Abstract

論 文 の 内 容 の 要 旨

論文題目　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）

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1. Introduction

Inside a cell, there are 10 nm-scale biological molecular machines called molecular motor protein. These proteins transform chemical energy obtained from ATP hydrolysis into mechanical work to fulfill their functions. Unlike artificial machines, these molecular machines have high energy conversion efficiency and ably use thermal fluctuation to generate unidirectionality. Therefor, understanding of these motor proteins expected to give us a guideline for designing nanomachines. To understand the mechanism how motor protein works, I'm studing "kinesin" which is related to cellar transport.

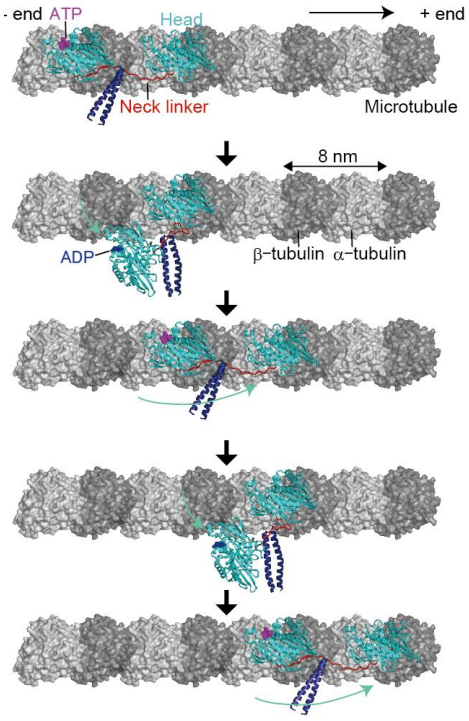

Fig.1 Model of kinesin movement

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, *Science*, 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, *Nature*, 1990][Svoboda, *Cell*, 1994].

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. The neck linker, which connects two heads, is pulled backward at the leading head and forward at the trailing head. Therefore, we hypothesized that the direction of the tension through the neck linker regulates kinesin's ATP hydrolysis cycle coupled with microtubule binding and dissociation. In this study, we will verify the above model and elucidation the mechanism of kinesin's unidirectional movement. To verify the model, we carried out following 3 experiments.

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, *Nature*, 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, we directly observed ATP binding to the leading head using single molecule FRET and fluorescent ATP analog (Fig. 2(A)).

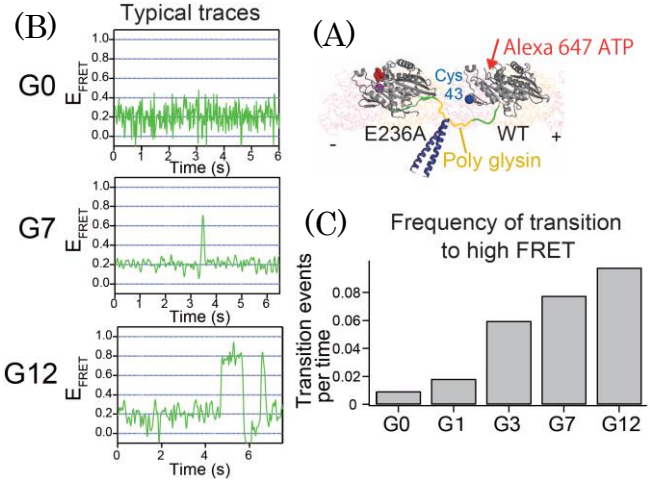

Fig.2 SmFRET observation of ATP binding

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, *J.B.C.*, 1997], indicating most ATP 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, *TRENDs in Biochem . Sci.*, 2003]. In this experiments, we mimicked forward and backward neck linker tension by using disulfide cross-linking between two cysteine residues inserted in the neck linker and the head. Then, we measured the rate constants of neck linker constraint mutants with stopped flow instrument.

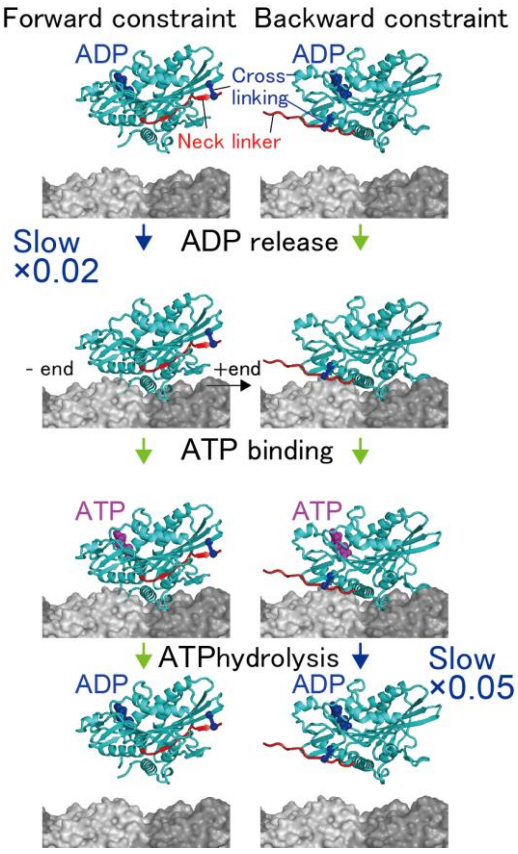

Fig.3 ATPase cycle of neck linker constraint mutants

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

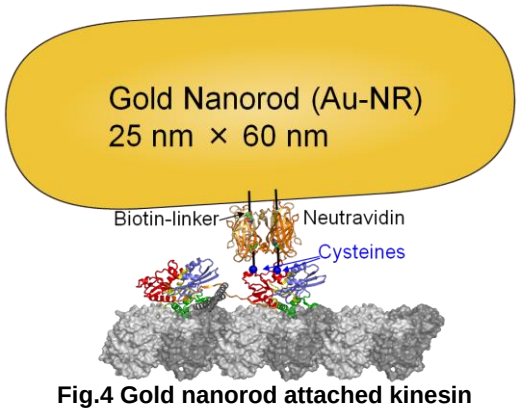

Fig.4 Gold nanorod attached kinesin

showed that kinesin's subdomein undergoes ~25 degree rotation up on ATP isomerization [Cao ,*Nat. commn.*, 2014] , suggesting that ATP-bound and -unbound states can be distinguished by monitoring the orientation of the head. Here we applied high-speed gold nanorod imaging to visualize the rotational motion of the kinesin head. 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 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 neck linker tension on subdomain rotation, we observed subdomain rotation of neck linker extended mutant. The result showed that the angle of nanorod changed more than once during one bound state of nanorod attached head. The result indicates that subdomain rotation is regulated by the neck linker tension.

4. Summary

In this study, we investigated the effect of the neck linker tension on ATP hydrolysis cycle and subdomain rotation. 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, kinesin's coordinated movement is regulated by the direction of the neck linker tension.