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We are aiming to create a 'bio-hydrogel' having biological functions, such as recirculating and self-repairing. We prepared herein F-actin hydrogel by covalent cross-linking with bi-functionalized PEG. We first evaluated the mechanical properties of the actin hydrogel by an oscillatory rheometry. It was found that the actin hydrogel (0.5 wt% actin concentration) has a storage modulus (G') about several kPa and a loss modulus (G'') about 10-20 Pa. This actin hydrogel showed reversible gel-sol transition by actin polymerization and depolymerization. Then we added oscillatory shear to this actin hydrogel by changing a strain from 10 to 1000%. When oscillatory strain is applied more than 20 %, the G' of the actin hydrogel immediately decreased three to five orders of magnitudes, indicating that the hydrogel transferred to a sol state under a large shear strain. When the large shear strain was removed, the values of G' were returned to 80 % within several minutes. In contrast, F-actin network without cross-linking were reported to be irreversibly weakened their storage modulus. Electron micrographs of cross-linked actin under a shear by pipetting appeared that filamentous actin structures were disassembled into oligomers or less, whereas the F-actin structures still maintained without cross-linking. These results suggest that the self-repairing of actin gel can rapidly occurred polymerization of disassembled actin perhaps by cross-link induced cooperative depolymerization of F-actin.

1P157 筋収縮系自励振動現象 (SPOC) の生理的意義
1D1520 Physiological significance of spontaneous sarcomeric oscillations (SPOC) in cardiac beat

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Cardiac sarcomeres exhibit spontaneous oscillations (SPOC) under intermediate activating conditions. We have reported that the period of sarcomeric oscillations in skinned myocardium correlates with that of resting heart rate in various animal species (2005, 2006). In the present study, we analyzed sarcomeric oscillations in isolated single cardiomyocytes of the rat, by using Qdots conjugated with anti- α -actinin antibody for clear visualization of the Z-lines. First, we measured the period and amplitude of SPOC at various sarcomere lengths (SLs) in skinned cardiomyocytes, and found that the amplitude of oscillations was inversely related to SL. We also conducted a SL measurement in intact cardiomyocytes at various stimulation frequencies, after transfection of Qdots into the cells. At low frequencies (e.g., 1 Hz), the shortening and relengthening of the sarcomere during a contraction cycle simply reflected the changes in $[Ca^{2+}]_i$. However, an increase in stimulation frequency to the physiological level (3-5 Hz) caused a phase shift of shortening and relengthening due to enhancement of the relengthening speed, resulting in the waveform being similar to what was observed during SPOC. These findings suggest that the intrinsic auto-oscillatory property of sarcomeres may contribute to the regulation of cardiac beat *in vivo*. We are now performing real-time single sarcomere imaging in the beating heart of the rat.

1P158 ウサギ骨格筋筋原線維におけるクロスブリッジ周期性と破断力の顕微計測
1D1535 Microscopic measurement of periodic rigor cross-bridge formation and their unbinding force in rabbit skeletal myofibril.

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Single molecule studies previously reported that the thin (actin) filament sterically regulates the myosin binding sites, called target zones. But it has been established that the periods of long helices of the thin filament and the thick (myosin II) filament are mismatched to each other, i.e., 36 nm and 42 nm, respectively, so that the question has been raised how the target zone is distributed, i.e., periodical or unperiodical. EM images and X-ray diffraction patterns of muscle fiber have provided the models on the interaction between the thick and the thin filaments, but direct measurements in live muscle are missing. Therefore, here we directly observed and measured the binding of single actin filament with myosin II on the A-band in skeletal muscle of higher vertebrates under optical microscope. Single bead-tailed actin filament was manipulated by optical tweezers, and the unbinding events of rigor cross-bridges due to the external force were detected. We found that the cross-bridge formation occurred periodically, and frequently observed the large gaps, which were attributable to the mismatch between the helical pitches of the two kinds of filaments. The distribution of the unbinding force had two peaks. The weaker and stronger peaks probably correspond to single-headed and double-headed binding, respectively.

1P159 二枚貝以外の軟体動物のキャッチ機構
Catch mechanism in mollusks other than bivalves

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Some muscles of bivalves (class Bivalvia) such as the smooth part of the adductor and the byssus retractor are known as "catch muscles" that can maintain high passive tension for long periods with little energy expenditure after their active contraction. The state of this high passive tension is called "catch" and it has been hypothesized that some special "catch mechanism" exists for the maintenance of the tension. We have developed an *in vitro* catch assay in which the catch state is reconstituted by using proteins purified from bivalve catch muscles. With this assay, we have revealed that the catch tension is due to binding of actin to myosin filaments which can be directly visualized under a light microscope. We have also found that twitchin, a connectin/titin-related protein associated with myosin filaments, is an essential key component for the catch. It was originally found in a nematode *Caenorhabditis elegans*, and was later found widely in many invertebrates. This raises the question whether animals having twitchin other than bivalves also have the catch mechanism. To answer this, we prepared synthetic thick filaments containing myosin and twitchin from the foot of a spiral shell *Monodonta labio* (class Gastropoda) and a chiton *Liolophura japonica* (class Polyplacophora), and performed the *in vitro* catch assay. We found that the catch mechanism, at least at the molecular level, exists also in these mollusks. The results suggest that the catch mechanism is distributed more widely in Animal Kingdom than we know.

1P160 複数の接触配置のアクチン 2 分子における高分子込み合い効果ポテンシャル
Calculated potential map for two G-actin molecules in contact with relative orientations through macromolecular crowding effect

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Potential of mean force between two G-actin molecules is important for studying a filament formation of actin molecules. We calculated potentials of mean force through macromolecular crowding effect between two G-actin molecules with a few relative orientations by using extended scaled particle theory (XSPT) in the previous meeting.

In this study, we have systematically calculated the potentials of mean force between two G-actin molecules in contact with 210 relative orientations in order to make a potential map for the molecules. The sites chosen for contact locations are 4, thereby 210 orientations are not enough to investigate a dimer formation, which is a first stage of an actin filament formation. However, this calculation can be extended to more orientations with a high-performance computer in a straight-forward way. The theoretical calculations of the potentials with an infinite number of crowding agents, actin molecules themselves in this work, require less computer time than numerical calculations with a finite number of crowding agents.

1P161 バクテリアのプロトン駆動型べん毛モーター固定子複合体 MotA/B の局在化の分子機構
Localization mechanism of the MotA/B complex to be the stator of the proton-driven bacterial flagellar motor

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The flagellar motor of *Salmonella enterica* is powered by proton motive force (PMF) across the cytoplasmic membrane. MotA and MotB form a transmembrane proton channel that acts as the stator of the motor to couple proton flow through the channel to torque generation. The C-terminal periplasmic domain of MotB is proposed to play an important role in targeting and stable anchoring of the stators to their binding sites of the motor. However, it remains unclear where their binding-sites are. Here, we constructed *Salmonella* strains expressing GFP-MotB and MotA-mCherry and investigated their subcellular localization by fluorescence microscopy. The D33N mutation in MotB, which interferes with the proton flow, did not affect the assembly of GFP-MotB into the motor, indicating that the proton conductivity is not required for stator assembly. Over-expression of MotA markedly inhibited wild-type motility, and it was due to the reduction in the number of functional stators. In agreement with this, MotA-mCherry was observed to co-localize with GFP-FlhG even in the absence of MotB. These results suggest that MotA alone can bind to the stator binding sites of the flagellar motor.

1P162 Research for sodium-driven flagellar hook-basal bodies of *Vibrio alginolyticus* with Zernike phase-contrast cryo-electron tomography

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Vibrio alginolyticus swims through liquid using flagella, which play the role of rotary machines similar to corkscrews. A flagellum consists of a filament, hook and basal body. The basal body is made of a rod and several ring structures called the L, P, MS, C and T rings. This study investigates the structure of the T ring which is a unique component of the sodium ion-driven flagellar basal body of *V. alginolyticus*. Using Zernike phase contrast (ZPC) cryo-electron tomography, we compared the 3D structures of purified hook-basal bodies (HBB) from a wild-type strain (KK148) and a deletion mutant lacking *motX* and *motY* (TH3), which are thought to form the T ring. The signal-to-noise ratio of the ZPC tomogram slices was greatly improved compared with conventional tomogram slices. Thus, we could observe the outline of the HBBs. In strain KK148, the T ring structure protruded from the periplasmic side of the P ring obliquely downward and formed a continuous ring-like structure around the axial rod. In strain TH3, the LP ring also existed, but no ring-like structure was observed on the periplasmic side of its P ring. Therefore, we measured the height and width of each ring. The diameter and axial dimensions of the T ring were 32.2±1.8 nm and 8.7±1.1 nm (respectively; n=50). The diameter of the LP ring was 48.3±5.5 nm (n=50) from strain KK148 and 47.8±5.0 nm (n=50) from strain TH3. The diameter of the MS ring was 28.1±3.6 nm (n=50) from strain KK148 and 26.5±3.9 nm (n=50) from strain TH3.

1P163 Visualizing transformation process of *Mycoplasma mobile* and *Escherichia coli*

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Mycoplasma mobile, a pathogenic bacterium glides on solid surfaces with a unique mechanism. To elucidate this mechanism, the genetic manipulation should be a powerful tool. However, this organism has not been transformed so far by the standard genetic vector and transformation methods including electroporation. In present study, we traced the process of transformation by electroporation visually in the single molecular level using fluorescence microscopy, for *E. coli*, *M. mobile* and *Mycoplasma pulmonis*, closely related to *M. mobile*. We visualized the transformation process for *E. coli* which could be transformed efficiently. After the electroporation, 55% cells incorporated the outside solution, 45% cells died, and 20% cells contained the plasmid. In *M. pulmonis* whose genes can be manipulated, after the electroporation, 90% cells incorporated the outside solution, 90% cells died, and 70% cells contained the plasmid. We examined *M. mobile* and found that the incorporation of plasmid is achieved, suggesting that the difficulty to transform *M. mobile* is caused after the transport of plasmid into the cells.

**1P164 ヒト肺炎マイコプラズマ滑走器官の電子顕微鏡による解析
The analyses of the attachment organelle of human pneumonia *Mycoplasma pneumoniae* by electron microscopy**

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Mycoplasma pneumoniae which causes human pneumonia binds to trachea epithelium through the membrane protrusion, the attachment organelle, and glides with an unknown mechanism. The attachment organelle featured with a rod-like cytoskeleton at its center is a complex organelle, and the information of this structure is critical for elucidating the gliding mechanism. The rod is known to be composed of three parts, a terminal button, segmented paired plates, and a wheel. However, the details of the rod and the other parts than the rod are still unknown. We used the following rapid and flexible methods to determine the fine structures; (i) observation by negative-staining after various treatments optimized for individual structures, (ii) stereo viewing by tilting the specimens, (iii) localizing ORFs fused with YFP, by using anti-YFP antibody. Consequently, we obtained the following information. (i) The obtained images are classified into top and side views. (ii) The terminal button is composed of 4 layers connected by fiber-like structures. (iii) The segmented paired plates are composed of 2 layers and the larger one can be divided into two. (iv) The rod and cell membrane is connected by a structure which wraps helically the rod when the cell membrane is removed. (vi) Irregular fibers are projecting to the proximal side from the wheel structure. (vi) Six proteins including *dnaN* and *HU* were found to be the components of specific parts of the rod.

**1P165 Na⁺ 駆動型べん毛モーターの回転速度の圧力依存性
Pressure dependence of the rotational speed of sodium-driven chimeric flagellar motors**

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The bacterial flagellar motor is a molecular machine that is driven by the electrochemical gradient of the specific ions across the cytoplasmic membrane. The rotational speed is strongly dependent on the rate of proton translocation through the stator complex in flagellar motors. To understand the detailed energy conversion mechanism, it is desirable to modulate the intermolecular interaction between the ion and stator complex. Application of pressure is one of the powerful methods for enabling to modulate ion-protein and protein-protein interactions. In this work, we performed a motility assay of sodium-driven flagellar motors at various conditions of pressure and sodium concentration. Sodium-driven chimeric motor was expressed in *Escherichia coli* (Sowa *et al.*, 2005), and the rotational speed was measured by following the position of a bead (0.72 μm in diameter), attached to a flagellar filament. The sodium concentration dependence of the speed at each pressure was well described by a Michaelis-Menten kinetics. The maximum speed decreased from 230 Hz at 1 bar to 29 Hz at 800 bar. On the other hand, the Michaelis constant increased from 1.3 mM at 1 bar to 6.6 mM at 800 bar. Similar relations were obtained by measuring the swimming speed of *Vibrio alginolyticus* VIO5 (Pof⁺ Laf⁻ Rif^r), which has a polar flagellum powered by a Na⁺-driven flagellar motor. The high pressure directly inhibits both the sodium ion binding and the subsequent reactions.

**1P166 クラミドモナスの外腕ダイニンが持つ構造特徴
Structural feature of *Chlamydomonas* outer arm dynein**

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Flagellar beating is generated by many kinds of dynein motor proteins. The dyneins are regularly arranged and produce force cooperatively along approximately 10 μm long axoneme for proper flagellar movement. However, how dyneins are incorporated into axonemes and permitted to behave cooperatively along axonemes is not clear.

We observed isolated outer arm dynein (OAD) from *Chlamydomonas* flagella, which has three heavy chains (HCs) (α , β and γ), using cryo-electron microscopy. In these observations, α and β HCs were in the same direction, while γ HC faced different direction from the others. These results were different from the previous reports: e.g., in deep-etch replica method, three HCs of isolated OAD were in the same plane, while in cryo-electron microscopy, three HCs on a microtubule were stacked. Our results suggested that γ HC should play a different role from α and β HCs. One plausible proposal is that γ HC play a key role in signal transduction between neighboring OAD in own cooperative manner. However, it is too difficult to obtain 3D structure of OAD because OAD have polymorphism. Thus we attempt to reveal 3D structure of OAD using electron tomography. Hereby, this will help for us to know the property of OAD itself controlling flagella beating.

**1P167 LIS1は細胞質ダイニンのリング状頭部の微小管に沿った運動を制御する
1B1240 Ring-sliding Walking of Cytoplasmic Dynein and Its Regulation by LIS1**

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To understand the cellular and subcellular movement, it is necessary to study cytoplasmic dynein motor regulation. Cytoplasmic dynein has a complex and huge structure (~1.2 MDa) containing two identical heavy chains. Meanwhile, *LIS1* was first identified as a mutated gene in classical lissencephaly patients. Recent studies have reported evolutionarily conserved roles of LIS1 in regulation of cytoplasmic dynein, but how LIS1 acts on dynein is unknown. In this study, we performed transmission electron microscope (TEM) tomography on cytoplasmic dynein bound to microtubules and succeeded in obtaining its nucleotide-dependent structural change. We here used purified cytoplasmic dynein from porcine brain and recombinant LIS1 expressed in insect cells. Then, three-dimensional (3D) structures were constructed from series of tilted negatively-stained electron micrographs. The cytoplasmic dynein and microtubule complex showed that the two ring-like motor domains with microtubule-binding domain (stalk) elongating to microtubule and tail domain. In the absence of nucleotide (post-power stroke state), two motor domains aligned side by side along microtubule axis. Treated with ATP and vanadate (mimicking pre-power stroke state), dynein molecules elongated vertically against microtubules and two motor domains were overlapped. When both LIS1 and dynein were incubated, interestingly, the two motor domains shrunk on microtubule with or without nucleotide, suggesting that nucleotide-dependent structural changes could be restricted by association of LIS1.