2P160 再構成キネシン繊維上での微小管滑り運動の方向と速度
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Direction and speed of microtubule moving along kinesin motors arranged on catchin thick filaments
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In the conventional *in vitro* motility assay, kinesin molecules were usually attached to glass surfaces, but their orientation was random. In this study, we have arranged the motor domains of kinesin by the use of the assembly property of a myosin rod-like protein, catchin (Yamada et al., *JMB*, 2000), obtained from the mussel. We prepared chimera proteins in which kinesin motor domain is fused to the C-terminal 829 amino acid residues of catchin and attached them onto the surface of catchin filaments. We observed movement of microtubules on these filaments by the *in vitro* motility assay. When the chimera protein contained the hinge 1 domain of kinesin, microtubules moved along the filaments in both directions toward and away from their center at the same speed (Taba et al., *Seibutsubutsuri* 43, S139, 2003). However, the chimera proteins lacking the whole hinge 1 and the C-terminal part of the neck helix moved microtubules toward the center of the filaments slightly but significantly faster than in the opposite direction. This fact suggests that the hinge 1 domain has a mechanical flexibility enabling interactions between kinesin motor domain and microtubules at various orientations. Furthermore, the difference in the movement speeds in the two directions, which is much smaller than that observed with actin filaments moving on myosin filaments (Sellers & Kachar, *Science*, 1990; Yamada *et al.*, *JB*, 1990), suggests that a substantial torsional flexibility resides also in the catalytic core and/or neck linker domains.

2P162 フォトクロミック分子を利用したキネシン ATPase 活性の光制御
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Photo-control of kinesin ATPase using photochromic compound.
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Azobenzene is a photochromic molecule that undergoes rapid and reversible isomerization between the *cis* and *trans* forms by ultraviolet (UV) and visible (VIS) light irradiations, respectively. Here, we introduced the SH-reactive azobenzene derivative 4-phenylmaleiminy (PAM) into the functional region of kinesin to reversibly regulate the ATPase activity of kinesin by photoirradiation. The 2 kinesin motor-domain mutants L249C and S275C, which contain a single reactive cysteine residue in the loops L11 and L12, respectively, were prepared. The loops were considered key region for the functioning of kinesin as the motor protein. PAM was stoichiometrically incorporated into the cysteine residue in the loop of the mutants. The PAM-modified S275C mutant exhibited reversible alteration in ATPase activity accompanied by *cis-trans* isomerization upon UV and VIS light irradiation. The ATPase activity exhibited by the *cis* isomer of the PAM bound to the mutant was 2 times higher than that of the *trans* isomer. Also, the PAM-modified L249C mutant exhibited reversible alterations in ATPase activity on UV-VIS light irradiation but produced the opposite effect on UV and VIS light irradiation. We have demonstrated that the ATPase activity of the motor protein kinesin is photoregulated using a photochromic molecule of an azobenzene derivative.

2P161 キネシンによる微小管の動的自己組織化 ―ATP 駆動型アクチュエーターを目指して―
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Dynamic self-assembly of microtubule complex by kinesin -toward ATP-fueled actuator-
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Establishment of *in vitro* motility assay has enabled us to elucidate physical properties of motor proteins. Based on this technique, a lot of applications such as combination with micro-fabricated surface, were reported. However, limited attempts have been carried out to integrate the motion of motor proteins. In this study, kinesin-microtubule system was employed to produce motile assemblies with highly ordered structure, which leads to ATP-fueled actuator. Kinesin is a protein runs along the rail of microtubule in eukaryotic cells. Microtubule has hollow cylindrical structure in diameter of 25nm. *In vitro*, microtubules show a sliding motion on the kinesin-fixed surface by coupling ATP hydrolysis. Here, biotin-labeled microtubules were driven in the same way, and cross-linked by streptavidin during sliding motion. After several hours running of this system, giant assembly of microtubule were observed. Linear assembly with a length of more than 100 μ m moved as fast as single microtubules. Ring shaped assembly were also observed, with diameters ranging from several to several tens micrometers. This ring shaped assembly kept rotation for at least 24h. Thus, using the motion of microtubule to form assembly, we have successfully fabricated the various shape and size of the assembly in this motility system.

2P163 光制御を目的とした二架反応性フォトクロミック分子を用いた分子モーターキネシンの分子間架橋
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Cross linking of kinesin using bifunctional photochromic compounds results in photo-regulation of ATPase activity
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As a photochromic compound changes its shape and property reversibly by UV/VIS light irradiation, it is expected that activity of the dimer kinesin cross-linked with bifunctional photochromic compounds could be photo-controlled by irradiation due to alternation of the steric coordination between the two kinesin motor domains. In the present study, we have crosslinked the kinesin using bifunctional thiol reactive photochromic compound, 4,4'-azobenzene-dimaleimide (ABDM) and tried to photo-control the ATPase activity. The five kinesin mutants A247C, T242C, A244C, L249C and A252C which have a single cysteine residue have been prepared. The mutants were cross-linked by ABDM at high efficiency 70-90%. The microtubules dependent ATPase activity of the cross linked kinesin mutants A247C, T242C, A244C, L249C did not change on alternate UV-VIS light irradiation. On the other hand, A252C cross-linked by ABDM showed significant alteration of ATPase activity between UV and VIS light irradiation.