm formation. *Salmonella* has several

flagella on the cell surface. Each flagellum consists of tens of thousands of flagellin molecules, allowing host cells to acquire both innate and adaptive immune responses to flagellin. Toll-like receptor 5 recognizes flagellin to activate the host immune system. Thus, the flagellum is also a considerable target to detect bacterial pathogens [1, 2].

The flagellum consists of basal body rings and an axial structure consisting of the rod, the hook, the hook-filament junction, the filament and the filament cap (**Figure 1**). The basal body rings are embedded within the cell membranes and act as a rotary motor powered by the trans-membrane electrochemical gradient of protons, namely proton motive force (PMF). The rod is directly connected to the basal body MS ring and acts as a drive shaft. The filament works as a helical propeller to propel the cell body. The hook exists between the rod and filament and functions as a universal joint to smoothly transmit torque produced by the motor to the filament. A type III protein export apparatus is located at the base of the flagellum to construct the axial structure beyond the cell membranes. A dozen stator units surround the basal body rings. The stator unit acts as a proton channel to couple the proton flow through the channel with torque generation. The flagellar motor regulates the number of active stator units in the

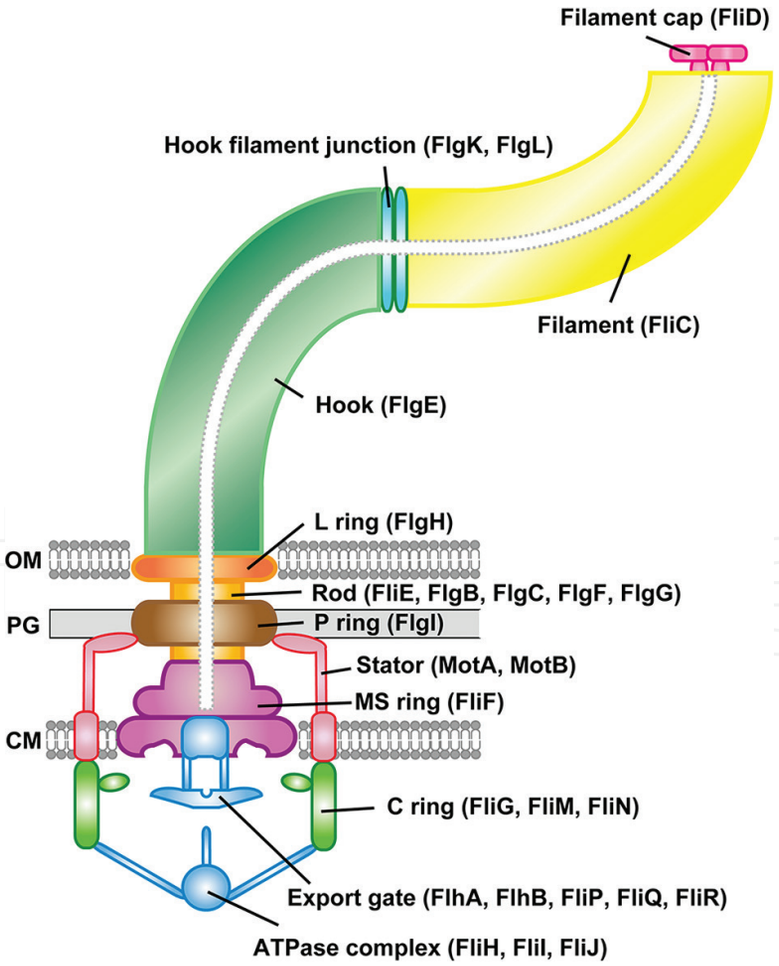

Figure 1. Schematic diagram of the bacterial flagellum. The name of each part and the component protein(s) is shown in black letters. OM: outer membrane, PG: peptidoglycan layer, CM: cytoplasmic membrane.

motor in response to changes in the environment [3–6]. In this chapter, we describe our current understanding of the structure and function of the *Salmonella* flagellum.

2. Structure of the flagellum

2.1. Basal body

The basal body consists of the C ring, the MS ring, the P ring and the L ring and the rod. The C, MS, P and L rings are located in the cytoplasm, the cytoplasmic membrane, peptidoglycan layer and outer membrane, respectively (**Figure 1**). FliF self-assembles into the MS ring in the cytoplasmic membrane [7]. Recently, it has been shown that a C ring protein FliG is required for efficient MS ring formation [8]. FliG, FliM and FliN assemble into the C ring onto the cytoplasmic face of the MS ring (**Figure 2**) [9]. The MS-C ring complex acts as a rotor of the flagellar motor. A stator protein MotA interacts with the C-terminal domain of FliG ($FliG_C$) [10], allowing the motor to spin at the maximum speed of about 300 revolutions per second.

The flagellar motor rotates in both counterclockwise (CCW) and clockwise (CW) directions. The C ring acts as a structural switch to change the direction of flagellar motor rotation [6].

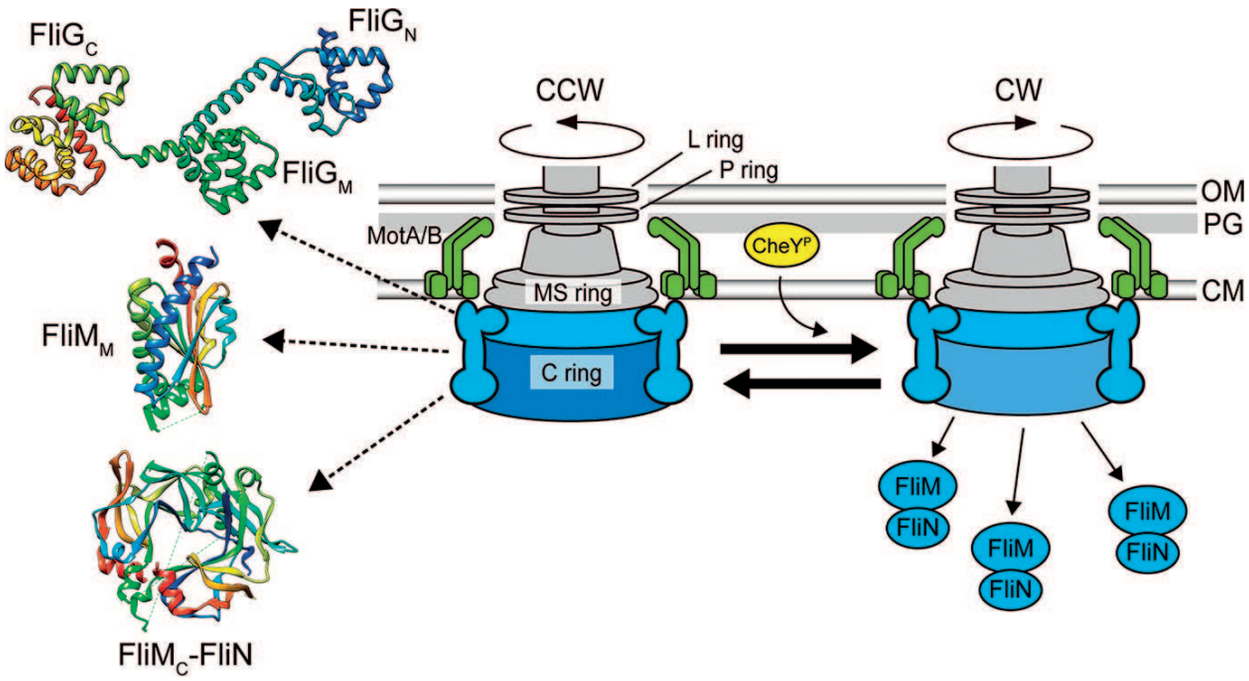

Figure 2. Structure and dynamic of the C ring. The C ring consists of FliG, FliM and FliN. FliG consists of three domains: $FliG_N$, $FliG_M$ and $FliG_C$. Since $FliG_C$ interacts with the stator protein MotA, $FliG_C$ is located at the upper part of the C ring. FliM binds to FliG through an interaction between $FliG_M$ and the middle domain of FliM ($FliM_M$) to form the continuous wall of the C ring. FliN binds to the C-terminal domain of FliM ($FliM_C$) to form the FliM/FliN complex. $FliM_C$ and FliN together form a spiral structure at the bottom of the C ring. The binding of CheY-P switches the direction of motor rotation from counterclockwise to clockwise directions and induces the dissociation of several FliM/FliN complexes from the C ring. Cα ribbon representations of FliG (PDB ID: 3HJL), $FliM_M$ (PDB ID: 2HPN) and the $FliM_C$-FliN fusion (PDB ID: 4YXB) are shown. CM, cytoplasmic membrane; PG, peptidoglycan layer; OM, outer membrane.

Phosphorylated CheY (CheY-P), which acts as a signaling molecule in a signal transduction network responsible for chemotaxis, binds to FliM and FliN, thereby inducing highly cooperative remodeling of the FliG ring structure. As a result, the motor can spin in the CW direction [11]. The FliG/FliN complex binds to the FliG ring through an interaction between $FliG_M$ and FliM to form a continuous wall of the C ring [12]. The CheY-P binding to FliM and FliN also induces the dissociation of several FliM/FliN complexes from the FliG ring, indicating that the C ring is a highly dynamic structure (**Figure 2**) [13].

FlgI assembles into the P ring around the rod. FlgI self-assembles into the L ring on the P ring to form the LP ring complex. Since the LP ring complex acts as a molecular bushing, the friction between the rod and the inner surface of the LP ring is postulated to be very small [5].

The rod is a helical structure consisting of three proximal rod proteins, FlgB, FlgC and FlgF and the distal rod protein FlgG [5]. Recent high-resolution structural analysis of the FlgG polyrod by electron cryomicroscopy and helical image analysis have shown that the FlgG rod is composed of 11 protofilaments [14]. FlgG consists of domains D0, Dc and D1, arranged from the inner to the outer part of the FlgG rod structure (**Figure 3A**). The N- and C-terminal α-helices form a coiled coil in the D0 domain to stabilize the entire rod structure [14]. Residues 46–63 in the Dc domain make the FlgG rod straight and rigid and so the rod can act as a drive shaft [14].

FliE is a basal body protein that interacts with FlgB [15]. Since FliE is the first export substrate to be transported by a type III protein export apparatus [16], FliE is thought to form the junction connecting the MS ring and the rod [15].

2.2. Hook

About 120 subunits of the hook protein FlgE form the hook structure at the tip of the rod. The hook is a short, curved tubular structure made of 11 protofilaments [17]. The hook protein is composed of four domains, D0, Dc, D1 and D2, arranged from the inner to the outer part of the hook structure (**Figure 3B**) [17]. The D0, Dc and D1 domains of FlgE are highly homologous to those of FlgG, thereby allowing the hook to be directly connected to the rod [17]. The axial packing of the subunits in the outer part of the tube made of the D1 and D2 domains is relatively loose [17]. The curvature and twist of the supercoiled structures presumably depend on the direction of intermolecular D2-D2 interactions along the protofilaments in the outermost part of the hook structure [18], and domain Dc plays a critical role in the polymorphic transformation of the supercoiled form of the hook structure [19]. The N- and C-terminal α-helices form a coiled coil in the inner core domain D0 in a way similar to the rod [17].

2.3. Hook-filament junction

FlgK and FlgL together form the hook-filament junction structure at the distal end of the hook structure. When these two proteins are missing, flagellin cannot form the flagellar filament at the hook tip and hence is excreted into the culture media [20]. So, the junction is a buffer structure to connect the hook and filament with distinct mechanical characteristics [21].

2.4. Filament

S. enterica has two distinct flagellin genes, *fliC* and *fljB*. About 30,000 subunits of flagellin form the filament at the tip of the hook-filament junction zone. The filament is a tubular structure made of 11 protofilaments in a way similar to the rod and hook. Flagellin consists of four domains, D0, D1, D2 and D3 (**Figure 3C**). Domains D0 and D1 form the inner and outer tubes of the concentric double-tubular structure, respectively. Hydrophobic interactions between domains D0 make the filament structure mechanically very stable. Domains D2 and D3 form the outer part of flagellin in the filament [22, 23].

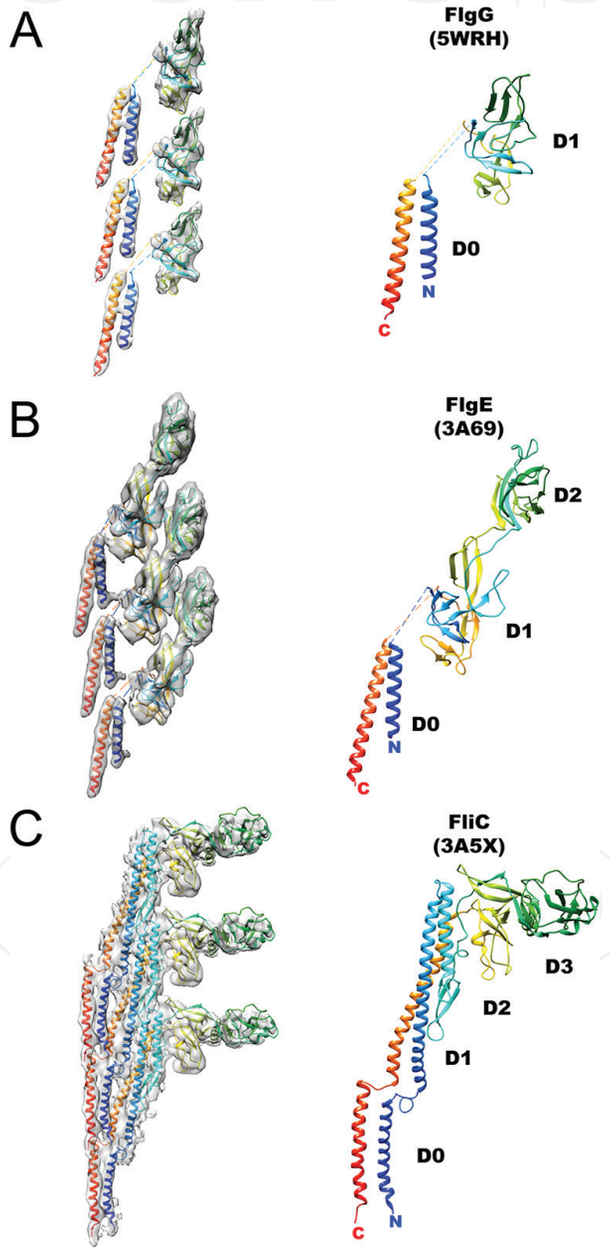

Figure 3. Protofilament structures of the rod, hook and filament. (A) Three subunits of the hook cut out from the EM density map (EMDB-6683) and an atomic model of FlgG (PDB ID: 5WRH) are shown. (B) Three subunits of the hook cut out from the EM density map (EMDB-1647) and a crystal structure of FlgE (PDB ID: 3A69) are shown. (C) Three subunits of the filament cut out from the EM density map (EMDB-1641) and an atomic model of FliC (PDB ID: 3A5X) are shown.

The filament switches between two distinct left- and right-handed supercoiled forms. When each motor spins in CCW direction, several left-handed helical filaments form a flagellar bundle, thereby allowing the cell to smoothly swim in liquid media. Quick reversal of the motor to CW rotation produces a twisting force that transforms the left-handed to the right-handed helical form in a highly cooperative manner. As a result, the flagellar bundle is disrupted and so the cell tumbles and changes the swimming direction [3]. The supercoiled filament forms can be produced by combinations of two distinct conformations and packing interactions of the L- and R-type protofilaments [24]. It has been proposed that conformational change of the β-hairpin in domain D1 is postulated to be responsible for the switching between the L- and R-type filaments [25].

2.5. Filament cap structure

The filament cap is composed of five copies of FliD and exists at the growing end of the filament to facilitate filament assembly [26, 27]. The FliD cap consists of a pentagonal plate domain as a lid and five axially extended leg-like domains [28]. Since there is a symmetry mismatch between the FliD cap with the five-fold rotational symmetry and the helical subunit array of the filament with 11 protofilaments, this symmetry mismatch is postulated to drive filament formation [28].

2.6. Type III protein export apparatus

Component proteins of the axial structure are transported via a type III protein export apparatus into the distal end of the growing flagellar structure [29]. The protein export apparatus has been visualized to be located at the flagellar base by electron cryo-tomography (ECT) and subtomogram averaging (**Figure 4**) [30–32]. The export apparatus is composed of a PMF-driven transmembrane export gate complex made of FlhA, FlhB, FliP, FliQ and FliR, and a cytoplasmic ATPase ring complex consisting of FliH, FliI and FliJ [29]. These proteins are highly homologous to those of the injectisome of pathogenic bacteria, which are involved in direct injection of virulence effector proteins into eukaryotic host cells [33]. Interestingly, the entire architecture of the cytoplasmic ATPase ring complex looks very similar to F-type and A-type rotary ATPases [34–36]. In addition, FlgN, FliS and FliT act as flagellar type III export chaperons to facilitate the export of their cognate substrates [29].

FliP forms a homo-hexamer [37]. FliO is required for efficient FliP ring formation although it is not essential for flagellar protein export [37]. FliQ and FliR are associated with the FliP ring [37], suggesting that FliP, FliQ and FliR together form a core structure of the export gate complex. FlhA and FlhB bind to the FliO/FliP/FliQ/FliR complex [37]. FlhA is also associated with the MS ring [37]. FlhA forms a homo-nonamer through its C-terminal cytoplasmic domains named $FlhA_C$ [8, 31]. FliO, FliP, FliQ and FliR are required for efficient assembly of nine FlhA subunits into the export gate complex inside the MS ring, suggesting that the assembly of the export gate complex begins with FliP ring formation with the help of FliO, followed by the assembly of FliQ, FliR and FlhB and finally that of FlhA [8, 37].

The cytoplasmic ATPase ring complex is composed of six copies of the FliH homo-dimer, six copies of the FliI ATPase and one copy of FliJ [34–36]. The C-terminal domain of FliH

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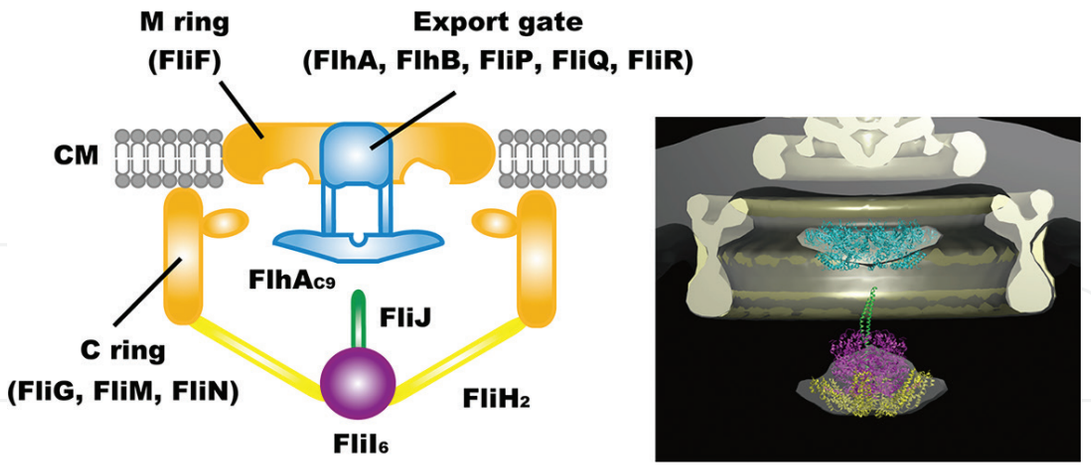

Figure 4. *In situ* structure of the flagellar type III export apparatus. A schematic diagram of cytoplasmic portions of the basal body (left panel). Name of each part of the basal body and component protein(s) are shown. Superposition of a cryoEM density map of isolated basal body on and docking of the atomic models of the $FlhA_{C9}$ ring and the $FliH_{12}$-$FliI_6$-FliJ ATPase ring complex and into the density map of *in situ* basal body (right panel). Cα ribbon representations of $FlhA_C$ (PDB ID: 3A5I), the $FliH_2FliI$ complex (PDB ID: 5B0O) and FliJ (PDB ID: 3AJW) are shown.

($FliH_C$) binds to the N-terminal domain of FliI ($FliI_N$) [38, 39]. FliJ binds to the center of the FliI homo-hexamer [35]. Interactions of the N-terminal domain of FliH ($FliH_N$) with FliN and FlhA anchor the ATPase ring complex to the flagellar base [40–42]. FliH and FliI also exist as the $FliH_2FliI$ complex in the cytoplasm [38]. The $FliH_2FliI$ complex binds to export substrates in complex with flagellar export chaperones [43, 44] and efficiently brings export substrates and chaperone-substrate complexes from the cytoplasm to the export gate complex [45].

FlgN, FliS and FliT are flagellar export chaperones specific for FlgK and FlgL, FliC and FliD, respectively [29]. They bind to the type III export apparatus proteins and facilitate docking and subsequent unfolding of their cognate substrates at the docking platform made of nine copies of $FlhA_C$ [46–48]. FlgN, FliS and FliT adopt a highly α-helical structure and undergo their helical rearrangements coupled with the association with and dissociation from their binding partners during protein export [49–51].

The flagellar type III protein export apparatus utilizes ATP hydrolysis by the FliI ATPase and PMF across the cytoplasmic membrane to drive flagellar protein export [52, 53]. The transmembrane export gate complex acts as a proton/protein antiporter to couple the proton flow through the proton channel of the export gate complex with protein export [54, 55]. FlhA forms part of a proton channel in the export gate complex [56]. ATP hydrolysis by the cytoplasmic ATPase ring complex is postulated to activate the export gate complex to drive flagellar protein export in a PMF-dependent manner [57].

2.7. Stator complex

The stator complex of the flagellar motor is composed of four copies of MotA and two copies of MotB [58]. The $MotA_4MotB_2$ complex acts as a proton channel to couple the proton flow with torque generation. MotA consists of four transmembrane helices, two short periplasmic loops and two extensive cytoplasmic regions. MotB consists of an N-terminal cytoplasmic

region, a single transmembrane helix and the C-terminal periplasmic domain termed $MotB_C$. The transmembrane helix of MotB forms a proton channel along with the transmembrane helices 3 and 4 of MotA [59]. A highly conserved aspartic acid residue, Asp-33 of MotB, which is located near the cytoplasmic end of its transmembrane helix, is involved in proton translocation [60]. $MotB_C$ binds to the peptidoglycan layer, allowing the $MotA_4MotB_2$ complex to act as an active stator unit in the flagellar motor [61]. The flagellar motor can accommodate a dozen $MotA_4MotB_2$ complexes around the MS-C rotor ring complex [62]. The $MotA_4MotB_2$ complexes alternate between localized and freely diffusing forms in response to changes in the environment such as PMF and external load [63, 64]. This indicates that a dozen $MotA_4MotB_2$ complexes do not permanently bind to the peptidoglycan layer.

3. Flagellar gene expression and assembly

3.1. Flagellar assembly

Flagellar assembly proceeds from more proximal structures to more distal ones [65]. FliF and FliG together assemble into the MS ring in the cytoplasmic membrane. During MS ring formation, FlhA, FlhB, FliP, FliQ and FliR together assemble into the transmembrane export gate complex with the help of FliO. Then, the FliM/FliN complex binds to FliG to form the C ring on the cytoplasmic face of the MS ring, followed by the assembly of the $FliH_{12}$-$FliI_6$-FliJ ring complex through interactions of $FliH_N$ with FliN and FlhA. Upon completion of the type III protein export apparatus at the flagellar base, FliE is translocated across the cytoplasmic membrane by the protein export apparatus and assembles at the periplasmic surface of the MS ring. Then, FlgB, FlgC, FlgF and FlgG assemble in this order to form the rod. Then, the LP ring complex forms around the rod. Upon completion of the basal body, FlgD forms the hook cap at the rod tip to support the assembly of FlgE into the hook structure. When the hook reaches its mature length of about 55 nm in *Salmonella*, the hook cap is replaced by FlgK. FlgK and FlgL self-assemble at the hook tip in this order to form the junction structure. Then, FliD forms the filament cap at the tip of the junction to promote the assembly of FliC into the filament that grows up to 15 μm long.

3.2. Flagellar gene expression

More than 70 genes are required for flagellar formation and function in *Salmonella*, and are organized into a transcriptional hierarchy of three promoter classes [66]. At the top of the hierarchy is the *flhD* master operon (class 1) which encodes two genes *flhD* and *flhC* that are required for the expression of class 2 and 3 operons. FlhD and FlhC together form the $FlhD_4FlhC_2$ complex to act as a transcriptional activator that drives the transcription from class 2 promoters. The class 2 genes encode proteins required for the structure and assembly of the hook-basal body (HBB). Also present in this class are the *fliA* gene whose product acts as a flagellum-specific sigma factor (σ^{28}) necessary for the transcription from class 3 promoters, and the *flgM* gene, of which product acts as an anti-sigma factor to inhibit the σ^{28} activity of FliA during HBB assembly. The class 3 operons contain genes required for flagellar filament formation, motility and chemosensory signal transduction [66].

3.3. Coordinating flagellar gene expression with assembly

The hierarchy of flagellar gene expression exactly parallels the flagellar assembly process [66]. The flagellar type III protein export apparatus couples the activation of class 3 genes with flagellar filament assembly. During HBB assembly, FlgM binds to FliA in the cytoplasm and prevents FliA from acting as σ^{28} to drive the transcription from the class 3 promoters [67]. Upon completion of HBB assembly, the protein export apparatus switches its export specificity from the hook protein FlgE to those required for filament formation, thereby terminating hook assembly and initiating the secretion of FlgM from the cytoplasm to the culture media. As a result, σ^{28} can transcribe the class 3 genes [68].

At least, two flagellar proteins, namely FlhB and FliK, are involved in export specificity switching of the flagellar type III protein export apparatus [69, 70]. The C-terminal cytoplasmic domain of FlhB ($FlhB_C$) acts as an export switch to switch substrate specificity of the protein export apparatus from FlgE to FlgM [71]. FliK is secreted via the protein export apparatus into the culture media during hook assembly [72] and acts an infrequent molecular ruler to determine the hook length of about 55 nm in *Salmonella* [73]. The N-terminal region of FliK ($FliK_N$) has the molecular ruler function [73] whereas the C-terminal domain of FliK ($FliK_C$) is responsible for the interaction with $FlhB_C$ to catalyze the export specificity switch [74].

4. Load-dependent energy coupling mechanism of flagellar motor rotation

The flagellar motor regulates the number of active stator units around a rotor ring complex in response to changes in external load [64]. $MotB_C$ acts as a structural switch to drive the assembly-disassembly cycle of the $MotA_4B_2$ complex in response to the load change [75]. A highly conserved Asp33 residue of MotB is involved in the load-dependent proton translocation mechanism of the $MotA_4B_2$ complex [76]. Highly conserved Arg90 and Glu98 residues in the cytoplasmic loop between transmembrane helices 2 and 3 of MotA ($MotA_C$) interact with highly conserved Asp289 and Arg281 residues in $FliG_C$, respectively [10]. It has been shown that the M76V, Y83H, A145E and E155K mutations in $MotA_C$ considerably affect load-dependent assembly and disassembly dynamics of the $MotA_4B_2$ complex. These suggest that the $MotA_4B_2$ complex itself acts as a load sensor and that $MotA_C$ acts as a load sensor that can detect changes in external load to regulate not only the number of active stator units in a motor but also its proton channel activity [77].

A plug segment consisting of residues 53 to 66 in $MotB_C$ suppresses undesirable proton leakage through a proton channel of the $MotA_4B_2$ complex prior to stator assembly into a motor [78]. Since the $MotA_C$-FliG interaction is also responsible for efficient assembly of the $MotA_4B_2$ complex around the rotor ring complex [79, 80], it has been proposed that this interaction induces the detachment of the plug segments from the proton channels, allowing $MotB_C$ to bind to the peptidoglycan layer. As a result, the $MotA_4B_2$ complex becomes an active stator unit to couple the proton flow with torque generation.

5. Conclusion

The flagellar type III protein export apparatus ensures the well-ordered export of flagellar proteins, thereby coupling flagellar gene expression with assembly. The export apparatus utilizes the energy derived from ATP hydrolysis by the FliI ATPase and PMF to efficiently couple the proton influx through the proton channel of the export gate complex with protein translocation into the central channel of the growing structure. But it remains unknown how the export apparatus coordinates flagellar protein export with assembly and how flagellar proteins are unfolded and transported by the export apparatus in a PMF-dependent manner. We are to look into these processes in much more detail to fully understand these intricate mechanisms.

The $MotA_4B_2$ complex is a load-sensor to regulate the number of active stators in a motor in response to external load change. To clarify the load-dependent energy coupling mechanism of the flagellar motor, we need to investigate more precise measurements of flagellar motor dynamics by biophysical techniques combined with genetic and biochemical approaches.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Erhardt M. Strategies to block bacterial pathogenesis by interference with motility and chemotaxis. Current Topics in Microbiology and Immunology. 2016;**398**:185-205

[2] Song WS, Yoon SI. Functional role of flagellin in bacterial flagellar assembly and immune receptor activation: Structure and application. Biodesign. 2016;**4**:98-107

[3] Berg HC. The rotary motor of bacterial flagella. Annual Review of Biochemistry. 2003;**72**:9-54

[4] Macnab RM. Type III flagellar protein export and flagellar assembly. Biochimica et Biophysica Acta. 2004;**1694**:207-217

[5] Minamino T, Imada K, Namba K. Molecular motors of the bacterial flagella. Current Opinion in Structural Biology. 2008;**18**:693-701

[6] Morimoto YV, Minamino T. Structure and function of the bi-directional bacterial flagellar motor. Biomolecules. 2014;**4**:217-234

[7] Ueno T, Oosawa K, Aizawa S. M ring, S ring and proximal rod of the flagellar basal body of *Salmonella typhimurium* are composed of subunits of a single protein, FliF. Journal of Molecular Biology. 1992;**227**:672-677

[8] Morimoto YV, Ito M, Hiraoka KD, Che Y-S, Bai F, Kami-ike N, Namba K, Minamino T. Assembly and stoichiometry of FliF and FlhA in *Salmonella* flagellar basal body. Molecular Microbiology. 2014;**91**:1214-1226

[9] Khan IH, Reese TS, Khan S. The cytoplasmic component of the bacterial flagellar motor. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**:5956-5960

[10] Zhou J, Lloyd SA, Blair DF. Electrostatic interactions between rotor and stator in the bacterial flagellar motor. Proceedings of the National Academy of Sciences of the United States of America. 1998;**95**:6436-6441

[11] Bai F, Branch RW, Nicolau DV Jr, Pilizota T, Steel BC, Maini PK, Berry RM. Conformational spread as a mechanism for cooperativity in the bacterial flagellar switch. Science. 2010;**327**:685-689

[12] Paul K, Gonzalez-Bonet G, Bilwes AM, Crane BR, Blair D. Architecture of the flagellar rotor. The EMBO Journal. 2011;**30**:2962-2971

[13] Branch RW, Sayegh MN, Shen C, Nathan VS, Berg HC. Adaptive remodeling by FliN in the bacterial rotary motor. Journal of Molecular Biology. 2014;**426**:3314-3324

[14] Fujii T, Kato T, Hiraoka D, Miyata T, Minamino T, Chevance F, Hughes K, Namba K. Identical folds used for distinct mechanical functions of the bacterial flagellar rod and hook. Nature Communications. 2017;**8**:14276

[15] Minamino T, Yamaguchi S, Macnab RM. Interaction between FliE and FlgB, a proximal rod component of the flagellar basal body of *Salmonella*. Journal of Bacteriology. 2000;**182**:3029-3036

[16] Hirano T, Minamino T, Namba K, Macnab RM. Substrate specificity classes and the recognition signal for *Salmonella* type III flagellar export. Journal of Bacteriology. 2003;**185**:2485-2492

[17] Fujii T, Kato T, Namba K. Specific arrangement of α-helical coiled coils in the core domain of the bacterial flagellar hook for the universal joint function. Structure. 2009;**17**:1485-1493

[18] Samatey FA, Matsunami H, Imada K, Nagashima S, Shaikh TR, Thomas DR, Chen JZ, Derosier DJ, Kitao A, Namba K. Structure of the bacterial flagellar hook and implication for the molecular universal joint mechanism. Nature. 2004;**431**:1062-1068

[19] Hiraoka KD, Morimoto YV, Inoue Y, Fujii T, Miyata T, Makino F, Minamino T, Namba K. Straight and rigid flagellar hook made by insertion of the FlgG specific sequence into FlgE. Scientific Reports. 2017;**7**:46723

[20] Homma M, Fujita H, Yamaguchi S, Iino T. Excretion of unassembled flagellin by *Salmonella typhimurium* mutant deficient hook-associated proteins. Journal of Bacteriology. 1984;**159**:1056-1059

[21] Homma M, Iino T. Location of hook-associated proteins in flagellar structures of *Salmonella typhimurium*. Journal of Bacteriology. 1985;**162**:183-189

[22] Yonekura K, Maki-Yonekura S, Namba K. Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. Nature. 2003;**424**:643-650

[23] Maki-Yonekura S, Yonekura K, Namba K. Conformational change of flagellin for polymorphic supercoiling of the flagellar filament. Nature Structural & Molecular Biology. 2010;**17**:417-422

[24] Yamashita I, Hasegawa K, Suzuki H, Vonderviszt F, Mimori-Kiyosue Y, Namba K. Structure and switching of bacterial flagellar filament studied by X-ray fiber diffraction. Nature Structural Biology. 1998;**5**:125-132

[25] Samatey FA, Imada K, Nagashima S, Vonderviszt F, Kumasaka T, Yamamoto M, Namba K. Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling. Nature. 2001;**410**:331-337

[26] Ikeda T, Asakura S, Kamiya R. "Cap" on the tip of *Salmonella* flagella. Journal of Molecular Biology. 1985;**184**:735-737

[27] Ikeda T, Oosawa K, Hotani H. Self-assembly of the filament capping protein, FliD, of bacterial flagella into an annular structure. Journal of Molecular Biology. 1996;**259**:679-686

[28] Yonekura K, Maki S, Morgan DG, DeRosier DJ, Vonderviszt F, Imada K, Namba K. The bacterial flagellar cap as the rotary promotor of flagellin self-assembly. Science. 2000;**290**:2148-2152

[29] Minamino T. Protein export through the bacterial flagellar type III export pathway. Biochimica et Biophysica Acta. 2014;**1843**:1642-1648

[30] Chen S, Beeby M, Murphy GE, Leadbetter JR, Hendrixson DR, Briegel A, Li Z, Shi J, Tocheva EI, Müller A, Dobro MJ, Jensen GJ. Structural diversity of bacterial flagellar motors. The EMBO Journal. 2011;**30**:2972-2981

[31] Abrusci P, Vergara-Irigaray M, Johnson S, Beeby MD, Hendrixson DR, Roversi P, Friede ME, Deane JE, Jensen GJ, Tang CM, Lea SM. Architecture of the major component of the type III secretion system export apparatus. Nature Structural & Molecular Biology. 2013;**20**:99-104

[32] Kawamoto A, Morimoto YV, Miyata T, Minamino T, Hughes KT, Namba K. Common and distinct structural features of *Salmonella* injectisome and flagellar basal body. Scientific Reports. 2013;**3**(3369)

[33] Galán JE, Lara-Tejero M, Marlovits TC, Wagner S. Bacterial type III secretion systems: Specialized nanomachines for protein delivery into target cells. Annual Review of Microbiology. 2014;**68**:415-438

[34] Imada K, Minamino T, Tahara A, Namba K. Structural similarity between the flagellar type III ATPase FliI and F1-ATPase subunits. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:485-490

[35] Ibuki T, Imada K, Minamino T, Kato T, Miyata T, Namba K. Common architecture between the flagellar protein export apparatus and F- and V-ATPases. Nature Structural & Molecular Biology. 2011;**18**:277-282

[36] Imada K, Minamino T, Uchida Y, Kinoshita M, Namba K. Insight into the flagella type III export revealed by the complex structure of the type III ATPase and its regulator. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**:3633-3638

[37] Fukumura T, Makino F, Dietsche T, Kinoshita M, Kato T, Wagner S, Namba K, Imada K, Minamino T. Assembly and stoichiometry of the core structure of the bacterial flagellar type III export gate complex. PLoS Biology. 2017;**15**:e2002281

[38] Minamino T, Macnab RM. FliH, a soluble component of the type III flagellar export apparatus of *Salmonella*, forms a complex with FliI and inhibits its ATPase activity. Molecular Microbiology. 2000;**37**:1494-1503

[39] González-Pedrajo B, Fraser GM, Minamino T, Macnab RM. Molecular dissection of *Salmonella* FliH, a regulator of the ATPase FliI and the type III flagellar protein export pathway. Molecular Microbiology. 2002;**45**:967-982

[40] González-Pedrajo B, Minamino T, Kihara M, Namba K. Interactions between C ring proteins and export apparatus components: A possible mechanism for facilitating type III protein export. Molecular Microbiology. 2006;**60**:984-998

[41] Minamino T, Yoshimura SDJ, Morimoto YV, González-Pedrajo B, Kami-ike N, Namba K. Roles of the extreme N-terminal region of FliH for efficient localization of the FliH-FliI complex to the bacterial flagellar type III export apparatus. Molecular Microbiology. 2009;**74**:1471-1483

[42] Hara N, Morimoto YV, Kawamoto A, Namba K, Minamino T. Interaction of the extreme N-terminal region of FliH with FlhA is required for efficient bacterial flagellar protein export. Journal of Bacteriology. 2012;**194**:5353-5360

[43] Thomas J, Stafford GP, Hughes C. Docking of cytosolic chaperone-substrate complexes at the membrane ATPase during flagellar type III protein export. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:3945-3950

[44] Minamino T, Kinoshita M, Imada K, Namba K. Interaction between FliI ATPase and a flagellar chaperone FliT during bacterial flagellar export. Molecular Microbiology. 2012;**83**:168-178

[45] Bai F, Morimoto YV, Yoshimura SDJ, Hara N, Kami-ike N, Namba K, Minamino T. Assembly dynamics and the roles of FliI ATPase of the bacterial flagellar export apparatus. Scientific Reports. 2014;**4**(6528)

[46] Bange G, Kümmerer N, Engel C, Bozkurt G, Wild K, Sinning I. FlhA provides the adaptor for coordinated delivery of late flagella building blocks to the type III secretion system. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:11295-11300

[47] Kinoshita M, Hara N, Imada K, Namba K, Minamino T. Interactions of bacterial flagellar chaperone-substrate complexes with FlhA contribute to co-ordinating assembly of the flagellar filament. Molecular Microbiology. 2013;**90**:1249-1261

[48] Furukawa Y, Inoue Y, Sakaguchi A, Mori Y, Fukumura T, Miyata T, Namba K, Minamino T. Structural stability of flagellin subunits affects the rate of flagellin export in the absence of FliS chaperone. Molecular Microbiology. 2016;**102**:405-416

[49] Evdokimov AG, Phan J, Tropea JE, Routzahn KM, Peters HK, Pokross M, Waugh DS. Similar modes of polypeptide recognition by export chaperones in flagellar biosynthesis and type III secretion. Nature Structural Biology. 2003;**10**:789-793

[50] Imada K, Minamino T, Kinoshita M, Furukawa Y, Namba K. Structural insight into the regulatory mechanisms of interactions of the flagellar type III chaperone FliT with its binding partners. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:8812-8817

[51] Kinoshita M, Nakanishi Y, Furukawa Y, Namba K, Imada K, Minamino T. Rearrangements of α-helical structures of FlgN chaperone control the binding affinity for its cognate substrates during flagellar type III export. Molecular Microbiology. 2016;**101**:656-670

[52] Minamino T, Namba K. Distinct roles of the FliI ATPase and proton motive force in bacterial flagellar protein export. Nature. 2008;**451**:485-488

[53] Paul K, Erhardt M, Hirano T, Blair DF, Hughes KT. Energy source of the flagellar type III secretion. Nature. 2008;**451**:489-492

[54] Minamino T, Morimoto YV, Hara N, Namba K. An energy transduction mechanism used in bacterial type III protein export. Nature Communications. 2011;**2**:475

[55] Morimoto YV, Kami-ike N, Miyata T, Kawamoto A, Kato T, Namba K, Minamino T. High-resolution pH imaging of living bacterial cell to detect local pH differences. MBio. 2016;**7**:e01911-e01916

[56] Minamino T, Morimoto YV, Hara N, Aldridge PD, Namba K. The bacterial flagellar type III export gate complex is a dual fuel engine that can use both H^+ and Na^+ for flagellar protein export. PLoS Pathogens. 2016;**12**:e1005495

[57] Minamino T, Morimoto YV, Kinoshita M, Aldridge PD, Namba K. The bacterial flagellar protein export apparatus processively transports flagellar proteins even with extremely infrequent ATP hydrolysis. Scientific Reports. 2014;**4**:7579

[58] Kojima S, Blair DF. Solubilization and purification of the MotA/MotB complex of *Escherichia coli*. Biochemistry. 2004;**43**:26-34

[59] Nishihara Y, Kitao A. Gate-controlled proton diffusion and protonation-induced ratchet motion in the stator of the bacterial flagellar motor. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**:7737-7742

[60] Sharp LL, Zhou J, Blair DF. Tryptophan-scanning mutagenesis of MotB, an integral membrane protein essential for flagellar rotation in *Escherichia coli*. Biochemistry. 1995;**34**:9166-9171

[61] Kojima S, Imada K, Sakuma M, Sudo Y, Kojima C, Minamino T, Homma M, Namba K. Stator assembly and activation mechanism of the flagellar motor by the periplasmic region of MotB. Molecular Microbiology. 2009;**73**:710-718

[62] Reid SW, Leake MC, Chandler JH, Lo CJ, Armitage JP, Berry RM. The maximum number of torque-generating units in the flagellar motor of *Escherichia coli* is at least 11. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**:8066-8071

[63] Leake MC, Chandler JH, Wadhams GH, Bai F, Berry RM, Armitage JP. Stoichiometry and turnover in single, functioning membrane protein complexes. Nature. 2006;**443**:355-358

[64] Lele PP, Hosu BG, Berg HC. Dynamics of mechanosensing in the bacterial flagellar motor. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:11839-11844

[65] Minamino T, Imada K. The bacterial flagellar motor and its structural diversity. Trends in Microbiology. 2015;**23**:267-274

[66] Chevance FF, Hughes KT. Coordinating assembly of a bacterial macromolecular machine. Nature Reviews. Microbiology. 2008;**6**:455-465

[67] Ohnishi K, Kutsukake K, Suzuki H, Iino TA. Novel transcriptional regulation mechanism in the flagellar regulon of *Salmonella typhimurium*: Anti-sigma factor inhibits the activity of the flagellum-specific sigma factor, σ^F. Molecular Microbiology. 1992;**6**:3149-3157

[68] Hughes KT, Gillen KL, Semon MJ, Karlinsey JE. Sensing structural intermediates in bacterial flagellar assembly by export of a negative regulator. Science. 1993;**262**:1277-1280

[69] Hirano T, Yamaguchi S, Oosawa K, Aizawa S. Roles of FliK and FlhB in determination of flagellar hook length in *Salmonella typhimurium*. Journal of Bacteriology. 1994;**176**:5439-5449

[70] Kutsukake K, Minamino T, Yokoseki T. Isolation and characterization of FliK-independent flagellation mutants from *Salmonella typhimurium*. Journal of Bacteriology. 1994;**176**:7625-7629

[71] Minamino T, Macnab RM. Domain structure of *Salmonella* FlhB, a flagellar export component responsible for substrate specificity switching. Journal of Bacteriology. 2000;**182**:4906-4919

[72] Minamino T, González-Pedrajo B, Yamaguchi K, Aizawa S, Macnab RM. FliK, the protein responsible for flagellar hook length control in *Salmonella*, is exported during hook assembly. Molecular Microbiology. 1999;**34**:295-304

[73] Erhardt M, Singer HM, Wee DH, Keener JP, Hughes KT. An infrequent molecular ruler controls flagellar hook length in *Salmonella enterica*. The EMBO Journal. 2011;**30**:2948-2961

[74] Kinoshita M, Aizawa S, Namba K, Minamino T. The role of intrinsically disordered C-terminal region of FliK in substrate specificity switching of the bacterial flagellar type III export apparatus. Molecular Microbiology. 2017;**105**:572-588

[75] Castillo DJ, Nakamura S, Morimoto YV, Che Y-S, Kamiike N, Kudo S, Minamino T, Namba K. The C-terminal periplasmic domain of MotB is responsible for load-dependent control of the number of stators of the bacterial flagellar motor. Biophysics. 2013;**9**:173-181

[76] Che YS, Nakamura S, Morimoto YV, Kami-ike N, Namba K, Minamino T. Load-sensitive coupling of proton translocation and torque generation in the bacterial flagellar motor. Molecular Microbiology. 2014;**91**:175-184

[77] Pourjaberi SNS, Terahara N, Namba K, Minamino T. The role of a cytoplasmic loop of MotA in load-dependent assembly and disassembly dynamics of the MotA/B stator complex in the bacterial flagellar motor. Molecular Microbiology. 2017;**106**:646-658

[78] Hosking ER, Vogt C, Bakker EP, Manson MD. The *Escherichia coli* MotAB proton channel unplugged. Journal of Molecular Biology. 2006;**364**:921-937

[79] Morimoto YV, Nakamura S, Kami-ike N, Namba K, Minamino T. Charged residues in the cytoplasmic loop of MotA are required for stator assembly into the bacterial flagellar motor. Molecular Microbiology. 2010;**78**:1117-1129

[80] Morimoto YV, Nakamura S, Hiraoka KD, Namba K, Minamino T. Distinct roles of highly conserved charged residues at the MotA-FliG interface in bacterial flagellar motor rotation. Journal of Bacteriology. 2013;**195**:474-481