of intact flagellar basal body. Comparison with the structure of a purified basal body revealed the density of the export platform and the ring structure derived from FliI within and below the C ring. The flagellar basal body of the $\Delta fliH/fliI$ -bypass mutant, which maintains flagellar protein export and motility in the absence of FliH and FliI showed that the two densities within and below the C ring were completely lost, suggesting that the platform structure is not essential for flagellar protein export.

2PS029 細菌べん毛モーターの固定子組込み機構に対する MotB(D33N)点変異の影響

Effect of the D33N mutation in MotB on stator assembly of the bacterial flagellar motor

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The bacterial flagellar motor converts the energy of proton translocation across the membrane through the proton-channel stator complex into torque. The MotA/B proton channel complex is firmly anchored to the peptidoglycan (PG) layer through the PG-binding domain of MotB to act as the stator of the motor. The stator units dynamically associate with and dissociate from the motor during flagellar motor rotation, and an electrostatic interaction between MotA and a rotor protein FliG is required for efficient stator assembly. However, the association and dissociation mechanism of the stator units still remains unclear. In this study, we analyzed the speed fluctuation of the flagellar motor of *Salmonella enterica* wild-type cells carrying a plasmid encoding a nonfunctional stator unit, MotA/MotB(D33N), which lost the proton conductivity. The wild-type motor rotated stably but the motor speed fluctuated considerably when the expression level of the MotA/B(D33N) stator was increased. Rapid accelerations and decelerations were also observed. These results suggest that the MotA/B(D33N) stator retains the ability to associate with the motor but rapidly dissociates from it as well. We propose that the stator dissociation process depends on the proton translocation through the proton channel.

2PS030 MS リングに突然変異が生じたサルモネラ菌べん毛モータの構造安定性と出力特性

Structural stability and rotational characteristics of the flagellar motor of *Salmonella* MS-ring mutant

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The bacterial flagellar motor is a rotary nanomachine fueled by the proton motive force. Two ring structures, MS ring and C ring, together act as a rotor, and responsible for torque generation by interacting with a stator complex consisting of MotA and MotB. It has been known that the flagella of a *Salmonella* FliF mutant are fragile as compared with those of wild-type strain, suggesting that the MS ring plays an important role in keeping stability of the motor structure. In this study, the mechanical strength and the rotational characteristics of the FliF mutant motor were analyzed to understand how MS ring is involved in the structural stability. Quantitative measurement of the strength revealed that the mechanical strength of the FliF mutant motor decreases up to a half of wild-type motor. However, both the swimming speed and the motor speed of the FliF mutant were almost same with those of wild-type strain. These suggest that the defect of MS ring can decrease the structural stability of flagellar motor without impairment of torque generation.

2PS031 The role of the flagellar C ring in hook-length control by FliK

Kaoru Uchida, Tatsuya Yamasaki, Shin-Ichi Aizawa (Grad. Sch. Pref. Univ. Hiroshima)

The flagellar hook has an average length of 55 nm, which is controlled mainly by FliK. We previously demonstrated that mutations of any one of the fliG, fliM, and fliN genes gave rise to short hooks in *Salmonella enterica* serovar Typhimurium. FliG, FliM, and FliN form the cytoplasmic ring (C ring) at the flagellar base, which is a multifunctional structure necessary for flagellar protein secretion, torque generation, and switching of the motor rotation. We also showed that hook length of the C-ring deficient mutants broadly distributes around 55 nm, suggesting a tight length-control by the C ring. In order to elucidate the role of the C ring in hook-length control, we introduced two

modified fliK constructs into C-ring mutants. A recombinant FliK with the size of 373 a.a. produced short hooks and the other recombinant FliK with the size of 570 a.a. produced long hooks, suggesting that FliK is dominant in hook length control even in the C ring mutants. However, the hook length in C-ring mutants varied in wider range than that of the wild type. We will be discussing the interaction between the C ring and FliK.

2PS032 べん毛モーター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

Satoshi Inaba, Shin-Ichi Aizawa (Prefectural University of Hiroshima)

FliG is one of the rotor components of the flagellar motor in *Salmonella enterica* serovar Typhimurium. It is said that five conserved charged residues (K264, R281, D288, D289, R297) in the C-terminal domain of FliG are directly involved in torque generation. These five charged residues formed a ridge on the surface of the domain. Among these residues, R281, D288 and D289 are belonging to the same α -helix. R281X and D288X/D289X gave rise to motility-defective mutants by substitution with either Thr or Ala. In order to examine the functional roles in detail of the residues R281 and R288/R289, we substituted these residues with all 20 amino acids.

Among variants of R281X, four (R281D, E, G, and P) were Mot⁺, and the rest swam at various speeds. Distribution of their rotational speeds continuously dispersed in a wide range, indicating that torque generated in the motor may change according to changes in conformation near the R281 site. Among D288X/D289X pair variants, three pairs (D288E/D289E, S/S, Y/Y) swam, while the rest were Mot⁺. To fill the gap between R281 and D289, we chose D284 and I285 and substituted them with 20 a.a. The variants I285D, R, T swarmed. The variants D284E, F, R, and V swarmed. These results indicate that D284 and I285 at the middle of the α -helix are not directly involved in torque generation. In summary, a pair of D288/D289 residues might be the major element of the torque generator, and that R281 residue might play a subsidiary role in the torque generator.

2PS033 Meaning of HAP1 in the length measurement of the hook

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In the bacterial flagella, the hook occupies a region between the rod and HAP1 in an average length of 55 nm to connect the filament to the basal body. The hook is composed of FlgE. We analyzed the hook in a temperature-sensitive flgE mutant of *Salmonella enterica* serovar Typhimurium. The mutant cells produced wild-type flagella when grown at 30 °C but short hooks without filaments at 37°C. We found by electron microscopy that the mutant hooks produced at 30°C had wild-type length (average length: 55 nm) in intact flagella, whereas they were shorter (45 nm) in the hook basal body (HBB) obtained by acid treatment of intact flagella. SDS-PAGE showed that the HBB preparation did not contain HAP1 that is supposedly attached on the distal end of wild-type hook, indicating that interaction between the mutant hook and HAP1 might become weaker by a mutation of the hook protein. As a consequence, we learned that the hook length that we have so far measured was length of the hook region including HAP1, and that authentic hook length was about 45 nm and HAP1 length was about 10 nm in the wild-type "hook". The HAP region (HAP1 and HAP3) in intact flagella is distinguishable from the hook and filament by its appearance and thin width. However, surface appearance of the HAP region resembles that of the hook than the filament, which led us to measure hook length including HAP1. We will show length distribution of HAP region in wild type and mutants.

2PS034 コフィリンのアクチン線維への協同的結合の直接観察

Direct observation of cooperative binding of cofilin to actin filaments

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ADF/cofilin proteins are ubiquitously distributed actin severing proteins in eukaryotic cells. Several biochemical and electron microscopic studies have suggested that cofilin binds to the actin filament in a cooperative manner. We directly observed the binding of single Alexa-labeled cofilin molecules to actin filaments in solution by a single molecule imaging technique. We analyzed the non-contiguous (isolated, non-cooperative) cofilin binding to an actin filament

using low concentration of cofilin (10 nM-100 nM), in which measurement of the binding was made on a short segment (600 nm in length) in the filament. The rate constant of cofilin binding to the actin filament was estimated at $0.06/\mu\text{M}^{-1}\text{s}^{-1}$, which agrees with the value obtained with biochemical techniques. The rate constant elevated to $0.18/\mu\text{M}^{-1}\text{s}^{-1}$ when the concentration of cofilin was increased to 10 μM . This result indicates that our single molecule measurements could detect the process of the cooperative binding of cofilin in solution. In low cofilin concentrations (10 nM-100 nM), long lasting cofilin bindings followed by a subsequent cofilin binding in the same segment (600 nm) of the filament were observed. While the rate constant of the preceding cofilin binding was $0.06/\mu\text{M}^{-1}\text{s}^{-1}$, the rate constant of the subsequent cofilin binding was elevated to $0.18/\mu\text{M}^{-1}\text{s}^{-1}$. This result suggests the binding of cofilin to the actin filament accelerates the binding of another cofilin to the neighboring (less than 600 nm) binding site on the actin filament.

2PS035 微小管結合蛋白質 4 を介したアクチンフィラメントと微小管の相互作用の解析

Analysis of microtubule-associated protein 4-mediated interaction between actin filaments and microtubules

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Microtubule-associated protein (MAP) 4 is a non-neural MAP that plays important role in the intracellular organization of microtubules. Recent study revealed that MAP4 also binds to actin filaments, but not actin monomer, at the C-terminal part of the Proline-rich region in the microtubule-binding domain (Matsushima et al., J.Biochem., 2012). Since the binding affinity of MAP4 for actin filaments was the same order with that for microtubules, we believed that the interaction of MAP4 to actin filaments was physiologically significant. In this study, we examined MAP4 functions when both actin filaments and microtubules (or unpolymerized tubulin) coexisted in a sample. To distinguish actin filaments and microtubules under fluorescence microscopy, these filaments were labeled with different fluorescent dyes. When microtubules were mixed with actin filaments that preincubated with MAP4, bundles consisted of both microtubules and actin filaments were observed. Moreover, when unpolymerized tubulin instead of microtubules was mixed with actin filaments that preincubated with MAP4, elongation of microtubules along the actin filaments was observed. These results suggest that MAP4 bound to actin filaments retain microtubule binding and microtubule assembly-promoting activities. On the other hand, when actin filaments were mixed with microtubules that preincubated with MAP4, bundles containing both actin filaments and microtubules were not observed, suggesting that MAP4 bound to microtubules lost actin filament-binding activity.

2PS036 血管平滑筋細胞から単離したストレスファイバの収縮特性の計測 Measurement of contractile properties of stress fibers isolated from vascular smooth muscle cells

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Several lines of evidence have suggested a critical involvement of nonmuscle myosin II in the fundamental cellular functions of proliferation, differentiation, and apoptosis. In nonmuscle cells, nonmuscle myosin II forms stress fibers (SFs) along with actin filaments and exerts contractile forces on the extracellular matrix. Compared to increasing reports studied at the whole cell level, the contractile properties intrinsic to SFs themselves remain poorly understood. In the present study, we characterized the contractile properties of single SFs isolated from cultured vascular smooth muscle cells. SFs were extracted from the cells treated with hypotonic buffers and detergents. Western blotting showed that main constituents of SFs were maintained in the isolated materials. We manipulated the isolated SFs by using two fine glass needles and measured their contractile forces under physiological MgATP concentrations. The results showed that SFs could generate forces of several tens of nano-Newtons. SFs suddenly relaxed at high MgATP concentrations, and some fragments of F-actin were dissociated from the SFs. Interestingly, we observed rotational motions of SFs under passive stretch as well as active contraction conditions. These results may be useful in the understanding the biophysical roles of SFs in cell behavior.

2PS037 IQGAP confines myosin-II to the cytokinetic contractile ring

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During cytokinesis, myosin-II concentrates at the equatorial cortex with actin filaments (F-actin) and is supposed to generate forces to divide the cell into two, which is called the contractile ring (CR) hypothesis. Several lines of evidence indicate F-actin-independent recruitment of myosin-II and its specific interaction with the equatorial F-actin, although their details are still unknown. We investigate the dual regulation of myosin-II localization using the fission yeast *S. pombe*. IQGAP Rng2 is implicated both in the F-actin-independent recruitment of myosin-II to cortical dots and in its sufficient concentration on CR F-actin. Rng2 is required for the dot localization of Myo2 (the essential myosin-II) via its tail. Rng2 shows a slower exchange rate and lower mobility than Myo2. Myo2 is inhomogenous and unstable in CR of the rng2 mutant and preferably associates with ectopic F-actin induced by overexpression of the actin-modulating moiety of Rng2, Rng2Ns. Biochemically, Rng2Ns slows actin-activated Mg-ATPase of myosin-II. These results suggest that Rng2 tethers the Myo2 tail to cortical dots and accumulates myosin-II to CR F-actin by modulating its ATPase-coupled turnover.

2PS038 キネシン Eg5 に対するフォトクロミック阻害剤を用いた細胞分裂の光制御

Photo-regulation of cell division using photochromic inhibitors for kinesin Eg5

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Previously it has been demonstrated that some ATP driven motor proteins kinesins are directly involved in regulation of the cell division cycle. Eg5 is one of the kinesin and its biochemical properties and structure were well studied. Interestingly Eg5 is activated in M phase of cell cycle and performs stabilizing and positioning of spindle. It is also known that Eg5 is overexpressed in tumor cell and induce significant. Monastrol and STLC, which are potent inhibitor specific for kinesin Eg5 ATPase activity, shut off mitotic division and result in apoptosis. Therefore, these inhibitors are attracting as anti-cancer drug. Azobenzene and spiropyran, a widely studied photochromic compound, can be reversibly isomerized between the cis and trans forms by ultra-violet (UV) and visible (VIS) light irradiation, respectively. We have recently demonstrated the Monastrol and STLC analogues composed of photochromic molecules inhibit ATPase activity Eg5 reversibly upon UV and VIS light irradiation in vitro. Therefore, it is strongly expected that the photochromic inhibitors are applicable to reversible photo-regulation of cell mitosis. In this study, we have examined the effects of the photochromic inhibitors for mammalian cells. Effects of the photo-chromic inhibitors were evaluated by survival number of mammalian cells, HeLa cells or A172 cells. The experimental results suggested that the effects of photo-chromic inhibitor for cell division were controlled by UV and VIS light reversibly.

2PS039 蛍光顕微鏡観察による細胞性粘菌由来のコフィリン、HMM と F-actin の協同的相互作用の解析

Analysis of cooperative interaction of Dictyostelium-derived cofilin and HMM to F-actin using fluorescence microscopy

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In crawling cells, cofilin and myosin II are localized in the anterior and posterior regions, respectively. It has not been understood the molecular mechanism of the differential localization so far. In the last meeting, we reported that cooperative binding of cofilin and HMM for F-actin was mutually exclusive, and concluded that the mutually exclusive binding might contribute to the differential localization of cofilin and myosin II. However, since the cooperative binding was analyzed by use of proteins derived from different origins, such as human cofilin, Dictyostelium HMM, and rabbit actin, it has been remained a concern that the cooperative interaction containing the mutually exclusive binding is not a physiological phenomenon. In this study, therefore, we used proteins derived from only Dictyostelium to analyze the cooperative interaction. To visualize cofilin and HMM, we prepared recombinant Dictyostelium cofilin (D-cofilin) and HMM (D-HMM) fused with mCherry and GFP, respectively. Actin was purified from Dictyostelium (D-actin) or rabbit (R-actin), and was labeled with fluorescent-phalloidins. The cooperative binding for 40 nM F-D-actin was observed in the range of 100-150 nM D-cofilin. The cooperative