SPOC). During SPOC the sarcomere length auto-oscillates, displaying slow shortening and rapid lengthening, and the lengthening phase propagates along a myofibril (SPOC-wave). Here we examined the effect of partial substitution of troponin in skeletal myofibrils (Terui, T. et al., *J. Gen. Physiol.*, 2008), prepared from rabbit psoas glycerinated muscle, with cardiac troponin (prepared from pig heart muscle), on the SPOC properties under the ADP-SPOC conditions, such as the period and the amplitude of the sarcomere length oscillation and the phase-diagram. Based on the obtained results and exploiting the fact that the behavior and the conditions of the occurrence of SPOC are different between skeletal and cardiac muscles, we discuss the new aspect on the role of troponin in the contraction of striated muscle.

3P142 Model simulation on an auto-oscillatory SPOC wave traveling along a striated myofibril

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It is widely accepted that muscle cells take either force-generating or relaxing state in an all-or-none fashion through the so-called excitation-contraction coupling. On the other hand, in a demembrated contractile apparatus, spontaneous oscillation of sarcomeres named SPOC frequently occurs at the activation level intermediate between full activation and relaxation. During SPOC, each sarcomere repeats slow shortening and quick extension spontaneously forming a saw-tooth waveform and the oscillation is propagated to adjacent sarcomeres along a myofibril. Recently, we have constructed a simple model that describes these three muscle states (relaxation, contraction and SPOC), focusing on the change in the myofilament lattice spacing during contraction. This model reproduces the main features of SPOC (e.g., the saw-tooth waveform of each sarcomere length oscillation) and other well-known properties of muscle, e.g., the length-dependent activation at intermediate Ca^{2+} concentrations. Namely, this model comprehensively explains the characteristics of striated muscle, but only represents a single sarcomere (to be more strict, a half sarcomere). Here we extend this single unit model to a multiple units model to investigate a more realistic situation, i.e., a myofibril, which is composed of a series connection of sarcomeres. In this model, the difference of the lattice spacings between adjacent sarcomeres is taken into account. Thus, we could simulate the SPOC wave traveling along a myofibril.

3P143 心筋ゲルを用いた自己組織化するクラゲ型水中遊泳マイクロバ イオロボットの創成

3D1235 The Construction of Self-organized and Frameless swimming Bio-Robots With Cardiomyocyte Gel.

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It is known that myocytes have great advantages compared to other conventional actuators, capable of using contracting motion powered by chemical energy, self-recovery in vivo and possibility of designing flexible micro-structure. Recently, actuator using myocytes has gained more interest. In this study, we focused on jellyfish's characteristics in order to create novel micro bio-robots without the need of frames. Based on the observation of jellyfish, phase differences of contractions between top of umbrella and bell edge structures of its body could be found. It is possible that this effect is responsible for generating temporal asymmetry of jellyfish motion between contraction phase and relaxation phase, which contributes to propulsion of the whole body. In this work, in order to confirm this phase effect, we analyzed the jellyfish-like model based on the phase differences by finite element method. From the results, it is convincing that the jellyfish motion is related to the phase difference. Then, cardiomyocyte can be built into jellyfish-like structure, focusing on the property of collagen gel which shrinks spontaneously in culture. According to this idea, a self-organized jellyfish-like structure can be achieved. The results show that we successfully developed the jellyfish-like structure by controlling the shape of gel mold. In conclusion, this study shows the initiation of constructing frameless micro bio-robot.

3P144 高密度昆虫細胞を用いたリコンビナントアクチンの高収量発現

Improvement of the yield of recombinant actin in the insect cell/baculovirus system to the high-density cell culture

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The insect cell/baculovirus system is widely recognized as a reliable technology suitable for the production of heterologous proteins, vaccines, and vectors

for gene therapy. Insect cells are usually cultured in the commercial serum-free media or TNM-FH containing FBS. To prevent nutrient depletion of the medium, baculovirus infection must be performed in the mid-logarithmic phase of cell growth at the density of $1-2 \times 10^6$ cells/ml. We have previously reported the method for the purification of the baculovirus-expressed recombinant actins, based on the affinity chromatography using a carboxyl-terminal half of gelsolin (G4-6). While the recombinant non-muscle actins were usually expressed at relatively high levels, some of the actin mutants were produced poorly even by high-titer viruses. Therefore, we developed the technique for the baculovirus propagation and expression with additional nutrients, which allow the expression of recombinant actin in the high-density insect cell culture. This method resulted in a 6-fold increase in the protein yields compared to conventional methods. Here we report in detail the effective method for the production of baculoviruses and recombinant actins.

3P145 Multiple actin structures in the filamentous state

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One of the most abundant proteins in eukaryotes is actin, a ubiquitous protein that plays a role in cell dynamics like cell migration. The dynamics of actin filament treadmill is regulated by two actin structural states: globular actin (G-actin) and filamentous actin (F-actin). Although it was reported that the structure for these states differ (Oda, et al, Fujii, et al), there is still much to resolve on the matter of their dynamics. Here we observed these dynamics under various conditions by using single-molecule FRET in combination with TIRF microscopy. We found that F-actin has at least two distinct states, that the population distribution of these states is dependent on the ionic conditions, and that these states are sensitive to actin binding proteins like myosin and actinin. We are currently investigating these distinct states by performing FRET measurements and observing various points along the actin filament in the presence of actin binding proteins like myosin, tropomyosin.

3P146 細菌べん毛モーター・スイッチ複合体の極低温電子顕微鏡による構造解析

Structural analysis of the flagellar hook-basal body with the C ring by electron cryomicroscopy.

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Many bacteria swim by rotating flagella, which function as helical propellers driven by reversible rotary motors that are embedded in the cell membrane. The flagellum consists of three parts: the filament, the hook, and the basal body. The basal body houses machinery for rotation and directional switching as well as for export of flagellar proteins, and its complex structure is composed of about 20 different proteins. The basal body contains at least five parts according to their functions: the rod, the LP ring, the MS ring, the C ring, and the type III protein export apparatus. The C ring is made of the switch complex proteins (FliG, FliM, FliN), which controls clockwise-counterclockwise (CW/CCW) switching of the motor rotation and sits on the cytoplasmic face of the MS ring. To understand the switching mechanisms in detail, we analyzed the C ring structures of CCW-bias and CW-locked mutants by electron cryomicroscopy (cryoEM). The CCW-bias and CW-locked phenotypes arise from single point mutation (V135L) and the deletion of residues 169-171 (Δ PAA) in FliG, respectively. The hook basal bodies from the CCW-bias and CW-locked strains were prepared from a *fliC* and *clpP* disruption *Salmonella* strain. CryoEM images of them were collected, and the C ring images were classified according to their rotational symmetry by using multi-reference alignment against reference 3D models with symmetries from 33- to 35-fold. We report conformational changes in the C ring observed by the structural comparison between the CCW-bias and CW-locked motor.

3P147 Structural study of the flagellar bundle of magnetotactic bacterium MO-1 by electron cryotomography

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Recently, a new magnetotactic bacterium called magneto-ovoid strain MO-1 was isolated from the Mediterranean sea. The bacterium has the flagellum of a unique architecture and swims at an unusually high velocity. Magnetospectrophotometry assay and genetic analyses of MO-1 have been conducted in an attempt to understand the motility mechanism but it remains unclear because the detailed structure of the flagellum is not yet available. Here,

we analyzed the MO-1 cells by electron cryomicroscopy and tomography to identify the unique architecture of this flagellar apparatus. We find that the MO-1 cell has two flagellar bundles, each extending from the hemisphere of the cell. Typically, each bundle is composed of a sheath, filaments and fibrils. The individual filaments are discernible only at the distal end of the bundle extending out of the sheath. The proximal part of the bundle consists of seven separated filaments and many fibrils that are enveloped by the sheath. The structure of the sheath appears to have a helical lattice formed by the subunits. The sheath can be easily detached from the cell, resulting in the dispersion of the filaments and impaired cell motility. Three-dimensional image reconstructions of the flagellar bundle embedded in vitreous ice clearly visualized that the bundle consists of several filaments and fibrils that wind around one another within the sheath. The flagellar filaments are attached individually to the cell with a disk-like structure at the base.

3P148 べん毛モーターの機械的強度測定
Experimental estimation of mechanical strength of flagellar motors

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Bacterial flagellar motors are the only known rotary motors that are used for locomotion by living things. Torque generated by the motors is in the order of 1 nN·nm. Thrust force generated by the rotation of a flagellar filament is in the order of 1 pN. Therefore, we can imagine that the motor should not be broken with the torque or force whose magnitude is comparable to or smaller than those values mentioned above. However, a *Salmonella typhimurium* mutant, SJW3060, has motors that are broken by their own power. Flagellar filaments of a lot of the mutant cells form huge bundles under viscous environment and the cells get away from the bundles by releasing the filaments. In this process, the motors are broken at the rod. To estimate the exact mechanical strength of the flagellar motors and then to consider the relationship between motor structure and its strength, we performed an experiment, a kind of breaking-down test. We applied viscous force on tethered cells by flow of polymer solutions. Both a wild type, SJW1103, and the mutant, SJW3060, were examined, and significant difference between them was observed in flow speed at which tethered cells unfastened.

3P149 細菌べん毛モーター固定子 MotA/MotB 複合体の分子モデリング
Molecular modeling of the stator complex MotA/MotB in bacterial flagellar motor

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Bacterial flagellar motor rotates with high energy efficiency. In case of *Escherichia coli*, the flagellar motor driven by protons has two components, called rotor and stator. The rotor located in the center of the motor is enclosed with ~ ten stators. The stator is thought to act as a torque-generate unit and compromises MotA and MotB proteins embodied in inner-membrane. Experimental studies have suggested a model of torque generation in which binding of protons across membrane to Asp32 of MotB induces structural changes of the stator, changing the interaction of MotA with the rotor. Therefore, the structural information on the stator is indispensable for the understanding of the torque generating mechanism which achieves high energy efficiency. However, the atomic-level structure of the stator complex MotA/MotB is still unclear because of its location in the membrane. In this study, we predicted the structure of the transmembrane regions of the stator using molecular modeling. The distance restraints derived from disulfide cross-linking experimental data were employed in simulated annealing and molecular dynamics calculations. These calculations enabled us to obtain structural models satisfying the restraint conditions. The result shows that the helices of the outer proteins MotA bend over MotB centered at the stator. We are attempting to identify the relationship of the residues around proton binding site to its function.

3P150 ケージドセリンを用いた大腸菌べん毛モーターの回転計測
Measurement of rotational behavior of flagellar motors in *E. coli* induced by caged serine

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Escherichia coli senses an extracellular stimuli by chemoreceptors to be transduced to the intracellular signaling molecule, which regulates the rotational direction of flagellar motors. In the presence of attractant, signal transduction is suppressed and motors keep rotating CCW. Recently, we showed that switching of multiple motors on the same cell is highly coordinated without extracellular stimuli. In this study, to investigate the behavior of multiple motors for the extracellular stimuli, we developed the measurement system

using caged serine photorelease to observe the response of rotational motion of motors at high temporal resolution. To measure the rotational behavior of motors, a polystyrene bead ($\varphi=0.5\mu\text{m}$) was attached to flagellar filament and the rotation of bead was recorded by high speed CCD camera at 1,250 frame/s. In the presence of caged serine, the rotational direction of motors was changed CCW only after the irradiation of violet light. Furthermore, duration of CCW rotation was depend on caged serine concentration and irradiation time of violet light. Without caged serine, there was no significant change in the switching behavior by the irradiation of violet light. These results suggest that caged serine was photolyzed by violet light and the released serine was sensed by *E. coli*. To control the generation of chemotactic signal more locally, we are constructing system of caged serine photorelease by focusing of violet light laser beam.

3P151 マイコプラズマのちぎれた頭 ~滑走装置の単離~
3D1250 "Chopped head" of *Mycoplasma* -Isolation of gliding machinery-

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Mycoplasma, a flask-shaped bacterium 0.8 μm long, glides on surface at a speed of 2-4.5 $\mu\text{m/s}$ by a unique mechanism. We proposed a working model, "power stroke" model, where the *Mycoplasma* repeatedly catches and releases sialic acid adsorbed on the solid surface, by "legs" which are sticking out from the base of membrane protrusion of *Mycoplasma*. Here, we isolated the gliding machinery. When the cells were treated by 0.1% Tween 60, the membrane protrusion, "head" was elongated from 0.8 μm to 2 μm in 30 min. The elongated head was detached from the cell body by sonication and collected by centrifugation. "Chopped head" showed glass binding and gliding motility by the addition of ATP. It suggests that the chopped head contains all components required for gliding. The gliding speed depended on the ATP concentration, consistent with the Triton model, "gliding ghost" of *Mycoplasma*. The chopped head glided at the maximum speed of 3.0 $\mu\text{m/s}$, comparative to the cell gliding under normal conditions. However, it detached from glass more easily than a living cell, which was prevented by the addition of 10% Ficoll, suggesting that the gliding mechanism is not highly processive.

3P152 Library antibodies inhibiting *Mycoplasma mobile* gliding suggest topology and movements of gliding proteins
3D1305 gest topology and movements of gliding proteins

Chie Kawaguchi, Shuhei Yoshii, Makoto Miyata, (Graduate School of Science, Osaka City University)

Mycoplasma mobile, a pathogenic bacterium glides on solid surfaces with a unique mechanism at an speed of 4.5 $\mu\text{m/s}$. Gli521 and Gli349, weighing 521 kDa and 349 kDa respectively, are featured with a transmembrane segment and involved in the gliding mechanism.

Previously, we isolated 14 and 40 monoclonal inhibitory scFvs from Human antibody library, respectively against Gli349 and Gli521. Inhibitory effects of these scFvs on gliding supported the assumptions that Gli349 plays a role of "leg" and Gli521 plays a role of "Gear". In the present study, we chemically synthesized Gli521 and Gli349 genes divided into 300 amino acid parts, expressed recombinant proteins, and decided the binding sites of scFvs by Western Blotting. The results show that scFvs against Gli521 bind to three parts of Gli521, and scFvs against Gli349 bind to one part of Gli349, suggesting that these parts are exposed cell outside and moving. Integration of the inhibitory effects and binding sites of scFvs suggests that the movements generated from ATP hydrolysis is transmitted from C-terminus to N-terminus of Gli521 and then from N-terminus to C-terminus of Gli349, resulting in repeated catch of the binding target on the solid surface by the leg.

3P153 高圧力下におけるべん毛モーターのトルク-スピード関係
Effect of pressure on the torque-speed relationship of bacterial flagellar motor

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The bacterial flagellar motor converts the specific ion flux across the cell membrane to the rotational direction. The torque generation is achieved by the intermolecular interaction between rotor and stator complexes. Little is understood about the detailed energy conversion mechanism. Here, we demonstrate a novel assay that controls the torque generation of the flagellar motor with high-pressure techniques. The torque-speed relationship of the motor was measured by tracking of polystyrene beads attached to flagellar filaments at pressure range of less than 800 bar. At ambient pressure (1 bar), the torque is approximately constant (at ~1500 pN nm) from stall up to a "knee" speed of ~150Hz, and then falls linearly with speed, extrapolating to zero torque at 280 Hz. As the pressure increased, both the knee and zero-load speeds decreased significantly, while the zero-speed torque was not affected. Similar relations were obtained by decreasing intracellular pH. Thus, our results suggest that applied pressure decreases the rate of proton translocation in the