2P179 N 末端領域変異単頭キネシンによる微小管のコークスクリュー運動

Corkscrew motion of a sliding microtubule driven by single-headed kinesins with mutations in N-terminal region.

Sinsuke Owada¹, Junichiro Yajima², Tomoko Masaie¹, Ayako Tatsuguchi¹, Takayuki Nishizaka¹,
(¹Dept Physics, Gakushuin Univ. ²Dept. of Life Sciences, Grad. School of Arts and Sciences, The University of Tokyo)

Conventional kinesin-1 is a processive molecular motor and tracks the protofilament structure of a microtubule (termed as MT) in a hand-over-hand fashion. A recent contribution of our group shows that non-processive single-headed kinesin and Eg5 do not follow the protofilament pitch: instead, they rotate MT with a pitch of $\sim 0.3 \mu\text{m}$ in motility assays (Yajima et al. Nat. Struct. Mol. Biol. 2008). Although the short-pitch rotations generated by the non-processive motors are markedly similar, there are no clues about structural basis which determines the $\sim 0.3 \mu\text{m}$ value. To address the question which part correlates the rotational motion, we quantify MT-corkscrew driven by single-headed kinesins with a mutation in its N-terminal region, which may form the cover-neck-bundle structure with the C-terminal neck linker (Hwang et al. Structure.2008), through tracking single quantum dots (QD) specifically attached to the surface of MT. QDs always showed sinusoidal oscillation, with $\sim 0.03 \mu\text{m}$ in amplitude in 2-D tracking, as a projection of 3-D left-handed corkscrew; pitch is obtained from rotational periodicity of a fit to oscillation. Average sliding velocities of WT and I9A mutant were estimated as 0.08 and $0.09 \mu\text{m/s}$, respectively. Notably, the pitch decreased with I9A mutation; $0.24 \mu\text{m}$ (86% of WT). This result suggests that MT corkscrewing motion will be modulated by N-terminal region in kinesin head. We are additionally testing the effects of other mutations in N-terminal region and will discuss their correlations to rotation mechanism.

2P180 F₁-ATPase に結合した蛍光性ヌクレオチドを高時間分解能で画像化する

High-speed imaging of single fluorescently labeled nucleotides bound to F₁-ATPase

Yu Hashimoto, Kengo Adachi, Tomoko Masaie, Takayuki Nishizaka, (Dept Physics, Gakushuin Univ)

F₁-ATPase is the sector of F₀F₁-ATP synthase that has catalytic sites responsible for the ATP synthesis reaction. An isolated $\alpha_3\beta_3\gamma$ subcomplex of F₁ is the world's smallest rotary molecular motor; its chemomechanical coupling has ultimately been scrutinized through a series of single-molecule studies (Nishizaka et al., 2004; Adachi et al., 2007). To unravel hidden chemical intermediate state(s) in each dwelling angle of the shaft, we improve time resolution of the measurement system to detect single fluorescently labeled nucleotides under an optical microscope. In our previous reports, the collection time has been typically 33 milliseconds (ms) under TIRF illumination using 60x objectives with 1.45 numerical aperture (NA). Here we simply increase the laser power, decrease the nucleotide concentration and replace the objective with 100x one with 1.49 NA. A collection time successfully reaches 4 ms to visualize single Cy3-EDA-ATP molecules bound to surface-immobilized $\alpha_3\beta_3\gamma$. Although validation of the improved set-up for direct observation of the chemomechanical coupling is still needed, this high-speed approach can be useful to monitor all intermediate states in single turnovers of F₁ with fluorescently labeled nucleotide and phosphate. Our final goal is to dissect characteristic behaviors of the $\alpha_3\beta_3\gamma(\Delta A275)$ mutant that shows nine dwell angles over one revolution (Hirakawa et al., 46th annual meeting, 2008).

2P181 新規イネキネシンのトリプトファンと蛍光 ATP アナログ間の FRET を利用した速度論的解析

Kinetic studies on the novel rice kinesin K23 utilizing FRET between the intrinsic tryptophan residue and fluorescent ATP analogue.

Nozomi Umez¹, Nobue Hanzawa², Kazunori Kondo², Toshiaki Mitsui³, Shinsaku Maruta^{1,2},
(¹Division of Bioinformatics, Graduate School of Engineering, Soka University; ²Department of Bioinformatics, Faculty of Engineering, Soka University; ³Graduate School of Science and Technology, Niigata University)

Kinesin is a motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesin converts chemical energy from ATP into mechanical force. Kinesin family is classified into some subfamilies. Some species of kinesin derived from vertebrate have been well studied on their characterization. However, not so many studies for kinesins of plants have been done yet. In this study, we have focused on rice kinesin K23. In the phylogenetic tree, K23 belongs to the kinesin-7 subfamily. K23 motor domain was expressed in *E. coli* BL21(DE3) and purified by Co-NTA column. K23 showed about 60

times lower microtubule dependent ATPase activity than that of conventional kinesins while its affinity for microtubule is similar. For the kinetic characterization of K23, we utilized fluorescent ATP analogue Mant-ATP. Mant-ATP did not alter its fluorescence intensity significantly during hydrolysis. However, we have successfully observed significant FRET between Mant-ATP and intrinsic tryptophan in K23. K23 has 3 intrinsic tryptophans (W21, W145, W165) in its motor domain. The K23 mutant W145F/W165F showed almost similar FRET efficiency to that of wild type of kinesin. Therefore, the FRET between K23 and Mant-ATP is due to W21 predominantly. Furthermore, we succeeded in kinetics characterization of KIF5A motor domain by W-Mant FRET. The KIF5A motor domain has point mutation of L25W. The W-Mant FRET technique may be available for other kinesin.

2P182 植物微小管の調製とイネキネシンとの相互作用

Preparation of microtubules derived from plant and its interaction with rice plant kinesin.

Seigo Iwata¹, Shinsaku Maruta^{1,2}, Kazunori Kondo², (¹Division of Bioinformatics, Graduate school of engineering, Soka University; ²Department of Bioinformatics, Faculty of engineering, Soka University)

Previously, we have expressed novel rice plant specific kinesins and studied their biochemical characterization. The plant kinesins showed very unique properties. Especially ATPase activities of the kinesins were relatively much lower than that of conventional kinesin. Moreover, the kinesins did not show motility activity on the microtubules prepared from porcine brain. Although the structure of tubulin is well conserved, it is demonstrated that the plant tubulin has different characteristics from tubulin derived from animal. Therefore, plant kinesin may be more compatible with plant tubulin than animal tubulin

In this study, we prepared the plant tubulin from the pollen of *Pear* and *Ginkgo biloba*. The tubulin was extracted from the acetone powder of pollen. Subsequently, the tubulin was purified with DEAE Sephadex A-50 chromatography, Sephacryl S-300 gel filtration, and Mono Q anion exchange chromatography. Polymerization of the purified plant tubulin to microtubule was monitored by measuring the increase of absorption at 350 nm.

Negative staining electron microscopic analysis of the assembled tubulin revealed the microtubule configuration. In preliminary experiments, the ATPase activity of rice plant kinesin K16 was activated by plant tubulin more significantly than animal tubulin. These results suggested that plant kinesin we have prepared may show motility activity on the plant microtubule.

2P183 光ピンセット法を用いたミオシンVのブラウニアンサーチの可視化と力学特性の解明

Visualizing and clarifying the mechanical properties of the Myosin V Brownian search with optical tweezers

Keisuke Fujita¹, Mitsuhiro Iwaki², Atsuko Iwane¹, Toshio Yanagida^{1,2}, (¹Graduate School of Frontier Bioscience, Osaka Univ.; ²Graduate School of Medicine, Osaka Univ.)

Myosin V moves processively along actin filaments by taking 36-nm steps in a hand-over-hand manner. These steps involve the rear head quickly releasing from the actin filament and then taking a 72-nm forward movement before rebinding. The 72-nm movement consists of two processes: a conformational change, called the "lever arm swing", and a diffusional process of the head, called the "Brownian search". The Brownian search is a short-lived state, only recently observed by gold nano particle imaging with high time resolution. It is thought that the Brownian search is important for the released head to find its next actin binding site and for the high energy efficiency of force generation. However, little is known about its mechanical properties. In particular, there are only limited reports describing the interaction between the head and actin during the Brownian search.

Here, we constructed a new optical tweezers system that incorporates a DNA linker to the myosin V based on a previous report (Guydosh et al, Nature 2009). In this experimental setup, the optical tweezers apply an external force to the Brownian search via the DNA linker. By using this measurement system, we succeeded to observe the Brownian search under a controlled load, finding that its behavior changes according to load. These results suggest that our unique system is capable of clarifying the mechanical properties of the Brownian search. At the meeting, we will discuss these mechanical properties and their implications on the acto-myosin V interaction.

2P184 F₁-ATPase 中で 1 個もしくは 2 個の β サブユニットが発生するトルク

Torque generated by one or two β subunit(s) in F₁-ATPase

Ei-ichiro Saita, Masasuke Yoshida, (ICORP ATP synthesis regulation project, JST)

F₁-ATPase (F₁), its minimum subunit composition as a rotary motor is $\alpha_3\beta_3\gamma$, hydrolyzes ATP accompanied by rotation of γ subunit. Catalytic site is mainly