a result, we could observe the stepwise transition of the fluorescence intensity, which indicates the dynamics of tRNA binding and release from immobilized ribosomes during translation. Our additional data would provide new insight for the ribosome translational mechanism during elongation process.

2P-127 尿素存在下でのアクチン繊維の安定性

Stabilization of actin filaments in the presence of urea

Kuniyuki Hatori and Naoki Nomura (Dept Bio-System, Graduate School of Science and Engineering, Yamagata Univ)

We examined the stabilization of actin filaments, the polymerization from monomers into the filaments and sliding movement on myosin molecules in the presence of urea which could act as a denaturant to modify the structure of proteins. Under a fluorescent microscopy, rhodamine-phalloidin-labeled actin filaments were observed for measuring its length distribution. The mean value of the filament length increased with an increase of urea concentration up to 0.8 M. The sliding velocity of actin filaments on myosin molecules gradually decreased as the urea concentration was increased, and then was completely suppressed at 0.8 M of the concentration. In addition, when the polymerization from actin monomers into the filament was directly observed, the polymerization rate rapidly decreased from 0.07 to 0.002 $\mu\text{m}/\text{min}$ in the concentration range of 0-0.8 M. Actin properties were found to be significantly influenced by the presence of urea at the specific concentration of 0.8 M. Once actin molecules form into the filament, the filamental structure may be reinforced by alteration in behavior of water molecules around the filament.

2P-128 X線繊維回折法によって明らかとなったフル、ノンオーバーラップ骨格筋のミオシンクロスブリッジの配向の違い

Different orientation of myosin crossbridges between full- and non-overlapped skeletal muscles studied by X-ray fiber diffraction

Kanji Oshima (1), Yasunobu Sugimoto (2), and Katsuzo Wakabayashi (2). (1: The Center for Adv. Med. Eng. and Info., Osaka Univ; 2: Div. of Biophys. Eng., Grad. Sch. of Eng. Sci., Osaka Univ.)

The intensity analysis of the X-ray diffraction patterns from the full- and non-overlapped skeletal muscles allows us to show the orientation of resting myosin crossbridges around the thick filaments. In the analysis we used the cylindrically averaged difference Patterson function ($\delta Q(r,z)$) to correct the sampling effects due to the irregular hexagonal arrays of thick filaments on the layer lines by cutting off the outer peaks ($r > 32 \text{ nm}$) on the $\delta Q(r,z)$ map. However, it was difficult to remove completely the sampling effect. Recently, we found that the inter vectors between crossbridges on neighboring thick filaments, which are facing with each other, contribute to the intermediate region ($20 < r < 32 \text{ nm}$) in the $\delta Q(r,z)$ map. We investigated whether the facing inter-crossbridge vectors can reproduce the difference in the corrected layer line intensities observed between full- and non-overlapped muscles. We found that the effect of the facing inter-crossbridge vectors was smaller than the observed intensity difference, indicating that the structure of a thick filament is significantly different between the full- and non-overlapped muscles.

In our best-fit models, the configuration of myosin crossbridges in the regular repeating region of crowns is similar in the full- and non-overlap muscles, but is somewhat different in the perturbed repeating region. In their end-on views, the two heads of a crossbridge form a horse's-like hoof structure in both muscles but they cross at the outer end in the full-overlap muscle.

2P-129 トレハロース存在下でのマグネシウムパラクリスタルの形成

Formation of magnesium-paracrystals in the presence of trehalose

Haseda K (1), Imai E (1) and Honda H (1). (1: Dept. Bioeng., Nagaoka Univ. Tech.)

Actin filament forms crystal-like structure in the presence of high concentration of magnesium ion, called Hanson-crystal. The formation of crystals was found in 1973 and the crystal was used to identify the periodicity of troponin-tropomyosin complexes binding to an actin filament (Ohtsuki and Wakabayashi, 1972).

Trehalose, a kind of disaccharide, is known to protect proteins from cold denaturation, because trehalose change the structure of water molecules including their hydrogen bonds. We have tried to form the Hanson crystals in the presence of trehalose in order to use the ordered structure of the crystal in vitro. Actin was mixed with 25 mM MgCl_2 and incubated at room temperature both in the absence and presence of 25 w/v trehalose to find that trehalose suppress the formation of the crystal. Adding MgCl_2 at the final concentration of 100 mM, the crystal was found to be formed even in the presence of trehalose however the crystal was contained some fragile structures.

In order to image the internal state of each actin filament within the crystal, we have mixed 0.005 mM of fluorescently labeled actin filaments into 1.0 mM of unlabeled filaments. Slight vibration-like movements were found in the labeled filaments even they were incorporated into the crystal. The observations suggest that the trehalose molecule should be incorporated into crystals in the early period of their formation enabling the slight movement of the filaments composed.

2P-130 トロポミオシン断片によるアクチンフィラメント協同性の回復

Retrieving cooperative properties of actin filaments by tropomyosin fragments

Yuki Nishio (1), Eiichi Imai (1), Hajime Honda (1) (1: dept of bioengineering, Nagaoka Univ. of technology)

The complexes of troponin and tropomyosin molecules were associated with actin filaments contributing to the facilitation of calcium regulation of muscle

contraction. Among them, tropomyosin has been focused to its important role on the regulation mechanism in spite of its simple structures. Tropomyosin molecules bind to actin filaments at the molar ratio of 1:7. Due to strong cooperativity, overlapping regions of both C- and N-terminous region was assumed to be important.

We have found that the dual role of tropomyosin in 1996, where tropomyosin could improve the actomyosin motility with a positive manner but actomyosin ATPase in a negative manner. Therewith we have considered that this dual role as an activity of tropomyosin and that α -helix, the major component of the protein, must be responsible for its activity.

We have digested tropomyosin molecules by proteinase K in order to get α - helical fragments that could bind to actin filaments. The activity was checked by *in vitro* gliding assay of abolished actin filaments which were composed of 80 of EDC-treated and 20 of intact monomers. About 30K fragment that could bind actin filament was found to retrieve the motile activity of actin filament. This observation could suggest that the simple α -helical structure could be essential for the tropomyosin activity and that C- and N-terminous overlapping might not be essential for this kind of activity belonging to the tropomyosin.

2P-131 カエル生筋収縮時のクロスブリッジ形成によるトロポニン分子の構造変化の測定

Measurements of structural changes of troponin molecules caused by crossbridge formation in live frog muscle during contraction

Tatsuhito Matsuo (1,2), Naoto Yagi (1). (1: Spring-8/JASRI, 2: Graduate School of Engineering Science, Osaka University)

X-ray diffraction is a unique method to study the muscle contraction on the molecular scale under physiological conditions. Our previous study showed that the intensity decrease of the troponin reflection at $1/38.5 \text{ nm}^{-1}$ (T1) was large during twitch and not enhanced by a second stimulation. Thus it is expected that the maximum x-ray intensity changes of T1 during twitch and tetanus at the same sarcomere length (SL) are similar. To test this prediction, the small-angle x-ray diffraction patterns from a live frog skeletal muscle were recorded at a time resolution of 1 msec using Spring-8 during both twitch and tetanus at SLs of 2.6, 3.0, and 3.4 μm . Contrary to the expectation, it was found that the intensity decrease of T1 during tetanus was smaller than that during twitch at 2.6 μm . At 3.0 and 3.4 μm , the intensity of T1 during tetanus did not decrease but increased and kept high values, while that during twitch did not. To explain these results, the electron density of a troponin molecule projected onto the filament axis was approximated by a gaussian function. And it was assumed that 1) during contraction the gaussian of troponin molecules in the A-band is broader than that in the I-band. 2) By Ca^{2+} binding to troponin, the gaussian of troponin molecules become sharper in both the A-band and the I-band. Based on these assumptions, the intensity changes of T1 can be explained qualitatively at all SLs. This result suggests that in the live muscle troponin molecules in the A-band may be fluctuated axially by crossbridge formation.

2P-132 再構成細いフィラメント上でのアクチンとトロポミオシン残基間の蛍光エネルギー移動測定：アクチン-トロポミオシン複合体モデル

FRET between Residues on Tropomyosin and Actin in the Reconstituted Thin Filament: Modeling the Actin-Tropomyosin Complex

Takahiro Saitoh (1), Yutaka Ueno (2), Chieko Kimura-Sakiyama (3) and Masao Miki (1) (1: Graduate School of Engineering, Univ. of Fukui; 2: National Institute of Advanced Industrial Science and Technology; 3: ERATO Actin-filament dynamics Project)

Fluorescence resonance energy transfer (FRET) between probes attached to F-actin and tropomyosin (Tm) was measured to study the spatial relationship between actin and Tm on the thin filament. We generated single-cysteine mutants of rabbit skeletal muscle α -Tm. The energy donor was attached to a single-cysteine residue at position 146, 153, 160, 167, or 174 on Tm, while the energy acceptor was located at Gln-41, Cys-374 or the nucleotide-binding site on actin. The transfer efficiencies for all cases did not change significantly upon removal of Ca^{2+} . Since the Tm molecule consists of two chains, it contains two energy donors in the single-cysteine mutant Tm molecule. While, an actin monomer in F-actin is located from the preceding one by an axial translation of 27.5 $\text{\AA}$ and a rotation of 166 degrees. Then, the energy acceptor attached to a specific site on actin should be considered for neighboring five actin monomers located near the energy donor on Tm. The transfer efficiencies were analyzed by taking into consideration the two donors and five acceptors system. Using the atomic coordinates of the crystal structures of Tm and actin, we searched for the disposition and orientation of these structures by minimizing the deviations of the calculated FRET efficiencies from the observed FRET efficiencies, in order to locate the Tm fragment (146-174) on an actin monomer with and without bound Ca^{2+} , as previously reported for the location of the Tn core domain on Tm (Kimura-Sakiyama et al. (2008) J. Mol. Biol. 376, 80-91).

2P-133 アクチンフィラメント中のアクチン1分子の速いゆらぎ

Rapid fluctuations of single molecules within actin filaments

Yousuke Taguchi (1), Katsuhiko Shimada (2), Eiichi Imai (1) and Hajime Honda (1). (1: Department of BioEngineering, Nagaoka University of Technology; 2: School of Design and Architecture, Nagoya City University)

Observation of single protein molecules functioning in a physiological manner should be one of the crucial goals for understanding protein functions in cells.