Molecular Organization and Assembly of the Export Apparatus of Flagellar Type III Secretion Systems

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Abstract The bacterial flagellum is a supramolecular motility machine consisting of the basal body, the hook, and the filament. For construction of the flagellum beyond the cellular membranes, a type III protein export apparatus uses ATP and proton-motive force (PMF) across the cytoplasmic membrane as the energy sources to transport flagellar component proteins from the cytoplasm to the distal end of the growing flagellar structure. The protein export apparatus consists of a PMF-driven transmembrane export gate complex and a cytoplasmic ATPase complex. In

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addition, the basal body C ring acts as a sorting platform for the cytoplasmic ATPase complex that efficiently brings export substrates and type III export chaperone–substrate complexes from the cytoplasm to the export gate complex. In this book chapter, we will summarize our current understanding of molecular organization and assembly of the flagellar type III protein export apparatus.

1 Introduction

Bacteria swim in liquid environments and move on solid surfaces by rotating a very long filamentous assembly called the bacterial flagellum. The flagellum consists of at least three parts: the basal body as a bidirectional rotary motor, the hook as a universal joint, and the filament as a helical propeller. Flagellar assembly begins with the basal body, followed by the hook and finally the filament. Fourteen flagellar proteins are transported via a type III protein export apparatus into the central channel inside the growing structure and assemble at the distal end (Macnab 2004; Minamino et al. 2008; Minamino 2014).

The flagellar type III protein export apparatus is composed of a transmembrane export gate complex made of FlhA, FlhB, FliP, FliQ, and FliR and a cytoplasmic ATPase complex consisting of FliH, FliI, and FliJ. These proteins are evolutionarily related to components of the virulence-associated type III secretion system (T3SS) of pathogenic bacteria, also known as the injectisome (Fig. 1) (Galán et al. 2014; Wagner et al. 2018).

FliG, FliM, and FliN form the C ring on the cytoplasmic face of the basal body MS ring made of a transmembrane protein, FliF. The C ring acts not only as the rotor of the flagellar motor but also as the switch for bidirectional motor rotation, allowing the flagellar motor to rotate both in the counterclockwise and in the clockwise directions (Berg 2003; Morimoto and Minamino 2014). FliM and FliN, which are well conserved in virulence-associated T3SS families, provide the binding sites for the cytoplasmic ATPase complex in complex with export substrates and export chaperone–substrate complexes (González-Pedrajo et al. 2006; Minamino et al. 2009; Lara-Tejero et al. 2011). In this book chapter, we will describe the structure and assembly of the flagellar type III protein export apparatus in *Salmonella enterica*.

2 Structure of the Transmembrane Export Gate Complex

FlhA, FlhB, FliP, FliQ, and FliR form the transmembrane export gate complex inside the MS ring (Minamino and Macnab 1999; Fukumura et al. 2017). A transmembrane protein, FliO, which is not conserved in virulence-associated T3SS families of pathogenic bacteria, is required for efficient assembly of the export gate complex inside the MS ring although it is not essential for flagellar protein

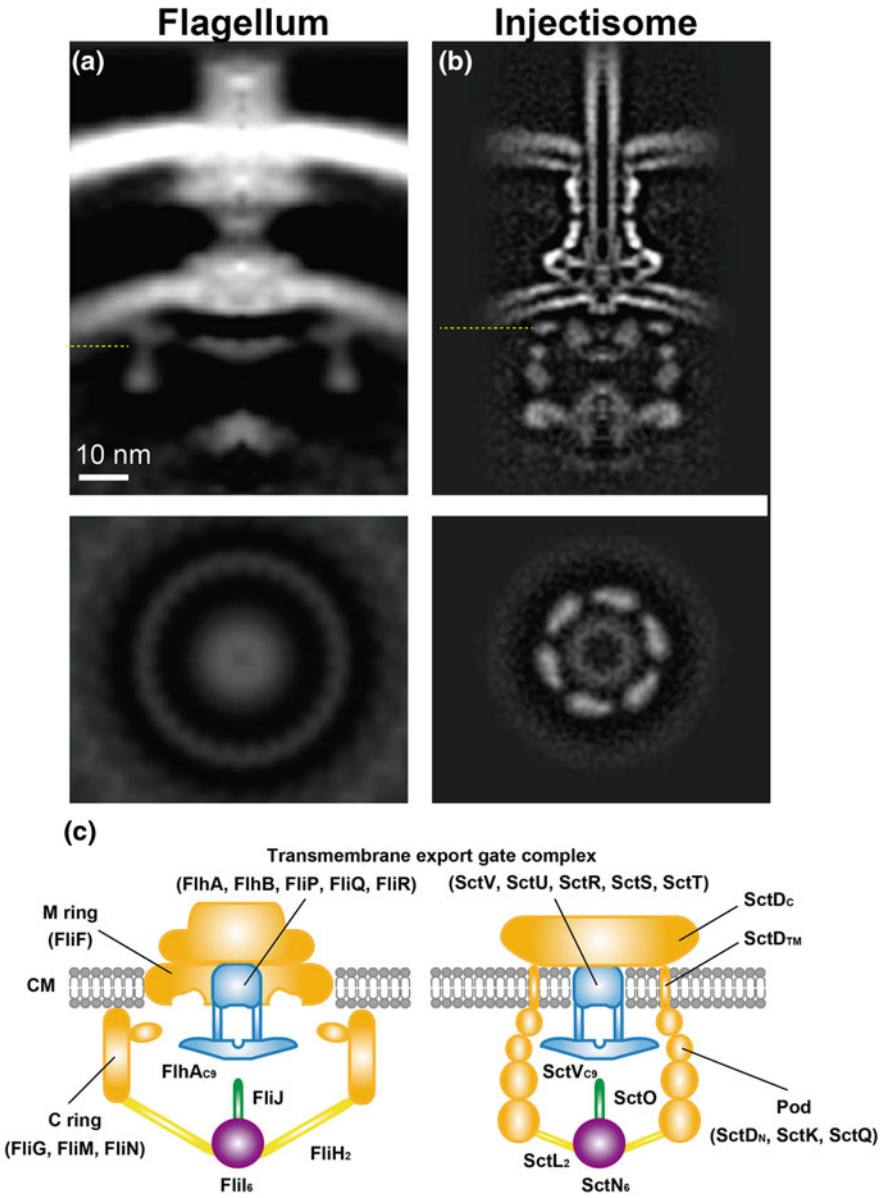

Fig. 1 Structural comparison between the flagellum and the injectisome. In situ structures of the basal bodies of the *Salmonella* flagellum (**a**) and injectisome (**b**) are visualized by electron cryotomography and subtomogram averaging. The central section maps of the flagellum (EMDB-2521) (**a**) and the injectisome (EMDB-8544) (**b**) after subtomogram averaging are shown. Upper panels, side view, lower panels, bottom view corresponding a cross section at height indicated by the dashed yellow line. **c** Schematic diagrams of cytoplasmic portions of the *Salmonella* flagellum (left panel) and injectisome (right panel). Name of each part of the basal body and component protein(s) are shown

export (Barker et al. 2010; Morimoto et al. 2014; Fukumura et al. 2017; Fabiani et al. 2017). The transmembrane export gate complex is powered by the proton-motive force (PMF) across the cytoplasmic membrane and facilitates unfolding and protein translocation across the cytoplasmic membrane (Minamino and Namba 2008; Paul et al. 2008; Minamino et al. 2011; Lee et al. 2014; Terashima et al. 2018).

2.1 FlhA Ring Structure

FlhA forms an ion channel to conduct protons and sodium ions and plays an important role in the energy coupling mechanism along with the cytoplasmic ATPase complex (Minamino et al. 2011, 2016; Morimoto et al. 2016; Erhardt et al. 2017). FlhA consists of a hydrophobic N-terminal transmembrane domain ($FlhA_{TM}$) and a large C-terminal cytoplasmic domain ($FlhA_C$) (Minamino et al. 1994). Genetic and biochemical analyses have shown that $FlhA_{TM}$ interacts with FlhB, FliF, and FliR (Kihara et al. 2001; Barker and Samatey 2012; Hara et al. 2011; Fukumura et al. 2017). $FlhA_C$ has been visualized by electron cryotomography (ECT) and subtomogram averaging to form a ring-shaped projection in the cavity within the C ring (Fig. 1a, c) (Abrusci et al. 2013; Kawamoto et al. 2013). Similar ring-like structures formed by the C-terminal cytoplasmic domain of a FlhA homologue of the injectisome, SctV, have been identified by ECT (Fig. 1b, c) (Kawamoto et al. 2013; Hu et al. 2015, 2017; Makino et al. 2016). $FlhA_C$ and $SctV_C$ form a homo-nonamer as part of the export gate complex (Fig. 2a) (Abrusci et al. 2013; Kawamoto et al. 2013; Morimoto et al. 2014). $FlhA_C$ interacts with FliH, FliI, FliJ, flagellar type III export chaperones, and export substrates and coordinates flagellar protein export with assembly (Minamino and Macnab 2000c; Bange et al. 2010; Minamino et al. 2012a; Kinoshita et al. 2013; Furukawa et al. 2016; Kinoshita et al. 2016; Inoue et al. 2018). This suggests that the $FlhA_C$ ring structure acts as a docking platform for these proteins and plays an important role in hierarchical protein targeting and export.

Crystal structures of $FlhA_C$ and $SctV_C$ have been solved by X-ray crystallography (Bange et al. 2010; Moore and Jia 2010; Saijo-Hamano et al. 2010; Worrall et al. 2010; Abrusci et al. 2013). $FlhA_C$ consists of four domains, D1, D2, D3, and D4, and a flexible linker ($FlhA_L$) connecting with $FlhA_{TM}$ (Fig. 2c). The crystal structure of $SctV_C$ derived from the *Shigella* injectisome forms a nonameric ring structure through D1–D3 and D3–D3 interactions (Abrusci et al. 2013). Because similar subunit interactions are observed in the crystal packing of the *Salmonella* $FlhA_C$ structure (Saijo-Hamano et al. 2010), D1–D3 and D3–D3 interactions are likely to be responsible for the $FlhA_C$ ring formation (Kawamoto et al. 2013). In addition to these interactions, interactions of $FlhA_L$ with domains D1 and D3 of its neighboring $FlhA_C$ subunit are involved in highly cooperative $FlhA_C$ ring formation in solution (Terahara et al. 2018). $FlhA_C$ adopts two distinct, open and closed conformations (Moore and Jia 2010; Saijo-Hamano et al. 2010; Worrall et al. 2010; Abrusci et al. 2013). A large open cleft between domains D2 and D4 is observed in

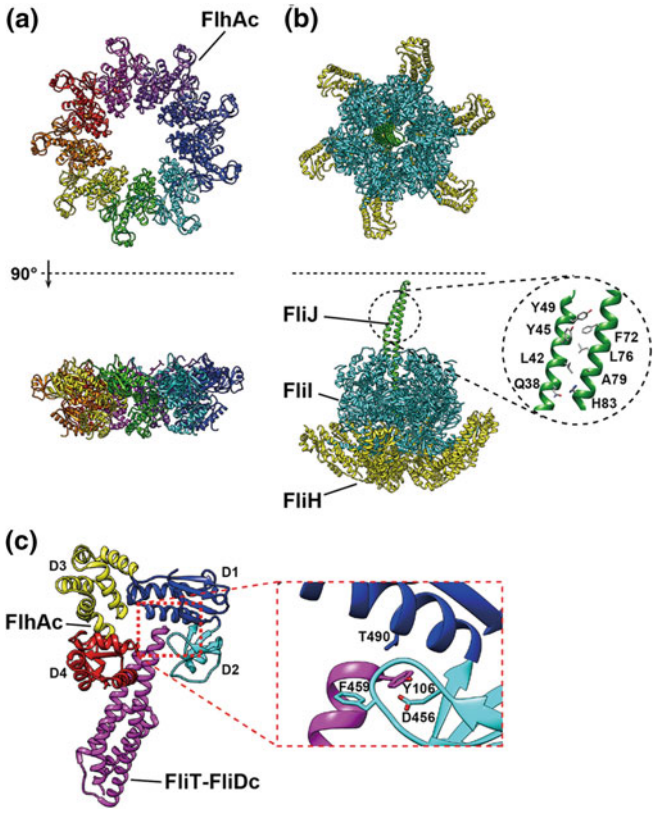

Fig. 2 Atomic models of the docking platform made of $FlhA_C$ and the cytoplasmic ATPase ring complex consisting of FliH, FliI and FliJ. **a** The $FlhA_C$ ring, which is involved in the interactions with FliH, FliI, FliJ, flagellar type III export chaperones and export substrates and **b** the $FliH_{12}FliI_6FliJ$ ring, which plays an important role in energy transduction. Cα ribbon representation of $FlhA_C$ (PDB ID: 3A5I), the $FliH_2FliI$ complex (PDB ID: 5B0O), and FliJ (PDB ID: 3AJW) is shown. Highly conserved Gln38, Leu42, Tyr45, Tyr49, Phe72, Leu76, Ala79, and His83 residues of FliJ (indicated as Q38, L42, Y45, Y49, F72, L76, A79, and H83, respectively) are responsible for the interaction with $FlhA_C$ and the FliJ–$FlhA_C$ interaction facilitates PMF-driven protein export by the transmembrane export gate complex **c** crystal structure of $FlhA_C$ in complex with a $FliD_C$–FliT fusion protein (PDB ID: 6CH2). $FlhA_C$ consists of four domains: D1, D2, D3, and D4. A highly conserved Tyr106 residue of FliT (magenta) binds to a conserved hydrophobic dimple at an interface between domains D1 and D2 of $FlhA_C$. Well conserved Asp456, Phe459 and Thr490 residues of $FlhA_C$ (indicated as D456, F459, and T490, respectively) are responsible for the interaction with Tyr108 of FliT

the open form, but the cleft is closed in the closed form. A conserved hydrophobic dimple containing Asp456, Phe459, and Thr490 resides is located at the interface between domains D1 and D2 and is directly involved in the interactions with the FlgN, FliS, and FliT chaperones in complex with their cognate substrates (Minamino et al. 2012a; Kinoshita et al. 2013). Crystal structures of $FlhA_C$ in

complex with the chaperone–substrate complexes have shown that the chaperones bind to the hydrophobic dimple of the open form of $FlhA_C$ (Fig. 2c) (Xing et al. 2018). Mutations at residues involved in the interactions of $FlhA_L$ with its neighboring $FlhA_C$ subunits in the $FlhA_C$ ring structure significantly weaken the interaction of $FlhA_C$ with the chaperone–substrate complexes, thereby reducing the probability of filament assembly at the hook tip (Terahara et al. 2018). This leads to a plausible hypothesis that interactions of $FlhA_L$ with the D1 and D3 domains of its neighboring $FlhA_C$ subunits may convert the $FlhA_C$ ring structure from the closed conformation to the open one, allowing the chaperone–substrate complexes to bind to the $FlhA_C$ ring.

2.2 *FlhB*

The type III protein export apparatus undergoes substrate specificity switching upon completion of hook assembly, terminating hook assembly, and initiating filament assembly. FlhB is involved in the substrate specificity switching along with a secreted molecular ruler protein, FliK, which is also secreted via the type III protein export apparatus during hook assembly (Minamino 2018). FlhB consists of a hydrophobic N-terminal domain and a relatively large C-terminal cytoplasmic domain ($FlhB_C$) (Minamino et al. 1994). Crystal structures of $FlhB_C$ and its homologue of the injectisome, $SctU_C$, have been solved by X-ray crystallography. $FlhB_C$ and $SctU_C$ contain two distinct: CN and CC polypeptides (Zarivach et al. 2008; Meshcheryakov et al. 2013). The $FlhB_{CN}$ polypeptide consists of a long α helix (α1) and a β strand (β1). The $FlhB_{CC}$ polypeptide is composed of three α helices (α2, α3, α4), three β strands (β2, β3, β4), and a highly flexible C-terminal tail (residues 354–383, $FlhB_{CCT}$). Four α helices surround a four-stranded β sheet, forming a globular domain (Meshcheryakov et al. 2013). $FlhB_{CCT}$ is dispensable for FlhB function, but its truncation results in autonomous substrate specificity switching of the type III protein export apparatus in the absence of FliK (Kutsukake et al. 1994). This suggests that $FlhB_{CCT}$ may contribute to the well-regulated substrate specificity switching. A highly conserved NPTH sequence lies on a flexible loop located between $FlhB_{CN}$ and $FlhB_{CC}$ (Minamino and Macnab 2000a; Fraser et al. 2003). $FlhB_C$ undergoes autocatalytic cleavage between Asn269 and Pro270 within the NPTH sequence to be split into $FlhB_{CN}$ and $FlhB_{CC}$ by a mechanism involving cyclization of Asn269 (Ferris et al. 2005; Zarivach et al. 2008). A conserved hydrophobic patch formed by Ala286, Pro287, Ala341, and Leu344 in $FlhB_{CC}$ is directly involved in interactions with the N-terminal region of the hook protein, which contains an export signal recognized by the type III protein export apparatus (Evans et al. 2013). Photo-cross-linking experiments combined with mutational analyses have shown that the C-terminal domain of FliK binds to $FlhB_C$, thereby terminating the export of the rod and hook-type proteins and initiating the export of the filament-type proteins (Kinoshita et al. 2017).

2.3 Core Structure of the Export Gate Complex

FliP, FliQ, and FliR form a core structure of the transmembrane export gate complex (Fukumura et al. 2017). The structure of purified FliP–FliQ–FliR core complex has been determined at 4.2 Å resolution by electron cryomicroscopy (cryoEM) and single-particle image analysis. The cryoEM structure of the core complex adopts a right-handed helical assembly composed of five copies of FliP, four copies of FliQ, and one copy of FliR (Kuhlen et al. 2018) (Fig. 3). Although FliP, FliQ, and FliR are predicted to have four, two, and six transmembrane helices, respectively (Ohnishi et al. 1997), they do not adopt canonical integral membrane topologies in the core complex (Kuhlen et al. 2018). FliP and FliR form a $FliP_5FliR_1$ complex, and four FliQ subunits are associated with the $FliP_5FliR_1$ complex on its outside. FliR is a structural fusion of FliP and FliQ and so compensates for a helical rise between the first and the fifth FliP subunits to stabilize the helical structure. The assembled $FliP_5FliQ_4FliR_1$ core complex has a central pore with a diameter of 1.5 nm, which seems to be the protein translocation channel (Kuhlen et al. 2018). The most distal part of the core complex is likely to interact with the most proximal end of the rod inside the basal body MS ring (Dietsche et al. 2016; Kuhlen et al. 2018). Biochemical analyses have shown that SctR, SctS, and SctT, which are FliP, FliQ, and FliR homologues of the injectisome, respectively, form a core structure in the injectisome in a way similar to the assembly of the $FliP_5FliQ_4FliR_1$ complex (Wagner et al. 2010; Zilkenat et al. 2016; Dietsche et al. 2016).

FliO consists of an N-terminal periplasmic tail, a single transmembrane helix and a C-terminal cytoplasmic domain (Barker et al. 2010). FliO forms a 5 nm ring structure with three flexible clamp-like structures that bind to FliP to facilitate FliP oligomerization (Fukumura et al. 2017). Highly conserved Phe137, Phe150, and Glu178 residues of FliP, which are functionally important, contribute not only to FliO–FliP interaction but also for FliP–FliP interaction (Fukumura et al. 2017). The FliO ring complex protects FliP from proteolytic degradation and promotes stable $FliP_5FliR_1$ complex formation (Fabiani et al. 2017). Overexpression of FliP restores motility of a *Salmonella fliO* null mutant to the wild-type level, suggesting that the FliO ring complex acts as a structural scaffold to facilitate the helical assembly of the $FliP_5FliR_1$ complex (Barker et al. 2010; Fukumura et al. 2017; Fabiani et al. 2017).

3 Cytoplasmic ATPase Ring Complex

The cytoplasmic ATPase ring complex is composed of twelve copies of FliH, six copies of FliI and one copy of FliJ (Fig. 2b) (Imada et al. 2016). The ATPase ring structure has been visualized at the flagellar base by ECT (Fig. 1a, c) (Chen et al. 2011). ATP hydrolysis by the ATPase ring complex activates the transmembrane export gate complex through an interaction between FliJ and FlhA, allowing the export gate complex to utilize PMF across the cytoplasmic membrane to transport

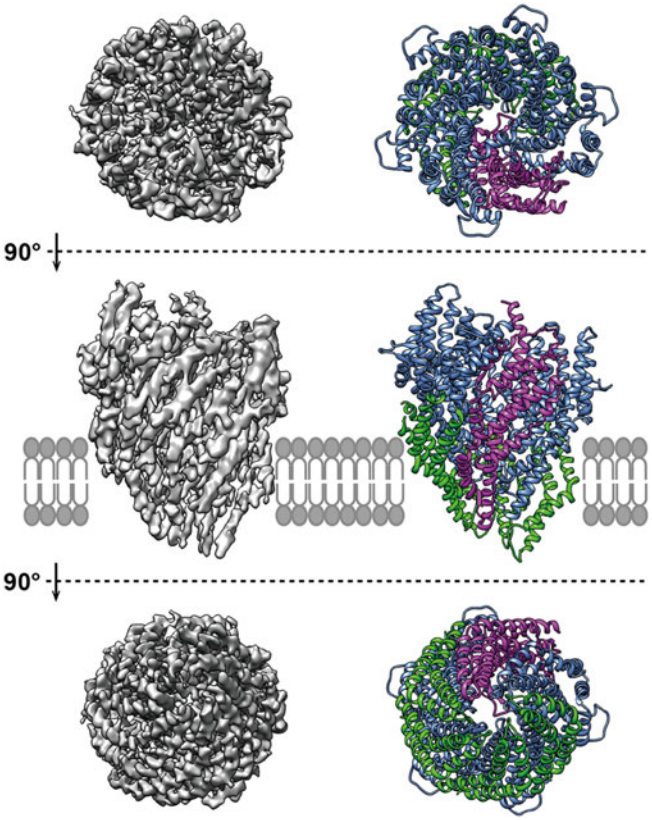

Fig. 3 CryoEM structure of the $FliP_5FliQ_4FliR_1$ complex. Left, cryoEM map of the $FliP_5FliQ_4FliR_1$ complex reconstructed from 98,000 particles with C1 symmetry (EMDB-4173). Right, Cα ribbon representation (PDB ID: 6F2D). Blue, FliP; green, FliQ; and magenta, FliR

flagellar proteins (Minamino et al. 2014). The $FliH_{12}FliI_6FliJ$ complex is structurally similar to F- and V-type rotary ATPases, suggesting that the flagellar ATPase ring complex is evolutionally related to these rotary ATPases (Imada et al. 2007; Ibuki et al. 2011; Imada et al. 2016).

FliI is a Walker-type ATPase and forms a homo-hexamer to fully exert its ATPase activity (Fig. 2b) (Fan et al. 1996; Claret et al. 2003). ATP binds to an ATP binding site located an interface between FliI subunits in a way similar to that found in F- and V-type rotary ATPases, and so FliI ring formation is required for ATP hydrolysis by the FliI ATPase (Kazetani et al. 2009). FliJ facilitates the assembly of FliI into the hexameric ring structure by binding to the center of the ring (Ibuki et al. 2011). Highly conserved, surface-exposed residues of FliJ, namely Gln38, Leu42, Tyr45, Tyr49, Phe72, Leu76, Ala79, and His83, are involved in the interaction with $FlhA_L$ (Fig. 2b) (Ibuki et al. 2013), and the interaction between FliJ and $FlhA_L$ coordinates ATP hydrolysis by the $FliI_6$ ring with proton-coupled flagellar protein export

(Minamino et al. 2014; Morimoto et al. 2016). FliH forms a homo-dimer (Minamino and Macnab 2000b) although the conformation of the two FliH monomers is different from each other (Minamino et al. 2002; Imada et al. 2016). The C-terminal domain of FliH binds to the extreme N-terminal region of FliI (Fig. 2b) (Minamino and Macnab 2000b; González-Pedrajo et al. 2002; Okabe et al. 2009; Imada et al. 2016). The FliH dimer binds to a C ring protein, FliN, and FlhA to allow the ATPase ring complex to efficiently localize to the flagellar base (González-Pedrajo et al. 2006; Minamino et al. 2009; Bai et al. 2014). Two conserved Trp7 and Trp10 residues of $FliH_N$ are directly involved in the interactions of FliH with FliN and FlhA (Minamino et al. 2009; Hara et al. 2012; Notti et al. 2015).

FliH and FliI also exist as a $FliH_2FliI_1$ complex in the cytoplasm (Minamino and Macnab 2000b; Minamino et al. 2001). Flagellar export chaperone–substrate complexes bind to the $FliH_2FliI_1$ complex through an interaction between the FliI ATPase and the chaperone (Thomas et al. 2004; Imada et al. 2010; Minamino et al. 2012b). More than six copies of FliI labeled with yellow fluorescent protein (FliI-YFP) are estimated to be associated with the basal body through the interactions of FliH with FliN and FlhA. Since FliI-YFP shows rapid exchanges between the flagellar basal body and the cytoplasmic pool, the $FliH_2FliI_1$ complex is thought to act as a dynamic carrier to bring export substrates and chaperone–substrate complexes from the cytoplasm to the $FlhA_C$–$FlhB_C$ docking platform of the transmembrane export gate complex (Bai et al. 2014; Terashima et al. 2018).

4 Sorting Platform

The C ring is composed of FliG, FliM, and FliN. The C ring acts not only as a rotor of the flagellar motor but also as a structural switch to change the direction of flagellar motor rotation (Fig. 1c) (Berg 2003; Morimoto and Minamino 2014). The C ring has 34-fold rotational symmetry (Thomas et al. 1999). FliG consists of three domains: N-terminal ($FliG_N$), middle ($FliG_M$), and C-terminal ($FliG_C$) domains (Lee et al. 2010). $FliG_N$ directly binds to the MS ring protein, FliF (Kihara et al. 2000). Crystal structures of $FliG_N$ in complex with the C-terminal portion of FliF ($FliF_C$) have been solved by X-ray crystallography (Lynch et al. 2017; Xue et al. 2018). Two α helices of $FliF_C$ are deeply inserted into a hydrophobic groove of $FliG_N$ formed by four α helices. Helix α4 of $FliG_N$ adopts two distinct conformations. One adopts an extended conformation whereas the other is divided into helices α4a and α4b. When the $FliF_C$–$FliG_N$ complex exists in a solution, the α4 helix adopts an extended conformation (Lynch et al. 2017). When FliG assembles into the FliG ring structure on the cytoplasmic face of the MS ring, the α4 helix induces a conformational change and so helix α4b interacts with helix α4a of its neighboring FliG subunit (Lynch et al. 2017; Xue et al. 2018). The $FliG_{MC}$ unit, which consists of $FliG_M$, $FliG_C$ and a helix linker connecting these two domains, adopts a compact conformation through an intramolecular interaction between $FliG_M$ and $FliG_C$, allowing FliG to exit as a monomer in solution (Baker et al. 2016;

Kinoshita et al. 2018a). In contrast, when the $FliG_{MC}$ unit adopts an extended conformation, intermolecular interactions between $FliG_M$ and $FliG_C$ promote the self-assembly of FliG into the ring structure (Baker et al. 2016; Kinoshita et al. 2018b). FliM and FliN form a stable $FliM_1FliN_3$ complex through an interaction between the C-terminal domain of FliM ($FliM_C$) and FliN (Notti et al. 2015; McDowell et al. 2016) and bind to the FliG ring through an interaction between the middle domain of FliM ($FliM_M$) and $FliG_M$ to form the C ring wall (Paul et al. 2011; Vartanian et al. 2012). Intermolecular interactions between $FliM_M$ domains are required for the formation of the continuous wall of the C ring (Park et al. 2006). $FliM_C$ and FliN together form a spiral structure at the bottom of the C ring (McDowell et al. 2016). It has been shown that surface-exposed hydrophobic residues of FliN, Val111, Val112 and Val113, are involved in the interaction with FliH (McMurry et al. 2006; Paul et al. 2006). Overexpression of FliI partially rescues the reduced ability of flagellar protein export by the *fliN* null mutant (McMurry et al. 2006), suggesting that the C ring is required for efficient recruitment of the $FliH_2FliI_1$ complex to the type III protein export apparatus for efficient flagellar protein export.

It has also been shown that the sorting platform of the injectisome contributes to a strict order of protein secretion by the type III protein export apparatus (Lara-Tejero et al. 2011). However, the structure and stoichiometry of the sorting platform of the injectisome are distinct from those of the flagellar C ring structure (Fig. 1) (Kawamoto et al. 2013; Hu et al. 2015; Makino et al. 2016; Hu et al. 2017). A FliM/FliN homologue of the injectisome, SctQ, forms six pod-like structures on the cytoplasmic face of the cytoplasmic membrane ring (Fig. 1b) (Hu et al. 2015; Makino et al. 2016; Hu et al. 2017). SctK, which is not conserved in the flagellar type III protein export system, associates with the pod-like structure (Hu et al. 2017). SctL, which is a FliH homologue of the injectisome, forms a linker connecting the pod and the ATPase ring complex made of a FliI homologue, SctN (Notti et al. 2015; Hu et al. 2017). It has been shown the sorting platform is highly dynamic structure during protein secretion (Diepold et al. 2015, 2017).

5 Assembly of the Type III Protein Export Apparatus

FliF assembles into the MS ring within the cytoplasmic membrane (Kubori et al. 1992; Ueno et al. 1992). Recently, it has been reported that FliG is required for efficient MS ring formation (Li and Sourjik 2011; Morimoto et al. 2014). This suggests that FliF and FliG together form the MS–FliG ring complex. FliP and FliR form a $FliP_5FliR_1$ complex in a FliO-dependent manner (Dietsche et al. 2016; Fukumura et al. 2017; Fabiani et al. 2017). FliQ is peripherally associated around the outside of the $FliP_5FliR_1$ complex and forms a helical $FliP_5FliQ_4FliR_1$ structure inside the MS ring (Kuhlen et al. 2018). FlhA and FlhB are associated with the $FliP_5FliQ_4FliR_1$ core complex (Fukumura et al. 2017). FlhA also binds to the MS ring directly (Fukumura et al. 2017). Since FlhA requires FliF, FliG, FliO, FliP,

FliQ, and FliR for its assembly to the flagellar basal body but not FlhB (Morimoto et al. 2014), it has been proposed that the assembly of the flagellar type III export gate complex begins with the formation of the $FliP_5FliR_1$ complex with the help of the FliO ring complex, followed by the assembly of FliQ and finally of FlhA and FlhB during MS ring formation (Fig. 4) (Wagner et al. 2010; Diepold et al. 2011; Dietsche et al. 2016; Fukumura et al. 2017).

The $FliM_1FliN_3$ complex forms the continuous wall of the C ring on the cytoplasmic face of the MS ring through the interactions between $FliG_M$ and $FliM_M$ (Paul et al. 2011; Vartanian et al. 2012). Finally, the cytoplasmic $FliH_{12}FliI_6FliJ_1$ ring complex is formed at the flagellar base through the interactions of FliH with FlhA and FliN (Fig. 4). Upon completion of the type III protein export apparatus, export substrates and flagellar export chaperone–substrate complexes are efficiently recruited via the cytoplasmic $FliH_2FliI_1$ complex to the type III protein export apparatus to be transported into the central channel of the growing flagellar structure.

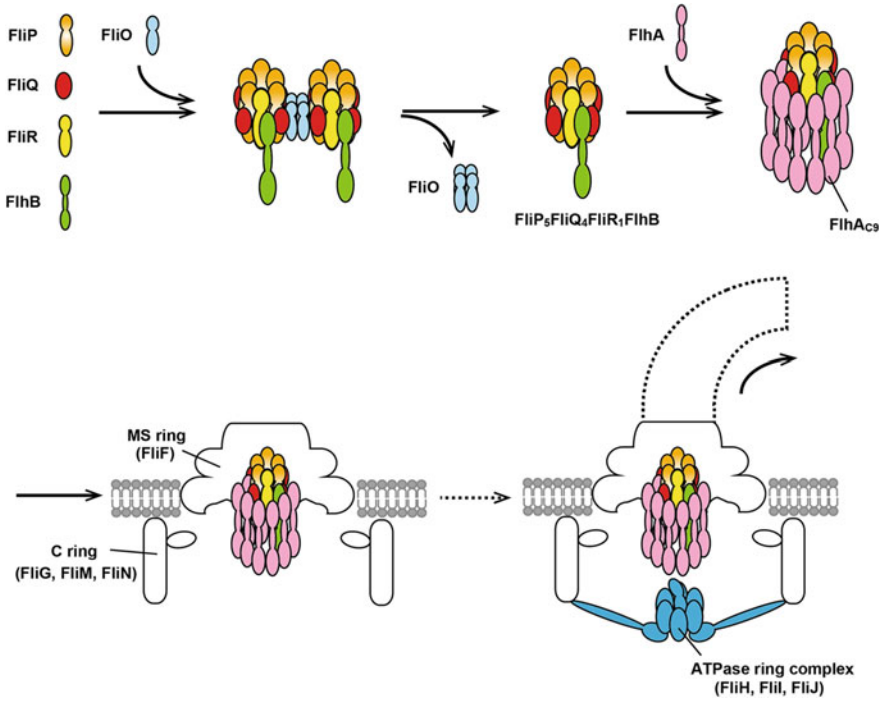

Fig. 4 Assembly mechanism of the type III protein export apparatus. FliP, FliQ, and FliR form a $FliP_5FliQ_4FliR_1$ complex with the help of the FliO complex, followed by the assembly of FlhB and finally of FlhA during MS ring formation in the cytoplasmic membrane. Then, the $FliM_1FliN_3$ complex binds to FliG to form the C ring on the cytoplasmic face of the MS ring. Finally, the cytoplasmic ATPase ring complex made of FliH, FliI, and FliJ is formed and is associated with the C ring through interactions between FliH and FliN

6 Conclusion

The type III protein export apparatus consists of a PMF-driven export gate complex made of FlhA, FlhB, FliP, FliQ, and FliR and a cytoplasmic ATPase complex consisting of FliH, FliI, and FliJ. The export apparatus utilizes ATP and PMF to efficiently couple the proton influx through the export gate complex with protein translocation into the central channel of the growing structure. Atomic structures of the C-terminal cytoplasmic domains of FlhA and FlhB, FliH, FliI, FliJ, and the $FliP_5FliQ_4FliR_1$ helical assembly have been solved. However, it still remains unknown how flagellar proteins are unfolded and transported by the PMF-driven export gate complex. High-resolution structural analysis of the entire protein export apparatus by cryoEM image analysis would be essential to advance our mechanistic understanding of the type III protein export process.

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