overlapped skeletal muscles by X-ray fiber diffraction

Kanji Oshima (1), Yasunobu Sugimoto (2), Katsuzo Wakabayashi (3) ((1) *The Center for Advanced Medical Engineering and Informatics, Osaka University*; (2) *Division of Biophysical Engineering, Graduate School of Engineering Science, Osaka University*; (3) *Division of Biophysical Engineering, Graduate School of Engineering Science, Osaka University*)

X-ray fiber diffraction is an indispensable technique for obtaining structural information on the molecular mechanism of muscle contraction. We have developed the technique to determine orientation of myosin crossbridges around the thick filament backbone in full- and non-overlapped skeletal muscles. The cylindrically averaged difference Patterson function was used to correct the sampling effects on the myosin-based layer-line intensities due to the hexagonal filament array. The corrected intensity difference in the layer lines between full- and non-overlapped muscles was ~17 %. To investigate whether the difference originates from the orientation of myosin crossbridges around their backbone or the order of the hexagonal array, we modeled the thick filament structure with the perturbed region and regular regions of crown repeats as reported previously (Oshima et al., JMB, 2007) to calculate the layer-line intensities of the single filament and of the hexagonal array of filaments. The difference in the intensity between these two models was ~13 %, which was less than the experimental value, indicating that the orientation of myosin crossbridges is significantly different in 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 full- and non-overlap muscles, but is different in the perturbed repeating region. In their end-on views, the two heads of a crossbridge form a horse shoe-like structure in both muscles but they cross each other at the outer end in full-overlapped muscle.

2P-108 ミオシン相互作用による Cys374 の疎水性

From the hydrophobicity of Cys374 of actin molecules to its interactions with myosin.

Takahiro Hayashizaki (1), Ryosuke Suzuki (1), Eiichi Imai (1), Hazime Honda (1) ((1) *Department of Bioengineering, Nagaoka University of Technology*)

Considering the water molecules surrounding an actin filament with their interactions with myosin, the hydrophobic properties of the residues or the myosin binding domain incorporating the residues supposed to be important in relation to the hydrolysis of ATP molecules. We have tried to detect the changes in the hydrophobic properties of Cy374 by a fluorescent probe, PyMPO ((1-(2-maleimidylethyl)-4-(5-(4-methoxyphenyl) oxazol-2-yl) pyridinium methanesulfonate), the fluorescent intensity of which was found to increase 20% upon polymerization and additionally upon the interaction with myosin molecules. However, the intrinsic fluorescent intensity of PyMPO is not rather strong enough to visualize the interacting positions with myosin along actin filament with considerable spatial accuracy. In order to concur with this problem, we have combined the fluorescent dye-cluster technique with this PyMPO dye. This technique should enable us to visualize the positions (or sites) along the actin filament which are just interacting with myosin molecules enabling the smooth movement in an in vitro motility assay. The results may bring us not only the spatial information about what derive the correlations between two molecules interacting with the same filament but also the influence of the energy of ATP-hydrolysis upon water molecules around the interacting site.

2P-109 多分子ミオシンシステムの運動モデル（測定プローブの影響を含めたエネルギー効率の観点から）

Mathematical model of multiple myosin system with measurement probes, focusing on energy efficiency.

Hiroto Tanaka (1) ((1) *NICT, PRESTO JST*)

Myosin is a molecular motor, which slides along actin filaments during ATP hydrolysis. Experimental results of single and multiple molecule measurements show that myosin can slide 15 – 200 nm, which is much larger than molecular size of myosin. To understand sliding mechanism of myosin system, we have developed the model of multiple myosin system with modifying potential function, and analyzed structure of the model. Our results suggest that energy consumption per unit step would be lower by connecting myosins, which is consistent with larger interaction length (IL) experimentally estimated previously (60 – 200 nm, with surface assay). In this study, we will show characteristic structure of potential function of the model to satisfy IL of single and multiple myosin system. Additionally, in order to verify the model with experimental data, here, we construct model including characteristics of measurement probes (ex. scanning probe (SP), optical tweezers (OT)), and simulate movement of myosin system attached to measurement probes (SP or OT). In order to take into account effects of measurement probes, we construct model of myosin system attached to probes via spring, and simulate movement of myosin system along periodic potential by Monte Carlo method. We test effects of spring constant and size of probes. As a result, sliding velocity with SP becomes slower than that with OT, then displacement generating process clearly observed with SP. We will show and discuss our model and experimental data at the meeting.

2P-110 Radial spokes は head-tail-joint のフレキシビリティに方向性を持つ The head-tail-joint flexibility of radial spokes depends on its direction

Youske Shimizu (1), Maki Yoshio (1), Kyo-hei Ito (2), Yang Pinfen (3), Sakakibara Hitoshi (1) ((1) *National Institute of Information and Communications Technology*; (2) *Department of Bioengineering, Nagaoka University of Technology*; (3) *Department of Biological Science, Marquette University*)

Radial spokes are major structure in the eukaryotic flagellum. The radial spokes protrude from outer doublet microtubules to the central-pair microtubules, supposing mechanical communication between outer and central apparatus. There are dyneins adjacent to the bases of the radial spokes, also supposing that the radial spokes have the regulatory roles in bending of flagellum. To obtain clues on the functions, we isolated the radial spokes from *Chlamydomonas* flagella and tested their properties. From negative-staining EM observations of mixture of radial spokes and microtubules, protrusions considered to be the radial spokes on the microtubules were observed. Binding of the radial spokes to microtubules was also confirmed by the co-sedimentation experiments. We then analyzed conformations by negative-staining EM and following single-particle analysis. The detail of the radial spokes, which consists of heart-shaped head of ~25nm in size, rod-shaped tail of ~30nm long, and tail-tip domain of ~10nm in size, was observed. Unexpectedly, we found that the head-tail joint is flexible and that the flexibility depends on the direction; angles of heads and tails distribute wider in one direction ($\pm 20^\circ$) than in the perpendicular direction ($\pm 10^\circ$). When the micrographs of the radial spokes were adopted into a tomographic image of the axoneme, the flexible way of heads and tails corresponded to cross-sectional direction of the axonemes and comparably rigid way corresponded to longitudinal direction, i.e., the direction of microtubule sliding. The directionality of head-tail-joint flexibility for the radial spokes would be correlated with the mechanical communications between outer and central apparatus, probably reflecting the state of microtubule sliding.

2P-111 CH ドメインを持つ新規イネキネシン O12 とアクチンとの相互作用

Interaction of the novel rice kinesin O12 that has the calponin homology domain with actin.

Nozomi Umez (1), Kazunori Kondo (2), Toshiaki Mitsui (3), Shinsaku Maruta (1) ((1) *Division of Bioinformatics, Graduate School of Engineering, Soka University*; (2) *Dept. of Bioinformatics, Faculty of Engineering, Soka University*; (3) *Graduate School of Science and Technology, Niigata University*)

Kinesin is a motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesin converts chemical energy from ATP into mechanical force. Kinesin family is classified into some subfamilies. Some species of kinesin derived from vertebrate have been well studied on their characterization. However, not so many studies for kinesins of plants have been done yet. In 2002, the genome sequences of rice japonica have been completed. Our preliminary bioinformatics analysis revealed at least more than 35 kinesins related protein encoded on the rice genome. O12 is one of the rice kinesin that has a calponin homology domain (CH domain). It is known that CH domain interacts with actin, which may be related with the regulation of the physiological function. In this study, interaction of the CH domain of rice specific kinesin O12 with actin was studied. The cDNAs of the O12 motor domain (O12MD) and O12 composed of motor domain and CH domain (O12CHMD) were cloned into expression vector pET15b or pColdI, and transformed into *E.coli* BL21 (DE3). There expressed O12 kinesins were purified by Co-NTA column. Coprecipitation experiment with actin revealed that CH domain binds to actin. The microtubules dependent ATPase activity of the O12CHMD was significantly reduced in the presence of actin. Interestingly, ATPase activity of the O12MD lacking CH domain also decreased at high concentration of actin. It has been shown that the microtubule dependent ATPase activity of the rice kinesin O12 is regulated by actin.

2P-112 新規イネキネシン K23 の特徴付け Characterization of the novel rice kinesin K23

Nobue Hanzawa (1), Nozomi Umez (2), Kazunori Kondo (1), Toshiaki Mitsui (3), Shinsaku Maruta (2) ((1) *Dept. of Bioinformatics, Faculty of Engineering, Soka University*; (2) *Division of Bioinformatics, Graduate School of Engineering, Soka University*; (3) *Graduate School of Science and Technology, Niigata University*)

Kinesins form part of a superfamily of microtubule motor proteins that are found in eukaryotic organisms. Kinesins perform many diverse functions such as intracellular transport, spindle formation and elongation in mitosis and meiosis, microtubule dynamics and signal transduction. Recent study of rice japonica kinesins and the completion of its genome sequence have lead to further research of its subfamilies. However, only a few kinesins have been characterized in rice. In this study, we have focused on K23 that is one of the rice-specific kinesin. Phylogenetically, K23 seems to belong to the branch of plants, in which kinesin-7 subfamilies also form part of. Its motor domain, starting from amino acid 36 to