1L1648 ネガティブ染色電子顕微鏡法による *Mycoplasma mobile* の滑走装置の観察

Gliding machinery of *Mycoplasma mobile* observed by negative-staining electron microscopy

Hiroki Yamamoto, Makoto Miyata (Grad. Sch. Sci., Osaka City Univ.)

Mycoplasma, a pathogenic bacterium, forms a membrane protrusion at a cell pole and glides in the direction of protrusion, by a unique mechanism. *Mycoplasma mobile*, the fastest species, glides at a speed up to 4.5 $\mu\text{m/s}$ with the maximum force of 27 pN. Three large proteins, Gli521, Gli349, Gli123, weighing, 521, 349, and 123 kDa, respectively, with the putative roles of crank, leg, and mount are involved in the gliding mechanism. Although the molecular shapes of isolated proteins have been elucidated, the gliding machinery has been only partly observed by freeze-fracture electron microscopy. In the present study, we reduced the background noises by using volatile buffer and the digested sialoprotein as the scaffold, and then succeeded in clear observations of gliding machinery by negative-staining electron microscopy, as follows. (i) The cells elongated in the volatile buffer with keeping gliding. Many flexible fibers about 50 nm long were observed on the cell surface. (ii) The fibers could be classified into two groups. One was aligned in 5 nm parallel to the cell membrane, with about 50 nm pitch, and the other extended softly from the cell membrane. (iii) The observation of mutants lacking either of Gli521 or Gli349 suggested that the parallel and extending fibers are, respectively, composed of Gli521 and Gli349, consistent with the molecular shapes, elucidated for the isolated proteins by electron microscopy.

13:00~17:00 M会場 / Room M

分子モーター 2

Molecular motor 2

1M1312 ジスルフィドクロスリンク法を用いたキネシン1のネックリンカーP45 のストレインセンサーとしての役割の解明

Strain-dependent regulation of kinesin-1 as studied by disulfide-crosslinking of the neck linker

Yamato Niitani, Xiao Ling, Michiko Nakajima, Michio Tomishige (Department of Applied Physics, School of Engineering, The University of Tokyo)

Kinesin is a dimeric motor protein that hydrolyzes ATP and moves along microtubules in a hand-over-hand manner. To walk by alternately moving two motor heads, the trailing head should detach from the microtubule prior to the leading head and the detached head should bind to the forward tubulin-binding site. To explain this mechanism, we hypothesized that ATP hydrolysis reaction of kinesin motor domain can be regulated depending on the direction of the tension posed to the neck linker: backward strain posed to the neck linker suppresses ATP hydrolysis and the forward strain posed to the neck linker suppresses ADP release. To test this hypothesis, we constrained the neck linker in the forward or backward extended conformation using disulfide-crosslinking between cysteine residues on the head and the neck linker and examined its effect on the microtubule affinity and ADP release kinetics. Single molecule fluorescent observation of GFP-fused monomeric kinesin showed that the dwell time on the microtubule in the presence of saturating ATP was increased by a factor of 15 when the neck linker was constrained in a backward extended conformation. Furthermore, stopped-flow measurement showed that ADP release rate after microtubule-binding decreased more than 100 fold when the neck linker was constrained in a forward extended conformation. These results support the idea that ATP hydrolysis cycle of kinesin's motor domain can be differently regulated depending on the direction of the neck linker extension.

1M1324 EPR を用いたネックリンカー伸長変異体キネシンの動的構造解析 Structural Dynamics of Mutant Kinesin with Lengthened Neck Linker by Distance Measurements Using Spin-labeling EPR

Shinji Takai¹, Masahumi Yamada², Tetsu Yasuda¹, Shinsaku Maruta², Toshiaki Arata¹ (¹Dep of bio. Sci., Osaka univ., ²Dep of Bioeng, Soka univ.)

The most popular hypothesis for kinesin movement is that the interaction between two domains, motor head and neck linker region, is likely to generate the bias which is require for the directional movement on microtubule. Previous EPR studies showed that the nucleotides changed (1) the mobility of side amino

acid chain and (2) the length of neck linkers, suggesting that the dock-undock conformation change occurs depending on the nucleotide condition.

In this research, we will induce a double spin label to the two domain of monomer kinesin, motor head domain (Cys 223) and neck linker domain (Cys 336), and measure the distance between two spin labeled domains by measuring the EPR spectrum broadening which is due to the interaction between two adjacent spin labels. This result showed that undock-to-dock conformation change of neck linker depends on the nucleotide condition clearly.

Then, two heads of dimeric kinesin seem to cooperate with each other, but it is not clear how it could. We will show the result of distance measurement by EPR in the case of dimeric kinesin to examine whether the cooperation between two heads occurs or not. It is likely that the rear attached head could interfere with the conformation change for front head by pulling the physical tension backward. We also discuss about this point based on the result of the experiment using genetically extended neck linker, in which the physical tension by neck linker is lost.

(1) Sugata et al. Biochem Biophys Res Commun. 314, 447-451 (2004).

(2) Sugata et al. J Mol Biol. 386, 626-636 (2009)

1M1336 Conformational change of α -1 helix of kinesin detected by electron paramagnetic resonance (EPR)

Satoshi Yasuda¹, Masafumi Yamada², Shinsaku Maruta², Toshiaki Arata¹ (¹Dept. of Biol. Sci., Grad Sch. of Sci., Osaka Univ., ²Divi. of Bioeng., Grad. Sch. of Eng., Soka Univ.)

We have used site-directed spin-labeling and electron spin resonance (ESR) spectroscopy to monitor a nucleotide dependent conformational dynamics of monomeric kinesin which is a neural motor protein moving on microtubule, a polymer of partner protein tubulin. We targeted α -1 helix of kinesin and nitroxide spin label was placed in cys-lite KIF5A (K349 monomer) at amino acid residue 61, 62, 66, 67, 70, or 72 on α -1 helix. C-terminal residues of this helix form a part of a nucleotide-binding pocket and N-terminal of this interacts with neck linker. Thus we studied conformational change of the helix caused by nucleotide binding or microtubule binding. Firstly, when we added microtubules (MTs) to spin-labeled kinesin, all spectra of each mutant were broadened. The same results were obtained under all nucleotide conditions. These data suggest that steric hindrance around the α -1 helix was increased by binding of kinesin to MTs. Secondly, when we added ADP or AMPPNP to spin-labeled kinesin in the presence of MTs, no spectrum change was detected. These data indicate that upon transition from the ADP form to AMPPNP form, no conformational changes occurred at this helix. Finally, when we added apyrase to spin-labeled kinesin in the presence of MTs, mobility of the residue 67 mutant increased, whereas mobility of the residue 66 mutant decreased. These data suggest that the α -1 helix may rotate around its long axis when kinesin releases ADP.

1M1348 全原子シミュレーションによるキネシン-微小管間の界面構造のリアインメント

The interface structure between kinesin and microtubule refined by all-atom MD simulations

Ryo Kanada¹, Tsukasa Makino², Michio Tomishige², Shoji Takada¹ (¹Grad. Sch. Sci., Univ. Kyoto, ²Dept. of Appl. Phys., Univ. Tokyo)

Kinesin is a molecular motor protein that can move along microtubules (MT) unidirectionally and processively by using ATP-hydrolysis free energy. Chemical reactions of kinesin, specifically ADP-release and ATP-hydrolysis, are significantly enhanced (affected) by the interaction with MT. However the mechanism for the enhancement of the catalytic functions by the interaction with MT is still unknown, because there is no available X-ray crystal structure for the kinesin-MT complex. To address this problem, using the recently solved crystal structure of nucleotide-free kinesin in solution (Makino et al.) and other structures, we first constructed kinesin-MT complex models under various nucleotide conditions based on rigid body fitting to the cryo-EM maps (Sindelar et al.). Then, we performed all-atom MD simulations to refine the initial models and investigated significantly altered regions by the interaction with MT. We expect the structurally affected regions having considerable relation with the mechanism of the enhancement. We compare the refined structures of various nucleotide states and try to find out the functionally important regions related to ADP release and MT-binding. We will report the detailed results in the meeting.

1M1412 フォトクロミック分子をキネシン特有のループ L5 に導入したキネシンの光制御