we prepared domain deletion mutants of FlgT and analyzed their effects on the basal-body ring formation. The results suggest that FlgT-N contributes mainly to construct the H-ring structure, and FlgT-M may mediate the T-ring association on the LP-ring. FlgT-C is not essential, but stabilizes the H-ring structure. Based on these results, we will discuss the assembly mechanism of the basal-body rings and the stator units of the sodium-driven flagellar motor.

3N1334 菌体細胞膜に埋まったビブリオ菌のナトリウムイオン駆動型べん毛モーター構造の電子顕微鏡による可視化

Visualization of *Vibrio* Na⁺-driven flagellar motor structure embedded in cell membrane by electron microscopy

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The flagellum is one of the biggest architecture in the bacterial organ. At the base of each flagellum, the ion-driven rotary motor is embedded in the membrane. *Escherichia coli* and *Salmonella* have H⁺-driven motor, and *Vibrio alginolyticus* has Na⁺-driven type. The flagellar motor is composed of basal body, C ring and stator. Basal body is the rotor part of the motor. *Vibrio* basal body has unique ring structures important for the function named T and H ring. C ring composed of FliG, FliM and FliN, is mounted on the cytoplasmic side of the basal body, and essential for torque generation by interact with stator. FliG participates most directly in the torque generation among them. Since, however, C-ring structure has isolated and identified just only from *Salmonella*, the C-ring structure of Na⁺-driven flagellar motor is still unknown. Previously, we isolated FliG associate with the basal body from the Na⁺-driven polar flagellum of *V. alginolyticus*. However, FliM and FliN dissociated during the C-ring preparation. Recent fluorescent analyses show the turnover of FliM and FliN in *E. coli* cells. It suggests that C ring is the fragile structure in bacterial cells. And in spite of the continuous structural studies of flagellar motor, structures of the stator proteins mounted on the motor have not been observed by electron microscopy. To visualize C ring and stator with basal body, currently, we attempt to observe flagellar motor embedded in *Vibrio* cell membrane by electron microscopy.

3N1346 溶液 NMR を用いたビブリオ菌べん毛の固定子タンパク質 FliG の動的構造変化の解析

Structural changes investigated by solution NMR in *Vibrio* flagellar rotor protein FliG

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In the bacterial flagellar motor, torque is generated by the interactions between rotor and stator. In the rotor, a C ring component FliG is mainly participated in torque generation. Recently, the crystal structure of whole FliG in *Aquifex aeolicus* was published. FliG consists of three (N, M, and C) domains that contain several α -helices. The paper suggested that dynamic conformational changes occur during rotational switching and torque generation. To investigate structural changes in FliG derived from *Vibrio* Na⁺-driven motor, we performed solution NMR. First we overproduced and purified full-length FliG, and measured its ¹H-¹⁵N HSQC spectrum to search an optimal condition for the measurement. We found that in pH 6.5 signal peaks were separated better than pH 8.0. Next, we made N-terminally truncated FliG variants, G122-FliG (MC-FliG) and G214-FliG (C-FliG), and measured ¹H-¹⁵N HSQC in pH 6.5 using NMR at 800 MHz. From these constructs, we could get much better peaks, which are possible to assign. Furthermore, we carried out DSC (Differential Scanning Calorimetry) measurements to examine their thermodynamic properties. Results suggested that FliG protein has domain structures, and G122-FliG might form a multimeric structure in room temperature. Currently we are trying to assign the peaks of FliG, and conformational dynamics will be examined using the FliG deletion derivatives.

3N1358 べん毛フックタンパク質 FlgE 変異体におけるフックの長さ制御

Hook-length control in *flgE* mutants

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In bacterial flagella, a flagellar protein FliK controls the length of the hook, which connects the filament to the flagellar basal body (BB) embedded into the membrane. The hook is composed of a single kind of hook protein (FlgE). A *flgE* mutant SJW2219 is temperature sensitive; cells produce normal flagella and actively swim when grown at 30C, whereas they became non-flagellated and thus non-motile at 37C. At 30C, most cells produced wild-type flagella. We found by electron microscopy that mutant cells grown at 37C produced intermediate structures of the flagellar hook-basal body (HBB) as many as wild type. About half of the population produced a few intact flagella/ cell, in which hook length was not different from that of the wild type. In addition, SDS-PAGE analysis showed that a considerable amount of mutant FlgE was secreted into the culture media. When we introduced modified *fliK* constructs into the mutant, the recombinant produced short and long hooks according to the size of FliK expressed from the constructs. Therefore, we conclude that FliK controls hook length in this *flgE* mutant, even though the polymerization rate of FlgE might be slower than that of wild type.

3N1422 べん毛モータのトルク発生器における帯電残基の性質

Characterization of the electrically-charged residues in the torque-generating unit of the flagellar motor

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The flagellar motor is composed with three kinds of proteins: FliG, FliM, and FliN, in *Salmonella enterica* serovar Typhimurium. It has been postulated that five conserved charged residues (K264, R281, D288, D289, R297) in the FliG C-terminal domain are directly involved in torque generation. We substituted these five residues with Thr individually and observed the behavior of the mutants. The D288T & D289T double-mutant strain was Mot⁻, while the rest were Mot⁺. To examine the functional role of the R281 residue that is the highly conserved one, we substituted this residue with all 20 amino acids and observed behavior of the constructs. Four substitutes (R281D, R281E, R281G, and R281P) were Mot⁻, while the rest could swim at various speeds on agar plates. Accordingly, their swimming speeds widely ranged from zero to wild-type speed: wild-type R > YQTSKCAH > V > WMFILN > DEGP = Mot⁻. As examined by the tethered cell method, the rotation speed of a single motor of the variants continuously dispersed in a wide range as follows; from wild-type speed R>T>S>Q>K>Y>C>A>W>V>(M, H, L, F, I) to zero speed (D, E, P, G), indicating that torque generated in the motor may fluctuate under different physiological conditions in the micro-environment of the site R281.

3N1434 Ascaris 精子をもちいたアメーバ運動装置の in vitro 再構成

Simultaneous reconstitution of MSP-based protrusion and retraction in the amoeboid sperm of Ascaris

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Crawling movement in eukaryotic cells requires coordination of the force for leading edge protrusion with that for cell body retraction. In *Ascaris* sperm, protrusion and retraction are powered by a motility system comprised of major sperm protein (MSP) in place of the actin apparatus typically associated with cell migration. By adjusting the concentration of cell-free sperm extracts, we reconstituted protrusion and retraction simultaneously in MSP filament meshworks, called fibers, that assemble behind plasma membrane-derived vesicles. As polymerization of filaments at the growing end of the fiber pushed the vesicle ahead (protrusion), filament rearrangement and disassembly pulled the rear of the fiber forward (retraction). Because nematode sperm do not use motor proteins to power retraction, we used these fibers to explore the mechanism of retraction by focusing on the cytoskeletal dynamics that occur during the transition from protrusion to retraction. Using a combination of fluorescent speckle microscopy, electron tomography, and advanced polarization microscopy, we found that although fibers progressively lost filaments during retraction the average length of the filaments did not change. The surviving filaments were able to move and reorient so that their packing density was conserved. To do so, fewer filaments packed into a smaller volume and this rearrangement appears to provide the driving force for retraction.

3N1446 ショウジョウバエ形態形成におけるアポトーシスの力学的寄与

P56 Active Mechanical Role of Apoptosis in Tissue Dynamics during *Drosophila* Morphogenesis

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