- 2P156 一分子 FRET 法によるキネシン二量体の ATP 結合待ち構造の解析
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Configuration of kinesin dimer during ATP-waiting state probed by single molecule FRET
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Kinesin, a dimeric motor protein with two motor domains (heads), has been shown to move along microtubules in a hand-over-hand manner. This mechanism shows that kinesin moves processively by taking two-head-bound intermediate and by exchanging two heads every 8 nm steps. However, the conformational state between steps is not well understood, and it is controversial whether kinesin takes one- or two-head-bound intermediate while it waits for binding of new ATP. To distinguish these two possibilities, we observed single molecule fluorescence resonance energy transfer (FRET) efficiencies between dyes attached on two kinesin heads. Two reactive cysteines were introduced into the front of one head and the rear of another head in a heterodimeric kinesin, and were labelled with Cy3/Cy5 dyes. In the presence of AMP-PNP (ATP-state), FRET efficiency showed bimodal distribution with peaks at 0 and 90% (two-head-bound). In the absence of ATP (with small amount of ADP) or for mutant heterodimer that can bind to microtubule only with one head, FRET efficiency distribution showed distinct peak around 30% (one-head-bound). When we observed FRET efficiency changes for kinesin moving processively under low ATP concentrations, where ATP binding is the rate limiting, we found that kinesin spent most of the time in 30% FRET state and briefly in 0 and 90% FRET states. These results support the idea that kinesin waits ATP-binding in a one-head-bound configuration.

- 2P158 2 分子を連結させたキネシン複合体の協調的運動の観察
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Coordinated processive movement of cross-linked two kinesin dimers
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Kinesin is a motor protein that hydrolyses ATP and moves processively toward plus-end of the microtubules. Single kinesin dimer has been shown to travel about 1 μm before detaching from the microtubule in vitro. Inside the cells, multiple kinesins and dyneins are thought to be attached to a cargo and might coordinate to execute efficient and regulated intracellular transport of the cargo. However, the mechanism for such coordination between cargo-transporting motors is not well understood and especially it is still unknown how two kinesin molecules attached to a cargo coordinate. To investigate the coordination mechanism between two motor proteins, we developed an in vitro motility assay to observe the movement of a complex composed of crosslinked two-kinesin dimers and we compared its motility to that of single kinesin dimers. Two kinesin dimers, separately labeled with Cy3 and Cy5, were specifically crosslinked using rapamycin-FK506 binding protein (FKBP) crosslinking system. By observing Cy3/Cy5 fluorescence simultaneously using total internal reflection fluorescence microscopy, we identified crosslinked "two-kinesin" molecules (about 10% of the fluorescent spots). The crosslinked two-kinesin complex showed similar velocity and slightly increased (1.5 fold) run length compared to those of single kinesin dimers. These results suggest that crosslinked two kinesin molecules might coordinate somehow not to interfere with the motility of each other.

- 2P157 単頭キネシンによる微小管の並進・回転運動機構の研究
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1pB08 Microtubule sliding and rotation driven by multiple single-headed rat kinesins
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Kinesin molecular motors are engines for long-haul transport of cellular components along microtubules. They work by converting chemical transitions into mechanical motion. Current models for their mechano-chemistry propose that mechanical force originates either in a directionally-biased selection of microtubule binding sites, or in a directional conformational change that follows microtubule binding. Here, we explore these competing proposals. To do this, we engineered kinesin-1 monomers that could be surface-linked via their N-termini, leaving the C-terminal neck linker dangling. We showed that N-linked kinesin heads drove microtubule sliding at similar rates to wild-type C-linked heads, even though their neck linker was disconnected so that it could not exert useful force. Neck-linker docking nonetheless remained essential for function: mutations that disrupted neck linker function profoundly inhibited microtubule sliding, super-activate the kinesin ATPase, and caused futile cycles of ATP turnover for N-linked heads as well as for C-linked heads. We also discovered that C-linked heads drove not only on-axis microtubule sliding, but also anticlockwise rotation of each microtubule around its axis. We can account for these behaviors on the simple assumption that kinesin heads select the closest tubulin dimer in the lattice, but only provided that the binding event produces a conformational change of the newly-attached head that shifts its N and C termini and/or that biases its subsequent detachment position towards the plus end.

- 2P159 3 次元光ピンセットによるバネ定数の計測
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Measurement of three-dimensional trap stiffness in optical tweezers
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Measurement of three-dimensional (3D) force generated by a single molecular motor is essential to understand its working mechanism because conformational changes of proteins which are related to the mechanical function and the efficiency of energy conversion could occur in any direction. To establish the system which enables measurements of forces along X-, Y- and Z-axis simultaneously under an optical microscope, 3D tracking method (Mizutani *et al.*, 44th Annual Meeting of the Biophysical Society of Japan, 2006) was applied to custom-made optical tweezers ($\lambda = 1.064 \text{ nm}$). To estimate 3D trap stiffness, the distributions ($\sim 200\text{-}500 \text{ nm}$) of trapped single beads ($\phi = 1 \mu\text{m}$) under thermal Brownian motion in 3D directions at low laser power was analyzed. The stiffness in XY-plane was 1.1 and 1.2 pN/nm/W, for NA 1.3 (plan flour Ph, Nikon) and 1.49 objectives (TIRF, Nikon), respectively, and increased by $\sim 10\%$ in the direction parallel to the polarization of the laser. In contrast, the stiffness along Z decreased by $\sim 80\%$, 0.2 pN/nm/W, for both NA 1.3 and 1.49 objectives and was almost constant within $2.5\mu\text{m}$ above the coverglass surface. Using the optical tweezers in which 3D force was calibrated in detail, we are in the process of examining 3D forces of single motor proteins such as kinesin-1.