which is equivalent to about 7100 amino acids and corresponds to about 780 kDa. 4000K-protein contains many Ig domains, two novel 47 and 46 residue repeats, and three PEVK-like sequence flanked by an Ig domain. We were unable to search similar proteins in the protein database. This 4000K-protein is assumed to exhibit elasticity in the obliquely striated muscle of polychaete *Neanthes* sp. To confirm that the cDNA was from 4000K-protein, we prepared a polyclonal antibody (PcGfam) using the recombinant protein of the unique region as an antigen. Immunoblot analysis showed that the antibody reacted only with the 4000K band and not with other proteins. This confirmed the sequence to be that of 4000K-protein cDNA.

2P-102 活性化レベルに依存する骨格筋自励振動現象の振舞い

The behavior of spontaneous oscillations of skeletal myofibril depending on the activation levels

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The contractile system of striated muscle generates spontaneous oscillations of force and sarcomere length (SL) at the intermediate levels of activation between contraction and relaxation, such as the coexistence of MgATP, MgADP and Pi in the absence of Ca²⁺ (ADP-SPOC; SPontaneous Oscillatory Contraction) and intermediate free Ca²⁺ concentrations under the physiological relaxing conditions (Ca-SPOC). The SL oscillation consists of slow shortening and rapid lengthening phases (saw-tooth waveform). In addition, the lengthening phase is transmitted to the adjacent sarcomeres along the myofibril (SPOC wave) (cf. Shimamoto, Y. et al., *Biophys. J.* 93, 4330-4341. 2007; see also Shimamoto, Y. et al., *Proc. Natl. Acad. Sci. USA*. Online, June 10, 2009).

Recently, we have succeeded in constructing a theory to describe the dynamic behavior of the contractile system of striated muscle at all the levels of activation (Sato, K., Ohtaki, M., Shimamoto, Y. and Ishiwata, S., manuscript in prep.): That is, the phase diagram, which is constituted of the relaxation, contraction and SPOC regions, was constructed as a function of activation levels and other physical parameters characteristic of the mechanical architecture of sarcomeres. Interestingly, the theory predicts that weak oscillation (small amplitude of SL oscillation distinct from the saw-tooth waveform), which is different from a typical SPOC pattern, appears in between the relaxation and the SPOC regions. Besides, the amplitude and the period of SL oscillation in the SPOC region increase as the activation level increases within the SPOC region. Therefore, we here try to examine whether these predictions by the theory on the dynamic behavior of myofibrils (sarcomeres) at various activation levels are correct.

2P-103 正常および心筋症ヒト心筋における SPOC の研究

A study of SPOC in failing and non-failing human cardiomyocytes

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In our laboratory, we have been studying the contractile mechanism of striated muscle, especially focusing on SPOC (SPontaneous Oscillatory Contraction). SPOC is an auto-oscillation phenomenon that occurs under intermediate activation conditions, namely, at intermediate concentrations of free Ca²⁺ under physiological solvent conditions (Ca-SPOC), or in the coexistence of MgADP and inorganic phosphate (Pi) under relaxing conditions (ADP-SPOC). We have reported that the period of sarcomere length oscillation is well correlated with that of the resting heart beat in various animal species (Sasaki, D. et al., *J. Musc. Res. Cell Motil.* (2005) 26: 93-101, *Biochem. Biophys. Res. Commun.* (2006) 343: 1146-1152). In this study, we examined the SPOC properties of human myocardium preserved by freezing at -200 °C (liquid nitrogen). Two types of samples have been used; (1) non-failing hearts aged 19-65 years old and (2) end-stage failing hearts of patients aged 15-63 years old. Preliminary experiments showed that in non-failing hearts the period of SPOC was longer with age. We also found that the period of SPOC was longer in the failing heart than in the age-matched non-failing heart. Thus, we have been studying the effect of end-stage heart failure on SPOC behavior (1) and the effect of aging on SPOC period of non-failing hearts (2). We will discuss the correlation between SPOC characteristics and the beating properties *in vivo*. This research has been approved by the Human Ethics Committee at Waseda University.

2P-104 分子動力学計算で探るミオシン構造状態の結合ヌクレオチドに対する応答

Molecular dynamics simulation on the allosteric response of myosin due to bound nucleotide

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Myosin II is an ATPase that drives muscle contraction in cooperation with actin. Crystal structure suggests that myosin exhibits drastic structural changes tightly coupled with the ATP hydrolysis, and it is thought that interaction with actin is controlled by the open-close transition of the actin-binding cleft. Furthermore, the

swinging of the lever-arm is supposed to produce the mechanical force. However, it is not clear whether these allosteric structural changes indeed exist in solution. Therefore we studied the influence of the nucleotide state on the structural state of myosin by molecular dynamics (MD) simulation in explicit water.

We used the crystal structure of scallop striated muscle myosin in the lever-arm-up and ADP-Pi-bound state. We then exchanged the ADP-Pi bound state for (1) ATP (2) ADP, and (3) nucleotide-free states. The subsequent relaxation processes were observed by MD simulation. We conducted an additional MD simulation using the crystal structure in the lever-arm-down and nucleotide-free state to probe the presumed post-power-stroke state. To attain statistical reliability, we performed eight (or sixteen) independent MD runs for each relaxation simulations (each MD run is 50-100 ns long).

From these relaxation simulations, we found an indication of nucleotide-dependent structural change in the actin-binding cleft. Loop1 also showed a nucleotide-dependent response: there is a tendency to form an alpha-helix in the ATP-bound state, which may trigger allosteric transitions. Even though the lever-arm-swing is expected according to the lever-arm model, it is not observed: our result instead showed that the lever-arm-down state is in the higher potential-energy state than the lever-arm-up state.

2P-105 筋原繊維内におけるミオシン II 動態の 1 分子直接観察

Direct observation of single myosin II molecule dynamics within a myofibril

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Muscle contraction occurs through the interaction between actin and myosin using the energy gained from ATP hydrolysis. The distance a single myosin molecule moves in relation to the energy from a single ATP is fundamental to understanding the mechano-chemical coupling of muscle contraction. Here, in order to examine how much a single myosin II displaces in muscle when using the energy from one ATP, we directly observed the movement of single myosin II molecules in a myofibril. Single sarcomeres were prepared from crab striated muscle. Some endogenous myosin regulatory light chains were exchanged with chicken smooth muscle myosin II, which were bright labeled with fluorescent dye cluster. single myosin molecule movement was imaged using TIRFM equipped with a sensitive CCD camera. Preliminary results showed 80-150nm displacements in both back and forward directions by myosin II parallel to the longitudinal direction of the myofibril suggesting a very large stepsize by myosin II in the myofibril. Now we are characterizing the movement of myosin further in detail.

2P-106 同位体スピラベルを利用した骨格筋アクチン - トロポミオシン間の高精度距離解析

Highly accurate distance analysis between tropomyosin and actin of skeletal muscle using isotopically substituted spin label

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Tropomyosin (Tm) is an α -helical, parallel coiled coil which binds to actin along the length of the thin filaments in muscle. Recently cryo-electron microscopic studies have identified distinct locations of Tm on the thin filament in the presence or absence of Ca²⁺. On the other hand, our previous site directed spin labeling (SDSL)-ESR studies using functional, reconstituted thin filaments have failed to detect any changes in the residual side chain mobility of Tm upon Ca²⁺-binding under physiological conditions of temperature and solvent. In this study, by using SDSL-ESR we have tried to measure the intersite distance between spin labels at a site on Tm (Cys13, Cys146, Cys160, Cys174, Cys230, Cys271 or Cys279; MTSL-¹³N-D₁₅) and a site on actin protomer (Cys374; MSL) at frozen state. The ESR spectrum was easily resolved into those from Tm and actin, because MTSL-¹³N-D₁₅ had two absorption lines and MSL three lines. The spectra corrected by subtraction of extra MSL-actin were compared with singly-labeled spectra and showed that the Tm was located very close to actin-Cys374 in the site of Tm-Cys13, 271 or 279 on the thin filament. In the absence of Ca²⁺, about a half of the spins were restricted in the range of 13-15 Å while the remaining longer than 25 Å. In the presence of Ca²⁺, the shorter distance distribution shifted to the range of 16-20 Å and might bind with the longer distribution. In contrast no spectral changes were observed in the other site of Tm. These results suggest that the region around the Cys374 in an actin protomer is blocked sterically by N- and C-terminal joint region of Tm fluctuating between two states against the binding of myosin heads.

2P-107 X線繊維回折法によるフル、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解析

Analysis of orientation of myosin crossbridges in full- and non-

overlapped skeletal muscles by X-ray fiber diffraction

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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.

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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視点から）

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

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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

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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.

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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

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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