Ryosuke Suzuki, Takahiro Hayashizaki, Yousuke Taguchi, Hajime Honda, (Department of Bioengineering, Nagaoka University of Technology)

When many myosin molecules are interacting with single actin filament, the estimation of the number and the location of interaction sites with myosin molecules along the single filament is not always easy either *in vivo*, such as myofibril, or *in vitro*, such as assays under fluorescence microscope. The one of the most powerful tool for the purpose should be the real-time visualization of the sites. We have been intended to introduce the environment-sensitive fluorescent dye into the Cys-374 residue of actin molecule, which is considered to be locating not only at the contact site of next actin monomer but also at the interaction sites of a myosin head during their sliding movement. Recently we have found that a hydration-sensitive dye, PyMPO ((1-(2-maleimidylethyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl) pyridinium methanesulfonate), might be the good candidate for the purpose. However, two problems still remained. One is weak emission intensity and another is fast photo-bleaching owing to the rather short excitation wavelength of PyMPO. In this presentation, we have concurred these problems by making a cluster of the dyes. The method for making the cluster and the visualized image of the interacting sites of actomyosin *in vitro* would be shown.

3P137 分子動力学・X線回折法による変異トロポニン導入心筋の構造解析 Molecular mechanism of hypertrophic cardiomyopathy caused by troponin mutation revealed by Molecular dynamics and X-ray diffraction study

Maki Yamaguchi¹, Masako Kimura¹, Shigeru Takemori¹, Tetsuo Ohno¹, Nobutake Akiyama², Masaru Watanabe³, Masatoshi Yumoto⁴, Naoto Yagi⁵, (¹The Jikei University school of medicine, Dept Physiology; ²The Jikei University school of medicine, Dept Molecular immunology; ³Tokyo Metropolitan University, Dept Frontier Health Science; ⁴The Jikei University school of medicine, Dept Anesthesiology; ⁵SPRING/JASRI)

E244D troponin T (TnT) mutant that causes hypertrophic cardiomyopathy is known to increase maximal tension of cardiac muscle fiber. To clarify how this mutation leads to the enhanced tension-development capacity, we performed molecular dynamics and X-ray diffraction experiments. Molecular dynamics at steady state showed that the electrostatic bond-formation between TnI and TnT involving the mutated amino acid was suppressed in E244D preserving its main-chain structure essentially identical to wild. Molecular dynamics with perturbation showed the connectivity of TnI to TnT was not different suggesting that the mutation has no significant effect on the signal transduction from TnI to TnT. Next, we carried out X-ray diffraction experiments on skinned muscle fibers in which endogenous troponin was replaced with wild or E244D TnT. Intensity of troponin reflection in both fibers suggested similar structural arrangement of E244D and wild TnT on thin filaments. While in E244D fibers, transition from resting to contracting states accompanied a larger intensity change of the myosin layer lines than wild fibers indicating that a larger fraction of myosin heads are recruited to contractile interaction in E244D fibers. These results suggest that the rearrangement of local electrostatic bonding triggered by the mutation probably brings abnormal interaction between TnT and tropomyosin to cause larger shift of tropomyosin allowing increased actomyosin interaction so as to enhance tension-development capacity in E244D mutant.

3P138 筋収縮における骨格筋ミオシンの非線形バネ弾性の実験的および理論的考察 3D1205 Experimental and theoretical considerations on the non-linear elasticity of skeletal myosins in muscle contraction

Motoshi Kaya, Hideo Higuchi, (Department of Physics, Graduate School of Science, University of Tokyo)

In muscle contraction, it has been proposed that an attached myosin in a positively-strain (working-stroke) region exerts a positive force that contributes to muscle shortening. Subsequently, the myosin is pulled into a negatively-strain (drag-stroke) region, exerting a negative force that opposes muscle contraction. Thus, the understanding of the molecular dynamics between the working- and drag-stroke myosins is one of key things to elucidate the molecular mechanism of muscle contraction. In this study, the novel technologies of the optical tweezers and fluorescence imaging were applied to obtain the elastic properties of single skeletal myosins in both the working- and drag-stroke regions for the first time. We found that the elastic properties of single myosins are non-linear associated with high stiffness in a working-stroke region and low stiffness in a drag stroke region. This biphasic elastic response implies that working-stroke myosins become stiffer to generate the high force with a small stretching, while drag-stroke myosins become softer to reduce the resistance force against the subsequent force generation done by other motors. Furthermore, we developed the analytical simulation model to

understand the molecular dynamics between 2-5 myosin motors, supporting our experimental findings. These results suggest that skeletal myosins are ingeniously designed to achieve the efficient and collective force generation without molecular interference, leading to the high mechanical efficiency of muscle contraction.

3P139 What the slight but the bright failures of 17 years ago really mean. Dedicated to Prof. Fujime.

Kouji Makino, (Graduate School of Integrated Science, Yokohama City University. A past student)

The method of Spudich and Watt is widely accepted as a standard protocol in the preparation of actin through muscle acetone powder. But this requires the sacrifice of the rabbits.

To prevent this problem, preparation from chicken sasami muscle were attempted. Originally this protocol was developed by H.Kondo in Nagoya University. The major differences were the followings.

A. Addition of the ATP to G.S. solution.

B. Triton X-100 treatment for the minced muscle.

In total, 5 time's trials with the combination of A and B were attempted, but the yields were between 0.8% and 2%, which were between a fifth and a tenth against the normal case. Major factor was the elution of the protein at the step of double distilled water wash and after the over night process.

Finally the protocol was established by H.K. with two modifications of the absence of the ATP and of the skip of O.N. process, and enables us to obtain acetone powder of almost 10% weight of the starting muscle.

After the long time passed, I'm considering the real meanings of 5 time's failures.

3P140 X線小角散乱による心筋筋関連トロポニン変異体の構造解析 Structural analysis of troponin mutants causing cardiomyopathy by small-angle X-ray scattering

Satoru Fujiwara¹, Yasushige Yonezawa², Fumiko Matsumoto¹, Toshiro Oda³, Soichi Takeda⁴, (¹Quantum Beam Science Directorate, Japan Atomic Energy Agency; ²Institute for protein research, Osaka university; ³RIKEN SPRING8 Center, RIKEN Harima Institute; ⁴National Cerebral and Cardiovascular Center Research Institute)

Various mutations in cardiac myofibrillar proteins cause Familial hypertrophic cardiomyopathy. Among these mutations, the mutations occurred in the regulatory protein, troponin (Tn), consisting of three subunits, TnC, TnI, and TnT, is particularly interesting because these mutations are directly related to the regulatory mechanism of muscle contraction. Here we focus the mutations in the coiled-coil region of TnT. These mutations, E244D and K247R in TnT, show an increase in the maximum level of ATPase activity while no changes in Ca²⁺ sensitivity occur [1,2], unlike other mutations in Tn showing changes in the Ca²⁺ sensitivity. As a first step towards elucidating the molecular mechanism underlying these functional aberrations, we carried out small angle X-ray scattering experiments on the solutions of Tn containing wild-type TnT, that containing TnT(E244D), and that containing TnT(K247R). In each sample, the scattering curve in the presence of Ca²⁺ was shown to be different from that in the absence of Ca²⁺, indicating the structural change due to Ca²⁺-binding to Tn. It was also shown that the scattering curves of Tn containing mutant TnT were different from those of Tn containing wild-type TnT, suggesting the structural changes of Tn induced by the mutations in TnT. The results of the detailed analysis, combined with the molecular dynamics simulations based on the crystal structure, will be described.

References

[1] Yanaga *et al.* (1999) *J. Biol. Chem.* **274**, 8806.

[2] Matsumoto *et al.* (2009) *Biochem. Biophys. Res. Com.* **382**, 205.

3P141 筋収縮系におけるトロポニンの役割：心筋トロポニン部分置換体の骨格筋自励振動 (SPOC) 現象に与える影響 Role of troponin in the contraction of striated muscle: The effect of partial exchange to cardiac troponin on SPOC in skeletal myofibrils

Shin Matsubara¹, Shin'ichi Ishiwata^{2,3}, (¹Department of Pure and Applied Physics, Waseda University; ²Department of Physics, Faculty of Science and Engineering, Waseda University; ³Waseda Bioscience Research Institute in Singapore (WABIOS))

To further clarify the regulatory mechanism of the contraction in striated muscle, we examined the effect of troponin (Tn), which regulates the cyclic interactions of myosin with the thin (actin) filaments, on SPOC (SPontaneous Oscillatory Contraction) in skeletal myofibrils. SPOC is an auto-oscillation phenomenon that occurs on the level of activation intermediate between contraction and relaxation, such as the intermediate level of [Ca²⁺] under physiological conditions in the presence of MgATP (Ca-SPOC) or at the coexistence of MgADP, MgATP and inorganic phosphate (Pi) in absence of Ca²⁺ (ADP-