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Molecular architecture of the flagellar export apparatus reveals membrane remodeling and conformational changes crucial for flagellar assembly

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Background

A highly conserved export apparatus, powered by the proton motive force (PMF) and ATP hydrolysis, is required to assemble bacterial flagella. The flagellar export apparatus consists of five integral membrane proteins (FlhA, FlhB, FliP, FliQ, and FliR) and three cytoplasmic proteins (FliH, FliI, and FliJ)(Macnab, 2004). The membrane components form an export gate for secretion of substrates, while the three cytoplasmic proteins form an ATPase complex that promotes the export process by binding and delivering substrates to the export gate. In order to determine the molecular events that govern the export of substrates, it is essential to understand the subunit organization of the intact export apparatus. FlhA is the largest membrane protein essential for coupling proton flow with the translocation of substrates into the central channel of the growing flagellum (Kihara et al., 2001; Minamino et al., 2010; Zhu et al., 2002). FlhA consists of an N-terminal transmembrane domain ($FlhA_{TM}$) and a C-terminal cytoplasmic domain ($FlhA_C$)(McMurry et al., 2004). A relatively small conserved cytoplasmic domain of FlhA (termed the FHIPEP domain) is also critical in *Salmonella* (Erhardt et al., 2017). FlhB, required for substrate specificity, regulates hook length and switching to flagellin secretion for filament assembly via an autocleavage event (Ferris et al., 2005; Fraser et al., 2003). FliP, FliQ, and FliR form the core complex (FliPQR) involved in protein secretion (Kuhlen et al., 2018). Cryo-EM structures of the purified FliPQR and FlhB complexes reveal an unexpected topology and orientation of the complex, with no canonical transmembrane regions. Instead, they form a helical structure that sits at the base of the basal body, mainly in the periplasm (Kuhlen et al., 2018; Kuhlen et al., 2020). Furthermore, cryo-electron tomography (cryo-ET) studies revealed that the cytosolic ATPase complex forms a hub-and--spoke structure attached directly to the flagellar C-ring (Qin et al., 2018)(Lin et al., 2015). Despite extensive structural characterization of the key protein components by X-ray crystallography, cryo-EM and cryo-ET, the subunit organization of the intact export apparatus remains incomplete.

Methods

We deployed cryo-ET and sub-tomogram averaging to visualize the intact export apparatus of periplasmic flagella in *Borrelia burgdorferi*, the causative agent of Lyme disease. In particular, *in situ* structure of the intact export apparatus is compared with those derived from specific export apparatus mutants.

Results

We determined high resolution *in situ* structure of the intact flagellar export apparatus in *B. burgdorferi*. Using export gate protein deletion mutants, we reveal that the inner membrane undergoes major remodeling to accommodate the assembly of the FliPQR complex (**Figure**). We provided evidence that the membrane remodeling is required for the assembly of the FlhA ring. Using two specific point mutants in FlhA, we showed how disruption of proton transport causes improper flagellar assembly.

Conclusions

We report that the FliPQR complex facilitates membrane remodeling, which is essential for the assembly of the FlhA ring and the formation of the export gate. We resolved full-length FlhA, resulting in identification of two nonameric rings connected by tubular density. Furthermore, we provided direct evidence that flagellar substrate exportation is dependent on the proton channel of FlhA and conformational change of the FlhA ring.

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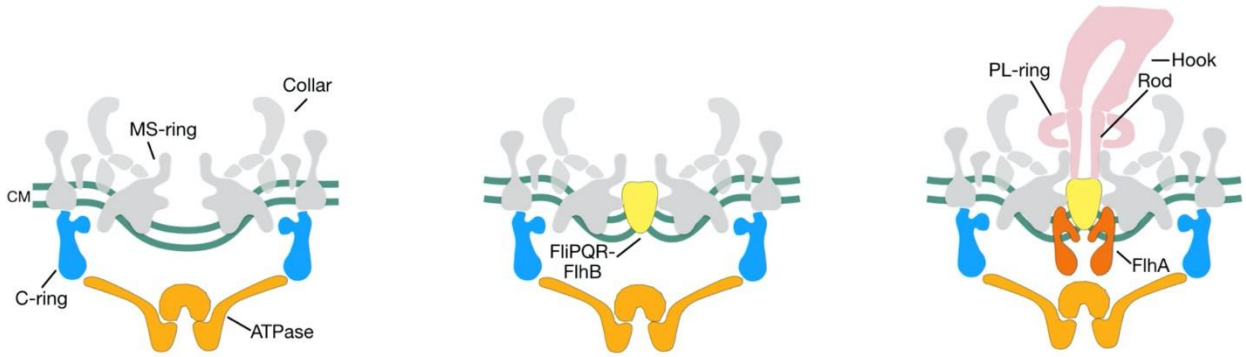

Figure 1. Figure. Models of the membrane-bound B. burgdorferi flagellar motor in three distinct conformations: single concave membrane (left) in the absence of the export gate complex, pinched membrane (middle) with the assembly of the FliPQR-FlhB complex, and FlhA docking in the pinched membrane (right). The membrane remodeling is critical for FlhA assembly and function.

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