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学位論文名	Studies on the mechanisms of assembly and activation of the MotA/B proton channel complex of the proton-driven bacterial flagellar motor （プロトン駆動型細菌べん毛モーター固定子複合体MotA/Bの局在化およびプロトン透過活性についての研究）
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論文内容の要旨

The bacterial flagellum, which is responsible for motility, is a reversible rotary motor powered by the electrochemical potential difference of protons or sodium ions across the cytoplasmic membrane. Five proteins, MotA, MotB, FliG, FliM, and FliN, are responsible for torque generation in the proton-driven flagellar motor. MotA and MotB form the

stator complex of the motor in the cytoplasmic membrane, which functions as a proton channel to couple proton flow to torque generation. FliG, FliM and FliN forms the C ring on the cytoplasmic side of the MS ring, which is composed of FliF, and acts as the rotor. Torque is generated by an electrostatic interaction between MotA and FliG. The proton-conductivity of the MotA/B complex is thought to be suppressed by a plug segment of MotB when the MotA/B complex is not assembled into a motor. However, it remains unknown how the stator complex is installed into the motor, how its proton-conductivity is activated, and how the proton flow through the proton channel is coupled to torque generation.

To clarify the regulatory mechanism of the proton-conductivity of the MotA/B complex, I measured the proton-conductivity of the plugged and unplugged MotA/B complex of *Salmonella* using a pH-sensitive green fluorescent protein, pHluorin, and showed that the proton-conductivity of the MotA/B complex not incorporated into the motor was two orders of magnitude lower than that of a complex that was incorporated and activated one. This leakage was, however, significant enough to change the cytoplasmic pH to a level at which the chemotactic signal transduction system responds.

To investigate the stator assembly mechanism, I constructed *Salmonella* strains expressing GFP-MotB and MotA-mCherry and studied their subcellular localization by fluorescence microscopy. I showed that the process of proton translocation through the channel is not required for stator assembly. I also showed that over-expression of MotA significantly reduced the number of stators in a rotating motor and hence considerably inhibited wild-type motility. These results suggest that MotA alone can be installed into the flagellar motor. And I showed that the electrostatic interaction of MotA with FliG is required for the efficient assembly of the stators around the rotor.

The proton influx coupled with flagellar motor rotation should be measured with high spatial and temporal resolution to further the understanding of the energy coupling mechanism. I introduced the M153R mutation to pH sensitive fluorescent protein pHluorin to improve the stability and brightness. And I constructed Salmonella cells with its flagellar motor components (MotB or FliG) fused to pHluorin(M153R) for measuring pH around the motor. Next I have developed an intracellular pH imaging system that can measure local pH using the pHluorin probe. This system can measure the intracellular pH distribution with a pH resolution of 0.02 and a time resolution of 3 msec.

論文審査の結果の要旨

申請者は、細菌のプロトン駆動型べん毛モーターにおいて、プロトン透過経路としても機能する固定子MotA/B複合体のプロトン透過活性について詳細な計測を行うとともに、蛍光イメージング法により細胞膜上に浮遊するMotA/B複合体がべん毛基部体を認識して結合し固定子となる機構を明らかにした。これらの結果は、細菌べん毛モーターのエネルギー変換の分子機構の解明だけにはとどまらず、生体超分子複合体がどのようにして共役イオンの熱ノイズレベルのエネルギーを高効率・高出力で力学的仕事に変換するのかという、生命科学の重要な謎の解明につながる成果である。また、モーター回転時に固定子のプロトン透過経路を細胞内に向けて流れ込むプロトン量を、モーターの入力エネルギー量として見積もることを目的とし、高時間空間分解能pHイメージングシステムの開発を行った。以上の通り、申請者はべん毛モーターの研究において高い成果をあげているため、学位に値するものと認める。