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. Magneto-spectrophotometry 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,