博士論文（要約）

Coordinated Catalysis and Walking Mechanism of Molecular Motor Kinesin

（分子モーターキネシンの頭部間協調性の仕組み）

新谷 大和

1. Introduction

Inside a cell, there are 10 nm-scale biological molecular machines called molecular motor protein that are essential agents of movement in living organisms. These proteins transform chemical energy obtained from ATP hydrolysis into mechanical work to fulfill their functions; rotary motors are involved in ATP synthesis or ion pumping, linear motors are involved in cargo transport or cell division and a certain motor protein also play a role on muscle contraction. Unlike currently available artificial machines, these molecular machines have high energy conversion efficiency and ably use thermal energy to generate unidirectionality. Therefore, understanding of these motor proteins is expected to give us a guideline for designing nanomachines that will work in an environment where the fluctuations due to thermal noise are significant. In this study, I tried to investigate the mechanism how motor protein works by studying kinesin motor protein which is related to cellar transport.

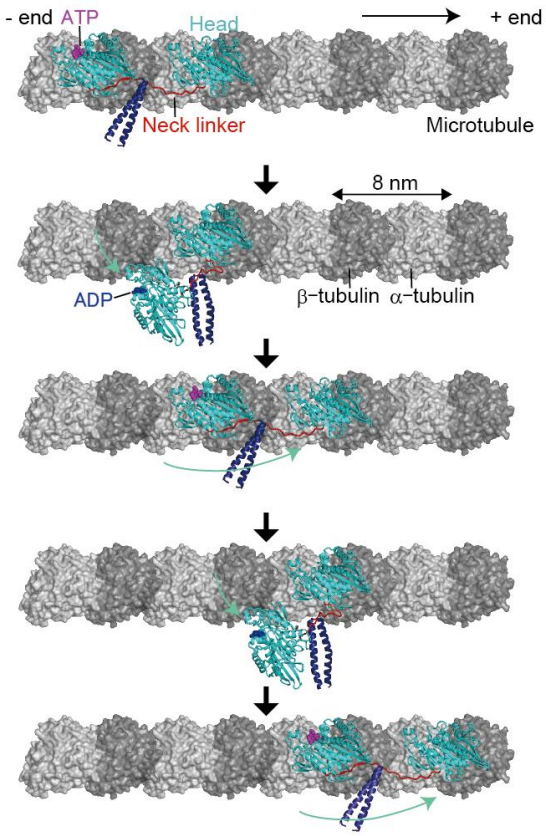

Fig.1 Model of kinesin movement

Kinesin-1 is a protein that transports cellar cargos along the rail called microtubule toward microtubule plus end hydrolyzing ATP. Kinesin moves in a hand-over -hand manner where it alternately moves two microtubule binding domains called “head” [Yildiz 2004](Fig. 1). During this bipedal movement, kinesin is known to take more than 100 steps without dissociation from microtubule with few backward steps [Block 1990][Svoboda 1993].

The mechanism how kinesin achieves processive bipedal movement is still unclear. This processive movement requires coordination of two heads. Because kinesin’s two heads are identical, the only element that can regulate two-head-coordination is the neck linker. Several previous studies actually demonstrated that orientation and tension in the neck linker have essential role for regulation of kinesin’s movement [Uemura 2003][Yildiz 2008], although which step in kinesin’s moving cycle is regulated by the neck linker is still unclear. In this study, I examine the effect of the neck linker orientation and strain on ATP hydrolysis reactions and also investigated the communication mechanism between the neck linker and ATP binding site. In order to understand these mechanisms, I carried out following three experiments and

understand the mechanism of kinesin's coordinated movement.

2. Experiments and results

2.1 Single molecule FRET observation of fluorescent ATP binding

During kinesin's bipedal movement kinesin alternately takes one-head-bound and two-head-bound state [Mori 2007]. In the two-head-bound state, trailing head should detach from microtubule prior to the leading head to prevent backward step. Two models have been proposed to explain the mechanism of preferential detachment of trailing head. One is front-head-gating model where the backward tension posed through the neck linker of the leading head suppresses ATP binding to the head. The other model is rear-head-gating model where the forward tension posed through the neck linker of the trailing head promotes ATP hydrolysis in the head. The difference between these models is whether ATP binds to the leading head. To distinguish these models, I directly observed ATP binding to the leading head using single molecule FRET and fluorescent ATP analog (Fig. 2(A)).

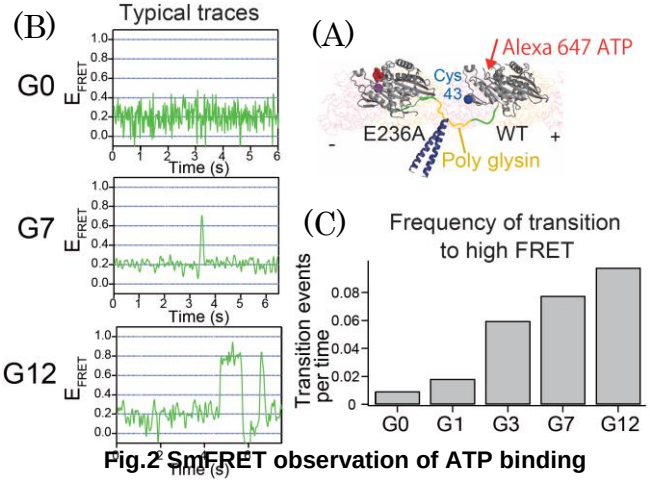

Fig.2 SmFRET observation of ATP binding

As a result, rarely observed ATP binding to the leading head in the presence of 200 nM fluorescent ATP. However, when the backward neck linker tension was reduced by insertion of poly-glysine between the neck linker and coild coil, the frequency of ATP binding to the leading head increased (Fig2. (B), (C)). These results indicate that Backward neck linker tension suppresses ATP binding to the kinesin head, although temporal resolution of FRET observation (20 ms) is relatively low compared to the kinesin's ATP off rate measured with stopped flow instrument (150 s^{-1}) [Ma 1997], indicating most ATP

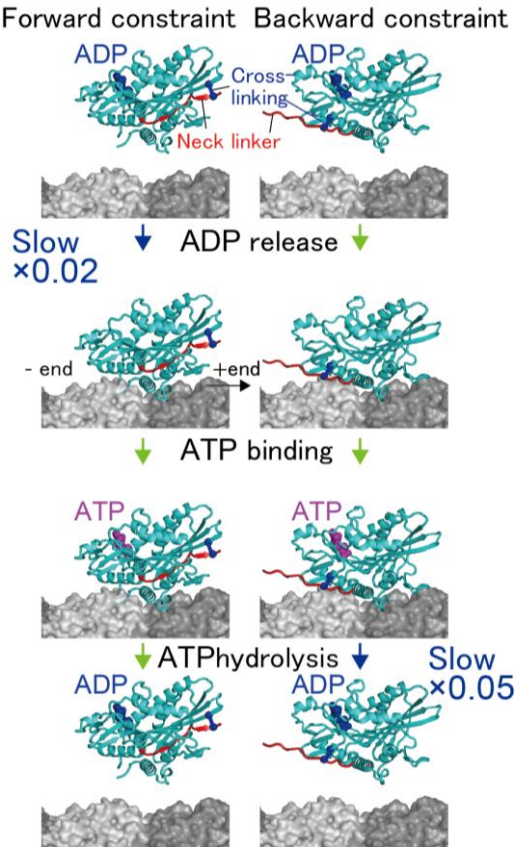

Fig.3 ATPase cycle of neck linker constraint mutants

binding events could not be observed.

2.2 Biochemical measurements using disulfide cross-linking and stopped flow instrument

Kinesin's ATP hydrolysis cycle in the head can be separated to two general steps; binding of the head to the microtubule coupled with ADP release and dissociation of the head from the microtubule coupled with ATP hydrolysis and following phosphate release. Rate constants of each step of monomeric and dimeric constructs in ATP hydrolysis cycle has been measured using stopped flow instrument, but the effect of neck linker tension on the each rate constant is unknown [Cross 2003]. In this experiments, I emimicked forward and backward neck linker tension by using disulfide cross-linking between two cysteine residues inserted in the neck linker and the head. Then, I measured the rate constants of neck linker constraint mutants with stopped flow instrument.

As a result, when the neck linker was constraint in the forward extended conformation, rate constants in the microtubule binding step were significantly decreased (Fig.3). On the other hand, when the neck linker was constraint in the backward extended conformation, rate constant of ATP induced microtubule dissociation was decreased. The results showed that ATP hydrolysis cycle is differently regulated depending on the direction of the neck linker tension, explaining coordination of the two head of walking kinesin.

3.1 Observation of subdomein rotation using gold nanorod

Above results showed that neck linker tension regulates ATP hydrolysis reaction in the head. However, the distance between the root of the neck linker and ATP binding pocket is about 2.5 nm, there should be some mechanism of communication between the neck linker and ATP binding pocket. Comparison of crystal structure between nucleotide-free state and ADP-AlF_4 state showed that kinesin's subdomein undergoes ~25 degree rotation up on ATP isomerization [Cao 2014] , suggesting that ATP-bound and -unbound states can be distinguished by monitoring the orientation of the head. Here I applied high-speed gold nanorod imaging to visualize the rotational motion of the kinesin head. The gold nanorod (60 nm × 25 nm) was attached to one of the heads at a fixed angle by introducing two reactive cysteines into the head and labeling them via avidin-biotin interactions (Fig. 4). Then the gold nanorod attached to a head of kinesin moving along microtubule was observed using dark-field microscopy with dot mirror at 100 μs temporal resolution, and the centroid

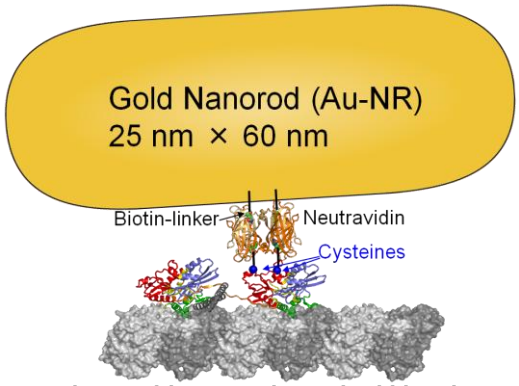

Fig.4 Gold nanorod attached kinesin

position and the angles in x-y and x-z planes (x-axis corresponds to the microtubule long axis) of the nanorod were determined by fitting the dark-field scattering image to simulated point spread function.

The observations showed that the angle abruptly changed while the labeled head binds to microtubule and then the angle reversed after the head unbound and displaced 16 nm toward plus end of the microtubule (after switching to the leading position). In the presence of saturating ATP, dwell times of pre- and post-rotation state were almost the same indicating that leading head mainly takes pre-rotation state and trailing head mainly takes post rotation state. Next, to examine the effect of the neck linker tension on subdomain rotation, I observed subdomain rotation of neck linker extended mutant. The result showed that the angle of the nanorod changed more than once during one bound state of the nanorod attached head. The result indicates that subdomain rotation is regulated by the neck linker tension.

4. Summary

In this study, to understand the mechanism of the neck linker tension based regulation of ATP hydrolysis reaction, I carried out the three experiments. Because backward tension suppresses subdomain rotation and ATP hydrolysis, detachment of the leading head from microtubule is also suppressed. In the trailing head, forward neck linker tension stabilizes subdomain rotation so that ATP dissociation is suppressed and hydrolysis is promoted, resulting in preferential detachment from microtubule. In this way, the tension dependent regulation of the subdomain rotation and catalytic activity explains the mechanism of kinesin's coordinated movement.

5. Reference

[Yildiz 2004] A. Yildiz, M. Tomishige, R. D. Vale and P. R. Selvin, Sience 303, 676-678, 2004.

[Block 1990]Block, S.M., Goldstein, L.S.B., and Schnapp, B.J., Nature 348: 348-352 (1990).

[Svoboda 1993] K. Svoboda, C. F. Schmidt, B. J. Schnapp and S. M. Block, Nature 365, 721-727, 1993.

[Uemura 2003] S. Uemura and S. Ishiwata, Nat. Struct. Mol. Biol. 10, 308-311, 2003.

[Yildiz 2008] A. Yildiz, M. Tomishige, A. Gennerich and R. D. Vale, Cell 134, 1030-1041, 2008

[Mori 2007] Mori, R. D. Vale and M. Tomishige, Nature 450, 750-754, 2007.

[Ma 1997] . Z. Ma and E. W. Taylor, J. Biol, Chem. 272, 717-723, 1997.

[Cross 2003] R. A. Cross, TRENDS in Biochem. Sci. 29, 301-309, 2004.

[Cao 2014] L. Cao, W. Wang, Q. Jiang, C. Wang, M. Knossow and B. Gigant, Nat. commun. 6364, 5364, 2014.