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論文内容の要旨

氏 名 (酒井 智史)	
論文題名	Functional analysis of the bacterial flagellar hook and rotor mutants. (細菌べん毛モーターの回転子およびフック変異体の機能解析)
<p>Peritricha, such as <i>Salmonella</i> and <i>Escherichia coli</i>, have 6 to 8 flagella in one cell and rotate each flagellum by a rotary motor at its base with the electrochemical potential of protons across the cytoplasmic membrane as the energy source. The cells swim linearly by rotating flagella in the counter clockwise direction, tumbles by rotating them clockwise, and move to environments suitable for growth by switching the swimming mode between “run” and “tumble” in response to the surrounding environment. In this study, to clarify the relationship between the structures and functions of proteins constituting the flagellum of <i>Salmonella</i>, I carried out "Mutant analysis of the rotor of the flagellar motor involved in torque generation and switching of rotation direction" and "Mutant analysis of the flagellar hook working as a universal joint that connects the flagellar basal body and the filament "</p> <p>In the first part, I carried out functional analyses of a mutant motor with its rotor proteins FliF and FliG being fused together after partial deletion in their joint portion. In this FliF-G partial deletion fusion mutant motor, the maximum torque was markedly reduced, and it was due to a reduction in the number of stators caused by the decrease in the diameter of the rotor that limits the space for the stator binding. The rotation direction was also strongly biased to the clockwise direction to reduce the motility, but this bias was suppressed by functional-reversion mutations that resulted in an increase in the rotor diameter. From these results, it was suggested that structural changes resulting in a change in the rotor diameter may be involved in the rotation switching mechanism of the motor.</p> <p>In the second part, I made mutants of the flagellar hook and analyzed their structures and functions to study the relationship between the structure and function. The hook is highly curved and has a bending flexibility to work as a universal joint. However, FlgE constituting the hook and FlgG constituting the rod, which is straight and rigid to work as a drive shaft, are highly homologous in their sequences and structures. I made deletion mutants of FlgE missing either domain D2 or the triangular loop of domain D1, which are the regions specific to FlgE and not present in FlgG, to study their roles. By the deletion of domain D2, the hook still kept the bending flexibility and was fully functional but lost the ability to form a supercoiled structure and became straight. The D1 loop was found to be involved in the interaction with the hook cap protein FlgD, and it was revealed that it plays an important role in hook formation by polymerization of FlgE.</p>	

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

申請者はサルモネラのべん毛を構成するタンパク質の構造と機能の関係を調べるため、「べん毛モーターのトルク発生と回転方向の切り替えに関わる回転子の変異体解析」および、「べん毛フックの変異体解析」を行った。

一つ目の研究テーマでは、べん毛モーターの回転子を構成するタンパク質FliFとFliGの一部が欠失して融合した変異型モーターの機能解析を行った。遊泳運動の観察と速度の計測、テザードセル解析による回転方向切り替えの挙動解析、ビーズアッセイによるトルク速度解析などにより、この変異型モーターでは回転方向の切り替えが頻繁に起こり、回転方向が時計方向に偏り、固定子の結合安定性が低下していることなどを解明した。そして、これらの機能変異をFlgGの結晶構造やべん毛基部体の構造と照らし合わせ、変異型FliGで欠失したN末端ドメインの役割を考察して、モーターの回転や回転方向切り替えにおけるこのドメインの役割を明らかにした。

二つ目の研究テーマでは、べん毛フックの変異体を作成して構造と機能の関係を解析した。フックは自在継ぎ手として働くため柔軟で曲がりやすいが、剛直で曲がりにくい基部体のロッドを構成するFlgGとフックタンパク質FlgEは、配列的にも構造的にも高い相同性を持つ。そこで、FlgGと比較してFlgEに特異的なD2ドメインとD1ドメインのループ構造の欠失変異体をそれぞれ解析し、各ドメインの役割を同定した。

これら一連の研究は当該分野に新たな進展をもたらしたので、博士号の学位授与にふさわしいと認める。