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論　文　内　容　の　要　旨

氏　名　（　山口智子　）	
論文題名	**Structural and functional analysis of the filament and bushing of flagellar motor** **（生体超分子べん毛モーターの繊維および軸受の機能・構造解析）**

論文内容の要旨

The bacterial flagellum is a motility organelle for bacteria to swim in liquid environments. The flagellum is composed of the basal body as a rotary motor, the filament as a helical propeller and the hook as a universal joint connecting them. It consists of about 30 different proteins, and these component proteins self-assemble in a highly regulated manner. The motor converts the electrochemical potential difference of cations across the cell membrane to mechanical work with almost 100% efficiency. The rotational speed of the flagellar motor is as high as 1,700 revolution per second, which is much faster than that of the Formula One racing car engine.

Salmonella enterica serovar Typhimurium (here after *Salmonella*) is a pathogenic bacterium and has two distinct flagellar filament component proteins (flagellin), FliC and FljB. FliC and FljB are antigenically different, and this is thought to help *Salmonella* to escape from host immune systems. Even though both flagellins contribute to bacterial motility and infection, FljB was less studied, and its structure remained unrevealed. To clarify structural and functional differences between FliC and FljB, I carried out structural and functional analyses. *Salmonella* cells producing only the FljB filament showed higher motility than those producing only the FliC filament under highly viscose conditions. To examine the reason of this functional difference, the FljB filament structure was analyzed at 3.6 Å resolution by electron cryomicroscopy single particle image analysis and was compared with the FliC filament. These two structures were nearly identical but the position and orientation of the outermost domain D3 of flagellin were distinctly different. Domain D3 of FljB was much flexible and mobile than that of FliC, suggesting that it plays an important role in optimizing the motility function in viscous environments as well as changing the antigenicity.

The LP ring complex is a part of the flagellar basal body and acts as a bushing of the motor. It is very stable against various chemical treatment such as acid and urea, and supports high speed rotation of the flagellar motor. The rod is a drive shaft that transmits the rotational force to the hook and filament. The LP ring spans the outer membrane (by the L ring) and the peptidoglycan (PG) layer (by the P ring) and surrounds the rod to support its rapid and stable rotation without much friction. Because the surface of the rod is highly negatively charged, it has been thought that the inner surface of the LP ring is negatively charged to produce electric repulsive force to minimize the frictional force between the rod and the LP ring. However, this raises another question of how the LP ring assembles around the rod against the repulsive force. To clarify how the LP ring assembles around the rod and acts as a bushing to support its high-speed rotation, the LP ring structure within the basal body and the P ring structure around the polyrod were analyzed by electron cryomicroscopy single particle image analysis at 3.5Å and 2.5Å resolution. The LP ring structure showed 26-fold rotational symmetry, and the L ring showed intricate intersubunit interactions of each subunit with up to six partners that explains the structural stability. The inner surface of the LP ring is charged both positively and negatively, and positive charges on the P ring and flexible residues of FlgI and FlgG presumably play important roles in the initial assembly process of the P ring around the rod. Therefore, the LP ring structure is optimized for both self-assembly and bushing function.

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論文審査の結果の要旨及び担当者

氏　名　（　山口　智子　）		
	（職）	氏　名
論文審査担当者	主査　特任教授　難波　啓一	
	副査　教　授　深川　竜郎	
	副査　教　授　中川　敦史	
	副査　准教授　福岡　創	

論文審査の結果の要旨

バクテリアはべん毛と呼ばれる運動器官により遊泳する。申請者はクライオ電子顕微鏡による単粒子像解析法を駆使し、構造解析が極めて困難であったべん毛繊維やべん毛モーター軸受の構造解析を次々と成功させ、2報の論文にまとめ国際学術誌に発表した。べん毛繊維では3.6 Å分解能の立体像マップを得て原子モデルを構築し、この成果をまとめた論文は国際学術誌Biomoleculesに掲載されて表紙を飾り、その表紙は当該学術誌の10th Anniversary Best Cover Awardsを受賞した。べん毛モーターの軸受LPリングでは1万3千枚ものクライオ電子顕微鏡像から6万4000個のべん毛基部体粒子像を抽出して解析し、3.5 Å分解能の3次元像から構成タンパク質FlgHとFlgIの原子モデルを構築した。各々26分子が構成するLPリングの構造はリングの内側に並んだ長い反平行βストランドが形成する巨大なβバレル構造が軸受の極めて滑らかな内壁を形成し、摩擦も摩耗もほぼゼロである分子軸受の機能メカニズムを明らかにした。

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。