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Molecular Architecture of Bacterial Flagellar Stator Revealed by Cryo-Electron Tomography

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Bacterial flagella are helical propellers turned by the flagellar motor, a remarkable nano-machine embedded in the bacterial cell envelope [1,2]. Powered by the proton gradient across the cytoplasmic membrane, the motor converts electrochemical energy into torque through an interaction between the rotor and the stator [3,4]. The stator of the flagella motor, composed of the membrane protein complexes (MotA/MotB), functions as a proton channel to couple proton flux with motor rotation and as an anchor that stabilizes the motor to cell wall. Mutations in *motA* or *motB* cause a paralyzed flagellum. Direct structural information of the stator and its precise location inside the flagellar motor has so far been limited. Recently, cryo-electron tomography (cryo-ET) of Lyme disease spirochete *Borrelia burgdorferi* revealed intact flagellar motor structure at 3.5nm resolution, yet the stator-rotor interface was not well defined because of the complexity [5]. In this work, a *motB* mutant was constructed by a newly developed non-polar gene inactivation system and was also complemented successfully (Fig.1a). The non-polar *motB*-cells synthesize periplasmic flagella but were paralyzed (Fig. 1b). The defect was corrected when the mutant was complemented (*motB*+) *in trans*. The flagellar motor structures of wild-type (WT), *motB* mutant (*motB*-), and *motB* complement (*motB*+) were reconstructed and analyzed comparatively. A stator ring composed by sixteen MotA/MotB complexes was structurally determined for the first time. It located peripherally around the rotor as shown in Fig. 2. The stator-rotor interaction provides new insight into the fundamental mechanism of flagellar rotation and bacterial motility.

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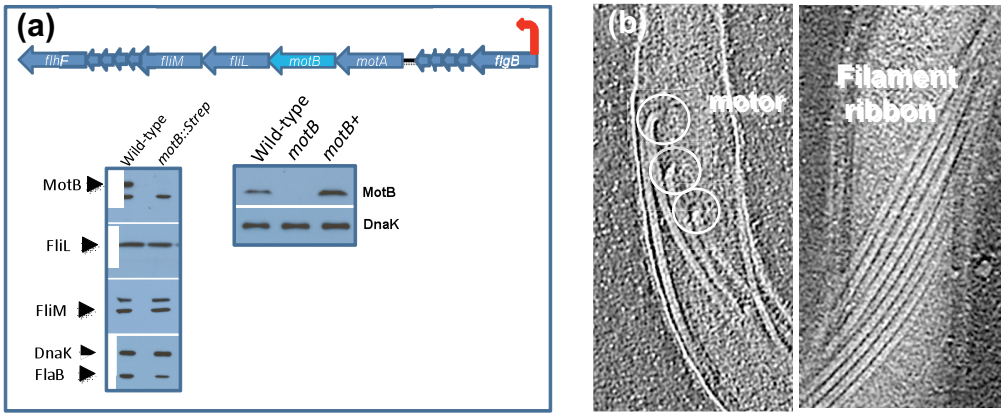

Fig. 1 (a) MotB protein is detected in the WT and the complemented *motB*+ cells but is inhibited in the mutant cells. No polar effect on downstream FliL or FliM synthesis is detected (FliL/FliM blots). Inactivation of *mot*B had a minor affect on FlaB synthesis (FlaB blot). Dnak is used as a loading control. (b) Tomograms of *motB*- cells. *motB*- cells are nonmotile and rod-shaped, but the flagella are assembled as a ribbon.

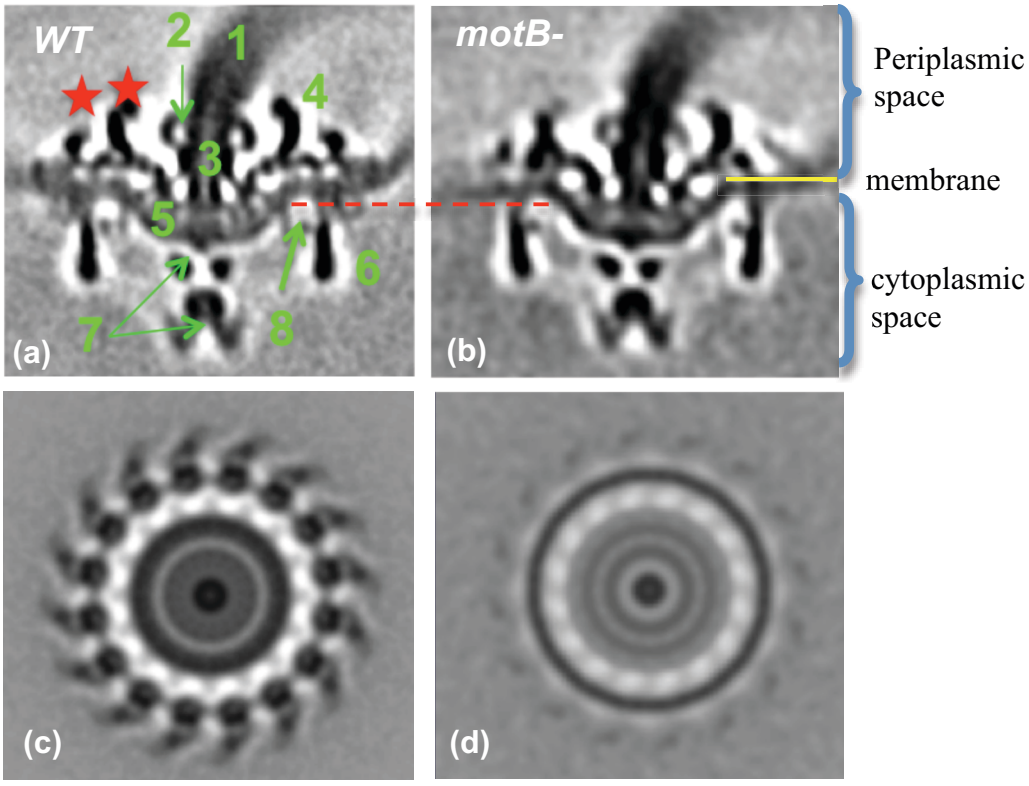

Fig. 2 Three dimensional density maps averaged WT and *motB*- motors. (a) and (b): side-view. The numbers in Fig. 2(a) represent: 1. hook, 2. P ring, 3. rod, 4. collar, 5. MS ring, 6. C ring, 7. export apparatus, and 8. FliG. The red stars indicate the "inner collar" (near the hook) and "outer collar". (c) and (d) plane-view of WT and *motB*- motors showing the slicers indicated by red dashed line. A ring composed by 16 subunits is assumed to be the stator ring in WT motor (c). This ring is not presented in *motB*- motor (d).

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