2P253**Effects of stretch and BTS on auto-oscillatory properties of contraction and Ca^{2+} concentration in cultured cardiac muscle**○Mutsuki Takeuchi¹, Shin'ichi Ishiwata²¹Grad Sch Pure/Appl Phys, Waseda Univ, ²Dept Phys, Sch Sci/Eng, Waseda Univ

A cardiac muscle cell beats spontaneously in response to the oscillation of Ca^{2+} concentration that is regulated by sarcoplasmic reticulum (SR). We previously showed that BTS (N-benzyl-p-toluene sulphonamide), which is a myosin II-ATPase inhibitor, disturbed and weakened contraction and oscillation of $[\text{Ca}^{2+}]$. Based on these results, we suggested the existence of a feedback loop between the contractile system and the SR system. Here we attempted to clarify the properties of the cross-talk between the SR system and the actomyosin contractile system by applying stretch stimulation to the cultured rat cardiac muscle cells in the presence or absence of BTS. First, we confirmed that BTS weakened both contraction and $[\text{Ca}^{2+}]$ oscillation in cultured cells. Additionally, we found that the 20-% stretching of a cultured cell in the presence of appropriate concentrations of BTS shortens the periods of both contraction and $[\text{Ca}^{2+}]$ oscillations and sometimes halts them, making both oscillations unstable. When the cultured cells were stretched after the contraction oscillation had almost completely been stopped by the addition of BTS, we could observe that both oscillations recovered, although they were weak and unstable. We plan to further develop this approach to characterize the relationship between the oscillatory contraction and the oscillation of Ca^{2+} concentration.

2P255**Influences of the hook length on the rotational vibration of a tethered cell**○Manami Hashimoto¹, Takuji Mashimo¹, Shin-Ichi Aizawa^{1,2}¹CREST JST, ²Pref. Univ. of Hiroshima

Bacterial cells can be tethered through flagella on the glass surface, using anti-flagellin antibody. A cell body tethered through one flagellum can rotate, through which the function of the individual flagellar motor can be observed. Torque is generated between two distinct structural units of the flagellar motor: the stator and the rotor. The stator is composed of MotAB complexes, while the rotor is composed of FliGMN complexes. We have constructed YFP-FliN (a rotor component protein) chimeras and observed them by fluorescent microscopy. Several fluorescent spots observed in a cell are presumably derived from the motor, because the tethered cells almost always rotate around one of those spots. The rotational axis is almost always located at one end of the cell body. Therefore, the rotating cells are gyrating with a tilting angle from the glass surface. When mutants that produce either short or longer hooks were tethered, the tilting angle became larger and the rotating axis vibrated. In many cases, even when a cell smoothly rotates, the rotating center vibrated and jumped in a circle with a diameter of 50-200 nm. These data indicate that the rotation of a tethered cell is a complex combination of the motor rotation and twisting power by the hook.

2P254**Strain Field Measurement of Cytoskeletal Actin Network in Lamellipodia of Crawling Fish Keratocytes**○Taiji Adachi^{1,2}, Kennedy O. Okeyo¹, Yoshimichi Shitagawa¹, Masaki Hojo¹¹Dept. of Mech. Eng. & Sci., Kyoto Univ., ²VCAD System Res. Prog., RIKEN

Cell motility is spatially and temporally regulated by interactions among mechanical and biochemical factors, through the regulation of dynamical function-structure relationships such as cytoskeletal reorganization. Experimental studies have been carried out on cell motility to clarify the molecular signaling processes, however, little work is available on quantitative evaluation of the mechanical factors such as strain field in the cytoskeletal actin network and their possible roles in the reorganization process. The aim of this study is to quantitatively evaluate strain field in the lamella region of crawling fish keratocytes and to reveal the mechanism for the regulation of directional cell motility relating to the biomechanical factors in crawling cells. We utilized Fluorescent Speckle Microscopy (FSM) to detect and track retrograde flow of fluorescent dye-labeled cytoskeletal actin structure, and Particle Image Velocimetry (PIV) to analyze the displacement field from the obtained time-lapse FSM images. Strain field of the actin network in the lamella region was then analyzed as a spatial gradient of the obtained displacement field. We found that a net compressive strain parallel to the crawling direction exists in the lamella region, suggesting that the depolymerization of actin filaments is mechanically regulated by compressive strain, which in turn leads to the regulation of directional motility.

2P256**Physicochemical and structural analysis of mutant FlhA, a subunit protein of the flagellar type III protein export apparatus of Salmonella**○Masafumi Shimada^{1,2}, Yumiko Saijyo-Hamano², Yukio Furukawa², Thoru Minamino^{1,2}, Katsumi Imada^{1,2}, Kei-ichi Namba^{1,2}¹Graduate School of Frontier Biosciences, Osaka University, ²Dynamic NanoMachine Project, ICORP, JST

The bacterial flagellum, a motile organelle made of a rotary motor and a helical propeller, is composed of almost 30 different proteins. For flagellar self-assembly, the axial component proteins are transported through the central channel of the growing flagellum to the distal end where they polymerize. The flagellar type III protein export apparatus composed of six transmembrane and three soluble proteins mediates protein export driven by the energy of ATP hydrolysis. FlhA (75 kDa) is the largest membrane component with a large cytoplasmic domain, FlhAc, which interacts with soluble components including the ATPase FliH. The ATPase regulator FliH is required for the efficient FlhAc-FliH interaction. The crystal structure of FlhAc shows a four-domain structure (Saijo-Hamano et al, unpublished). We isolated temperature sensitive mutants (ts-FlhA) with a mutation in domain D1 and six suppressor mutants (sts-FlhAc). In our previous studies, we characterized the difference in the thermal stability between ts-FlhAc and sts-FlhAc, which clearly indicated that the loss of function is due to unfolding. We have now carried out limited proteolysis at 30 degree and 42 degree and found that these mutants are degraded into small fragment quickly at both temperatures and domain D1 is relatively unstable. We have also isolated FlhA(V404M) as a suppressor for FliH null mutation and solved the crystal structure. On the basis of structural comparison with the wild-type FlhAc, we will discuss how the FlhAc-FliH affinity is increased by this mutation.