2P197

The head of myosin class-V motor moves along an actin filament with regular, ~5.5 nm steps.

○Takuya Okada^{1,2}, Hiroto Tanaka^{1,2}, Atsuko Hikikoshi Iwane^{2,3}, Kazuo Kitamura⁴, Mitsuo Ikebe⁵, Toshio Yanagida^{1,2,3}

¹Laboratories for Nanobiology, Graduate School of Frontier Biosciences, ²Formation of soft nano-machines, CREST JST, ³Department of Physiology and Biosignaling, Graduate School of Medicine Osaka University, ⁴Department of Neuroscience, Graduate School of Medicine Osaka University, ⁵UMASS

Class-V myosin is a processive motor that transports organelles in cells. Myosin-V has two heads followed by long necks and strides along actin helical repeats in a hand-over-hand fashion. The large stride is thought to be composed of an initial step driven by conformational changes in the front head while attached to actin followed by a diffusive search for the forward target by the rear head. The initial step has been extensively examined by structural and mechanical studies but the diffusive search mechanism is not well understood yet because the diffusive movement is too fast to observe. Here we measure the diffusive movement of a single myosin-V head along F-actin by scanning probe nanometry that detects the movement slowed by attaching the head to a relatively large probe. The myosin-V head undergoes directional diffusion with ~5.5 nm substeps. The step size (~5.5 nm) is independent of the neck length (2 and 6 IQ motifs) (Okada et al, Biophys. J, 2006). A two-headed myosin-V showed processive movement with ~36 nm. Furthermore we observed an intermediate step within each 36 nm step as previously observed by optical trapping nanometry at high loads or in the presence of BDM. The intermediate steps are further resolved to several (3 - 4) ~5.5 nm substeps. In this system, a single actin filament was used (Okada et al., Nippon seibutubuturigakkai, 2002). These results strongly indicate that the rear head searches for the forward target by directional diffusion on the actin subunits according to a potential slope along the actin helix.

2P199

Conformational Changes of Inner-Arm Dynein f from *Chlamydomonas* Coupled with Phosphorylation of Its Intermediated Chain IC138. III

○Hitoshi Sakakibara¹, Stanley A Burgess², Yukako Sakai¹

¹Kobe Advanced Research Center, NICT, ² Institute of Molecular and Cellular Biology, University of Leeds

An inner-arm dynein f (dynein-f or the II complex) of *Chlamydomonas* is a double-headed dynein composed of two different heavy chains, three intermediated chains, and light chains. It has been shown that an axonemal casein kinase I (CKI) phosphorylates the 138K intermediated chain (IC138) and that this phosphorylation of IC138 is involved in regulation of flagellar activity in phototaxis. To elucidate the mechanisms how IC138 regulates activities of dynein f, we examined conformations of dynein-f molecules with IC138-phosphorylation by negative-staining electron microscopy and single-particle image processing. We didn't observe large conformational changes coupled with the phosphorylation of IC138. However, it appeared that averaged images of two heads with IC138-phosphorylated dynein-f are dimmer than those of dynein-f without phosphorylation when we aligned dynein-f images based on the tail images, suggesting that dispositions of heads were varied. We then examined disposition of two heads. The results indicate that variation of heads' dispositions is increased couple with IC138-phosphorylation. We have examined as well conformations of dynein-f of *mia2*, a photo-taxis mutant that has IC138-phosphorylated dynein-f. Preliminary results are consistent to those with dynein-f, IC138 of which are phosphorylated *in vitro*. These suggest that IC138-phosphorylation increases flexibility of necks. We speculate that this flexibility increasing on necks would modify the interaction between an adjacent B-tubule and dynein-f in the axoneme.

2P198

High-resolution observations of the movement of single kinesin molecules using FIONA

○Keishi Kikuchi, Shuichi Onishi, Yuya Miyazono, Hiroaki Fujiwara, Hisashi Tadakuma, Michio Tomishige

Dept. of Applied Physics, Faculty of Engineering, Univ. of Tokyo

Kinesin is a motor protein that moves processively along microtubules using ATP hydrolysis energy. Kinesin has been proposed to walk by alternatively exchanging two motor heads in a hand-over-hand manner. In this model, the detached trailing head should bind preferentially to the forward tubulin-binding site. To elucidate the mechanism for such preferential binding, we thought that high-resolution observation of the movement of detached head would be essential. Optical trapping assays allow high-resolution measurements for the center-of-mass movement of kinesin, but not for the movement of individual heads. Recent single molecule fluorescence observation revealed 16 nm stepping motion of individual kinesin heads. However the temporal resolution was too low to detect an intermediate state between steps (sub-step). Here, we labeled kinesin with Quantum Dot (Qdot), and observed two-dimensional movement of Qdot-kinesin using FIONA (3 nm spatial resolution at 2 ms exposure, under the best condition), to detect the sub-step. As a first step, we evaluated the effect of Qdot labeling on kinesin's activity. Qdot was attached to the tail or the head of kinesin, and the movements of Qdot-kinesin along microtubules were observed. The velocity measured under various ATP concentrations showed typical Michaelis-Menten kinetics. Furthermore, the velocity and run length of Qdot-kinesin were almost similar to those reported previously, confirming that Qdot-labeling does not affect the processive motility of kinesin.

2P200

Simultaneous observation of myosin V and polyacrylamide bead along actin tracks using two colors TIRFM

○Yuta Saito¹, Naoki Nagura¹, Shin'ichiro Tanaka¹, Toshio Ando^{1,2}

¹Dept. of Physics, Kanazawa Univ., ²JST/CREST

Myosin V is a processive motor whose movement along actin tracks has recently been demonstrated to take place in a hand-over-hand (HOH) manner. However, phenomena that cannot be explained by the HOH mechanism have been observed and therefore we still do not know whether myosin V uses only the HOH mechanism or also uses an additional type of mechanism which does not clearly appear. We previously observed that positively charged polyacrylamide beads (approx. 3 nm in diameter) labeled with fluorophores moved along actin tracks when myosin V and ATP were present in the solution. The bead size seems too small to be "kicked" by myosin V heads moving in HOH. In addition, we found that the bead surprisingly moved even in high Ca²⁺, whereas myosin V whose head domains were labeled with fluorophores moved in low Ca²⁺ but not in high Ca²⁺. The speed of the bead movement in low Ca²⁺ was similar to that of myosin V, while in high Ca²⁺ it was slower but the movement took place very steadily in one direction. Because this bead behavior was unexpected at all, we repeated the experiment many times with different preparations of the sample, and could confirm this behavior in each experiment. To scrutinize this surprising behavior, we are now trying to observe the bead and myosin V simultaneously using a newly constructed two colors TIRFM. At the meeting, we will report results from previous experiments and the two colors TIRFM experiment.