Medical & healthcare, Drug development

Membrane voltage-dependent activation mechanism of the bacterial flagellar protein export apparatus

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Abstract

Proton motive force (PMF) across the cytoplasmic membrane consists of the electric potential difference and the proton concentration difference. Many motile bacteria employ the flagellar type III secretion system (fT3SS) to construct a supramolecular motility machine, the flagellum, on the cell surface. The fT3SS is composed of a transmembrane export gate complex powered by the PMF and a cytoplasmic ATPase ring complex. If the ATPase ring complex does not work properly under a given environmental condition, the export gate complex remains inactive. However, when membrane voltage exceeds a certain threshold value, the export gate complex becomes an active protein transporter to drive proton-coupled protein export. This observation suggests that the export gate complex has a voltage-gated activation mechanism.

Background & Results

The fT3SS consists 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. ATP hydrolysis by the FliI ATPase activates the transmembrane export gate complex through an interaction between FliJ and FlhA, allowing the export gate complex to utilize the electric potential difference, which is measured as membrane voltage, to couple the proton flow through the FlhA proton channel with protein translocation through a polypeptide channel formed by FliP, FliQ, and FliR. However, it remains unknown how membrane voltage is utilized for flagellar protein export. One *Salmonella* mutant with FliI that cannot work as a flagellum-specific ATPase in the fT3SS showed no visible flagellar filaments at an external pH value of 7.0. However, when this mutant strain was cultured at an external pH value of 8.5, many cells produced a few flagella. Because an upward shift of external pH from 7.0 to 8.5 increased membrane voltage significantly but decreased total PMF in *Salmonella* cells, our observations strongly suggest that the protein transport activity of the export gate complex is quite low at external pH 7.0 but increases markedly with increasing membrane voltage. Furthermore, an increase in membrane voltage significantly stabilized the FlhA-FliJ interaction, allowing FlhA to open its proton channel to conduct protons to be coupled with flagellar protein export.

Significance of the research and Future perspective

In addition to clarifying how membrane voltage is used for flagellar protein export, this is the first report describing a membrane voltage-dependent activation mechanism used for a biological function other than voltage-gated ion channels. These discoveries are expected to enable drug screening for the treatment of infectious diseases to replace conventional antibiotics by directly targeting the injectisome of pathogenic bacteria, which is functionally and structurally identical to the fT3SS.

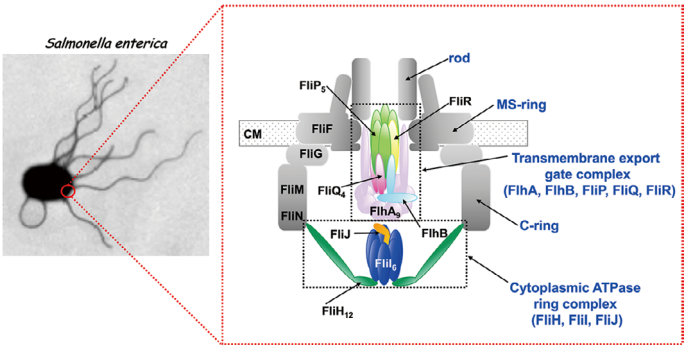

Figure 1. Electron micrograph of *Salmonella* cell and schematic diagram of the bacterial flagellar type III protein export apparatus, which is located at the base of the flagellum.

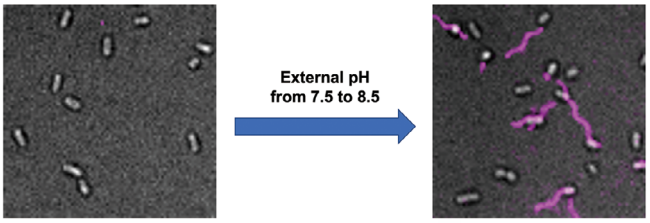

Figure 2. Capability of the *Salmonella* mutant with FliI, which cannot work as a flagellum-specific ATPase in the flagellar type III protein export apparatus, to form flagella.

Patent

Treatise Minamino, Tohru; Morimoto, Yusuke; Kinoshita, Miki et al. Membrane voltage-dependent activation mechanism of the bacterial flagellar protein export apparatus. Proceedings of the National Academy of Sciences of the United States of America. 2021, 118 (22), e2026587118, doi: 10.1073/pnas.2026587118

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Keyword bacterial flagella, type III secretion system, membrane voltage, bacterial infection