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学位論文名	Common architecture between the flagellar type III ATPase complex and F_1-ATPase revealed by the structure of FliJ （FliJの構造より明らかとなったべん毛3型ATPase複合体とF_1-ATPaseの構造的相同性）
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論文内容の要旨

The flagellum is a motile organelle composed of the basal body rings and the tubular axial structure. The axial component proteins synthesized in the cytoplasm are transferred into the central channel of the flagellum by the flagellar type III protein export apparatus for self-assembly at the growing end. The export apparatus is composed of six transmembrane proteins (FlhA, FlhB, FliO, FliP, FliQ, FliR) and three soluble components (FliH, FliI, FliJ). FliI is the ATPase and forms a hetero-trimer complex with a dimer of its regulator FliH in the cytoplasm. The $FliH_2$-FliI complex recruits export substrates to the proton-driven export gate made of the transmembrane proteins and helps the initial entry of the substrate into the gate. FliJ is an essential component for protein export. Since FliJ interacts with flagellar axial proteins and prevents their premature aggregation in the cytoplasm, FliJ has been postulated to be a general chaperone for the substrates. However, it remains unknown how these reactions proceed within the cell.

To elucidate the function of FliJ and molecular mechanism of flagellar protein export across the cell membrane, I determined the crystal structure of FliJ at 2.1 Å resolution. FliJ has a remarkable structural similarity to the γ subunit of F_oF_1-ATPsynthase. Structure-based sequence alignment revealed regions that are conserved between them. It has already been shown that the FliI structure closely resembles those of the α/β subunits of F_1-ATPase and that FliI forms a hexameric ring structure. These similarities suggest that FliI and FliJ may form a complex just like F_1-ATPase. I therefore analyzed the interaction between FliJ and FliI by electron microscopy and biochemical techniques. FliJ formed a complex with the FliI hexameric ring and facilitated the ring formation and the ATPase activity of FliI. These observations suggest that the flagellar protein export apparatus involves a mechanism similar to that of F_1-ATPase and that the flagellum and F_oF_1-ATPsynthase are evolutionally related.

The conserved region between FliJ and the γ subunit is highly conserved among the homologs and corresponds to the surface interacting with the ε subunit of F_1-ATPase, suggesting that FliJ may have binding partner corresponding to the ε subunit. To identify the function of these conserved residues, site-directed mutation experiments were performed for eight highly conserved, surface-exposed residues of FliJ. The F72A and L76A mutations abolished the interaction between GST-FliJ and FlhA. In the recent paper, it has been shown that an interaction between FliJ and a gate-forming protein FlhA facilitates an inward-directed proton flow through the gate. These results suggest that Phe-72 and Leu-76 of FliJ are essential for the interaction with FlhA to facilitate proton translocation-coupled protein export through the gate.

論文審査の結果の要旨

細菌べん毛は、細胞膜に固定された回転モーターと細胞外に伸びるらせん型プロペラからなる運動器官である。その形成は、べん毛基部にあるタンパク質輸送装置が、細胞内で合成されたべん毛構成タンパク質をべん毛軸に沿って貫通する細長いチャネルを通して先端に輸送し、構成タンパク質が先端に順次結合することで進行する。

申請者は、このべん毛特異的タンパク質輸送機構において重要な役割を担っているにもかかわらず、詳細な機能がまったく未知であったFliJのX線結晶構造解析を行い、F_oF_1-ATP合成酵素のγサブユニットとの予想外の構造類似性を発見した。そして、得られたFliJの構造情報をもとに、輸送装置ATPaseであるFliIとの複合体形成を予測し、電子顕微鏡によりそれを見事に証明した。この結果は、べん毛特異的タンパク質輸送装置とF_oF_1-ATP合成酵素が、複合体全体としても構造的および機能的に高い相同性を持つことを示唆した。

本審査会では、申請者が行ったべん毛輸送装置の構造に関する新しい発見と、これに基

づいた遺伝学的・生化学的実験の成果を極めて高く評価し、学位の授与に値すると考える。