3PS001 IC138 リン酸化によるクラミドモナスダイニン-f テールの形態変化 Configuration changes of *Chlamydomonas* dynein-f tail coupled with IC138 phosphorylation

Hitoshi Sakakibara, Kazuhiro Oiwa, Hiroaki Kojima (KARC, Nat. Inst. Inf. Com. Tech.)

Inner-arm dynein-f of *Chlamydomonas* is a hetero-dimeric dynein which has two heavy chains (α and β), three intermediate chains and light chains on the tail. It has been shown that an axonemal casein kinase I phosphorylates the 138K intermediate chain (IC138) and that this phosphorylation is involved in regulation of flagellar activity. To elucidate the regulation mechanisms of dynein-f functions through the IC138 phosphorylation, we examined molecular configuration changes of dynein-f coupled with IC138 phosphorylation by negative-staining EM and following single-particle analyses. For the analyses, we picked up a dynein-f density map from the tomogram of axoneme, prepared projections of the map from various angles, and used tail parts of the projections as the templates for alignments. First, we found that the distribution of the two heads' positions spread and distance between the α and β heads became closer coupled with IC138 phosphorylation. Second, in ~10% classes, we found that the tail showed forked structure which consisted of thick stem and the thin branch linking to the beta head. This thin branch became smearing in phosphorylation conditions, suggesting that the branch positioning became unstable. From these observations, we suppose that the IC138 phosphorylation could first affect the thin branch positioning, then the β -head position should be shifted. In axonemes, position shift of the β head could affect the interactions of dynein-f and adjacent microtubule to change their operation mode.

3PS002 鞭毛・繊毛運動の研究に向けたヒト軸系ダイニンの発現系の開発 Expression and purification of human axonemal dynein for studying cilia/flagella beating

Akane Furuta, Ken'ya Furuta, Misako Amino, Hiroaki Kojima (Bio ICT lab, NICT)

Motile cilia and flagella are crucial component for wide variety of cells. Previous studies have shown that the mutation in axonemal dyneins often impairs beating of cilia, leading to serious diseases known as primary ciliary dyskinesia. Normal axonemal dyneins in the cilia and flagella generate force that is highly regulated in time and space to generate regular waveform. Given the timescale of the regulation which is much shorter than that of signal transductions, it is natural to expect that individual dynein molecules act as a mechanical sensor for external loads, forming feedback loop to generate the oscillations. However, how dynein responds to external loads is unclear. To address this problem, it is critical to measure the force of both normal and mutated axonemal dyneins upon an applied external load. These assays require specific probes and mutations at desired sites on the axonemal dynein. A limiting issue in the study of axonemal dynein is that the recombinant proteins are not available so far. Here we used a transient expression system in HEK293 cells to express human recombinant axonemal dyneins. We isolated full-length and truncated human inner arm dyneins (DNAH2 and DNAH7) in a soluble form. Currently, we are working with functional analysis of these recombinant axonemal dyneins.

3PS003 Tubulin polyglutamylation regulates axonemal motility by modulating the function of a specific inner-arm dynein species

Tomohiro Kubo¹, Toshiki Yagi², Ritsu Kamiya¹ (¹Department of Biological Sciences, Graduate School of Science, University of Tokyo, ²Department of Cell Biology and Anatomy, Graduate School of Medicine, University of Tokyo)

Tubulin polyglutamylation is a post-translational modification that affects various microtubule-dependent cellular functions. The tpg1 mutant of *Chlamydomonas* lacks a homologue of mouse TTLL9 (a tubulin polyglutamylase) and has a reduced level of polyglutamylated alpha tubulin in the outer-doublet B-tubule. It displays lowered flagellar motility because of deficient interaction between inner-arm dynein and doublet microtubules (Kubo et al. Curr. Biol. 20, 441-445, 2010). *Chlamydomonas* has seven major species of inner-arm dyneins, designated dynein a-g. Dynein f (dynein II) is a two-headed dynein containing two heavy chains, while all others are one-headed. In the present study, we produced double mutants of tpg1 and various inner-arm-deficient mutants, and found that the tpg1 mutation did not affect the lowered motility of mutants lacking inner-arm dynein e, while it greatly reduced the motility of mutants lacking dyneins other than dynein e. This observation

suggests that tubulin glutamylation most sensitively affects the function of dynein e. The exceptionally basic microtubule binding site (the stalk tip) of dynein e may account for its high sensitivity to the tpg1 mutation, which should greatly change the microtubule surface charge.

3PS004 高速 AFM による細胞質ダイニンの機能動態の観察 High-Speed-AFM Observation of Processive Movement of Cytoplasmic Dynein

Shuji Fujita¹, Karunakaran Aathi², Uchihashi Takayuki^{1,3}, Vale Ronald D.², Toshio Ando^{1,3} (¹College of Science and Engineering, Kanazawa University, ²Department of Cellular & Molecular Pharmacology, University of California, San Francisco, ³Bio-AFM Frontier Research Center, College of Science and Engineering, Kanazawa University)

Cytoplasmic dynein is a microtubule-based motor protein which transports a cargos toward microtubule minus end. So far, several studies based on structural analysis and single molecule observations have been reported to reveal dynein's mechanisms¹⁻³. For examples, the structural analyses revealed the manners in which the mechanical elements interacts with the ATPase ring and a long-range allosteric communication takes place between the primary ATPase site and the microtubule-binding site. The single molecule analysis revealed the step-wise motions during the processive movement and what the stepping behavior varied as a function of interhead separation. However, there is no study by which direct observation of the conformational state of the functional dynamic because of a lack of technique. While we have recently succeeded in directly visualizing hand-over-hand movement of myosin V.⁴) This observation provided straightforward and detailed information on how myosin V moves along an actin filament. Here, we applied to high-speed AFM to observation of cytoplasmic dynein moving along microtubules. In this presentation, we will discuss what information about the dynein's mechanisms can be gained with high-speed AFM.

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- 2) M. A. DeWitt, A. Y. Chang, P. A. Combs, A. Yildiz, Science 335, 221-225 (2011).
- 3) T. Kon, T. Oyama et al., Nature 484, 345-350 (2012).
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3PS005 鞭毛軸系ダイニンによって駆動される微小管配列の振動運動 Oscillation of a microtubule array driven by axonemal dynein Susumu Aoyama, Yuichi Hiratsuka (Sch. Matl. Sci., JAIST)

Flagellar axoneme produces high speed oscillatory movements, which is essential for many eukaryotes to survive. Details of the mechanism of flagellar beating is not clearly elucidated, although some studies have shown that the minimal elements of oscillation is motor protein dynein and microtubules in an axoneme. To solve the mystery of flagellar movements, we tried to fabricate oscillatory units by arranging microtubules and dynein molecules with some accessory structures. In an axoneme, nine microtubules are arranged in the same polarity, and the minus ends of them are tightly bound with each other. We fabricated such microtubule arrays by polymerizing tubulin from polarity-arranged microtubules fixed on a glass surface. Polarity of microtubules was arranged by kinesin motility guided by a micro-track. A typical microtubule array was composed of five to fifteen microtubules. Next, we made microtubules on which axonemal dynein molecules were aligned, and incorporated them into microtubule arrays. In the microtubule array, dynein-bound microtubules interacted with polarity-arranged microtubules and displayed cyclical attachment and detachment. As a next step of this study, we are now trying to produce bending oscillation in our experimental system by loose cross-links between microtubules. We expect that the basis of flagellar beating can be produced in a simple microtubule-array system driven by dynein motors.

3PS006 Structural and Biochemical Properties of the Outer-Dynein-Arm Docking Complex (ODA-DC) in *Chlamydomonas* flagella Mikito Owa¹, Takahiro Ide¹, Ritsu Kamiya^{1,2}, Kenichi Wakabayashi¹ (¹Dept. Biological Sciences, Grad. school of Science, Univ. Tokyo, ²Dept. Life Science, Faculty of Science, Gakushuin Univ.)

Outer dynein arms in cilia and flagella are bound to specific loci on outer-doublet microtubules with a regular spacing of 24 nm. Their binding to the doublet microtubules is mediated by the outer dynein arm docking complex (ODA-DC) present at the base of the outer arm. In *Chlamydomonas*, the ODA-DC is composed of three subunits, DC1 (ODA3), DC2 (ODA1) and DC3

(ODA14). ODA-DC binds to specific loci on the outer-doublers of mutant axonemes that lack outer arm dynein, suggesting that it is responsible for the binding of outer arm dyneins to specific sites. To understand the basis for this crucial function of the ODA-DC, we examined the structural and biochemical properties of ODA-DC using both recombinant and native proteins. Low-angle rotary shadowing indicated that the recombinant ODA-DC has an oval shape, ~25 nm in length. The recombinant ODA-DC binds to cytoplasmic microtubules in an all-or-none manner, suggesting a cooperative binding event. Chemical crosslinking of axonemes from a mutant lacking outer-arms revealed that the native ODA-DCs associate with each other on the doublet microtubules. These data suggest that the periodic and cooperative binding of the ODA-DC molecules on the doublet microtubules is based on an end-to-end association of the ODA-DCs. Furthermore, chemical crosslinking also revealed that the ODA-DC is associated with three uncharacterized novel proteins on the axoneme. These proteins possibly specify the binding site of the ODA-DC on the outer doublet microtubules.

3PS007 クラミドモナス軸系微小管上の外腕ダイニンの組み立てに必要なタンパク質間相互作用の解析

Analysis of protein-protein interactions required for the assembly of outer arm dyneins on the axonemal microtubules in *Chlamydomonas*

Takahiro Ide¹, Mikito Owa¹, Kaoru Yoshida³, Manabu Yoshida², Ritsu Kamiya⁴, Ken-ichi Wakabayashi¹ (¹*Dept. Biological Sciences, Grad. School of Science, Univ. Tokyo*, ²*Misaki Marine Biological Station, Graduate School of Science, University of Tokyo*, ³*Biomedical Engineering Center, Toin University of Yokohama*, ⁴*Department of Life Science, Graduate School of Science, Gakushuin University*)

Outer arm dynein (OAD) in cilia and flagella are bound to specific loci on outer-doublet microtubules with a regular spacing of 24 nm. Previous studies showed that OAD has two sites for binding to microtubules: intermediate chain 1 (IC1) and the outer dynein arm docking complex (ODA-DC) located at the base of OAD. Studies using *Chlamydomonas* mutants have shown that OAD cannot bind to the microtubule at all without the ODA-DC, and the ODA-DC can bind to MTs only in a reduced amount without OAD. These data suggest that the two sites have weak binding affinities to microtubules, and therefore they possibly cooperate with each other to enable strong OAD attachment to microtubules. To test this idea, we quantified binding affinities of IC1, IC2 (the other intermediate chain located at the base of OAD) and the ODA-DC using recombinant proteins, and also tested whether there is a direct interaction between ICs and the ODA-DC. The recombinant IC1 and IC2 were found to form heterodimers, and the IC1-IC2 heterodimer further formed a complex with ODA-DC in vitro, in which IC2 was in contact with DC1 (an ODA-DC subunit). In addition, the binding affinities of ICs and ODA-DC to cytoplasmic microtubules, measured with a quartz crystal microbalance (QCM), were similar to each other. From these results, we propose a new model of protein-protein interactions for the docking of outer dynein arms to the axonemal microtubules.

3PS009 *In vitro* analyses on two adjacent inner-arm dyneins, subspecies e and c

Youské Shimizu, Hiroaki Kojima, Kazuhiro Oiwa, Hitoshi Sakakibara (*Advanced ICT Research Institute, NICT*)

Subspecies of inner-arm dyneins are aligned periodically and specifically in flagella. Though each of dynein subspecies "e" and "c" is able to be purified as monomer, these two dyneins are adjacent in flagella so that there may be interaction and/or cooperation between them. To study the properties, dynein e and c were highly purified. ATPase activity of dynein e ($k_{\text{cat}} \sim 0.3$) was low compared with that of dynein c ($k_{\text{cat}} \sim 1.8$). MT-binding affinity of nucleotide-sensitive binding domain of dynein e was higher than that of dynein c. *In vitro* motility assay indicated that dynein e was relatively slow ($V_{\text{max}} \sim 2 \mu\text{m/s}$) and dynein c was relatively fast ($V_{\text{max}} \sim 17 \mu\text{m/s}$) in MT-sliding. To obtain clues on the mechanism of spontaneous work of two dyneins, we analyzed properties of mixture. From the MT-sliding by the mixture of dyneins, it was suggested that slow dynein e did not cause a drag force on fast MT-sliding by dynein c. On the other hand, direct interaction between these dyneins was not confirmed, so the interaction was thought to be limited or weak. Dynein e and c may be working in flagella in cooperation with each other.

3PS010 生細胞内における温度分布のイメージング

1YS0915 Imaging of temperature distribution in a living cell

Kohki Okabe¹, Seiichi Uchiyama¹, Noriko Inada², Yoshie Harada³, Takashi Funatsu¹ (¹*Grad. Sch. Pharm. Sci., Univ. Tokyo*, ²*NAIST*, ³*CeMs, Kyoto Univ.*)

Temperature is a fundamental physical quantity that governs every biological reaction within living cells, and temperature distribution reflects cellular thermodynamics and function. In medical studies, the cellular pathogenesis of diseases (e.g., cancer) is characterized by extraordinary heat production. Therefore, intracellular temperature imaging of living cells should promote better understanding of cellular events and the establishment of novel diagnoses and therapies. However, imaging of temperature distributions in living cells has never been achieved. Here we demonstrate the first intracellular temperature imaging based on a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy (FLIM). The spatial and temperature resolutions of our thermometry were at the diffraction limited level (200 nm) and 0.2 degree Celsius, respectively. The intracellular temperature distribution we observed indicated that the nucleus and centrosome of a COS7 cell both showed a significantly higher temperature than the cytoplasm and that the temperature gap between the nucleus and the cytoplasm differed depending on the cell cycle. The heat production from mitochondria was also observed as a proximal local temperature increase. These findings demonstrate an intrinsic connection between temperature and organelle function. Thus, our intracellular temperature imaging has a significant impact on the comprehension of cell function and will provide insights into the regulatory mechanisms of intracellular signaling.

3PS011 走査電子顕微鏡を用いた非染色生物試料の高コントラスト・低ダメージ観察方法

A high-contrast and low-damage observation method of the unstained biological samples by scanning-electron microscope

Toshihiko Ogura (*AIST Biomedical Research Institute*)

Scanning electron microscope (SEM) is a useful technique for the investigation of surface structure of biological samples. To allow observations under the high vacuum conditions of SEM, many preparations of biological samples have been developed, e.g., glutaraldehyde fixation, negative staining, the Sputter-Cryo technique, and coating with gold or osmium. These preparations have some positive effects on the biological samples; for instance, they enhance contrast, reduce damage, and are uncharged up by the electron beam. However, these preparations require many steps and/or special equipments. Therefore, a more convenient preparation method for biological samples is needed. Here, we show that the unstained biological samples under a thin film give a high contrast detected by the secondary electron (SE) signal at a low accelerating voltage. The unstained biological samples are attached to the lower surface of a 30-50 nm thin film. The high-contrast images are created from the SE signal under the thin film. These are very low damage to the samples, and the images are similar to the transmission image. Our novel method can be easily utilized to observe various biological samples of bacteria, viruses and protein complexes.

3PS012 低温電子顕微鏡による細菌べん毛キャップ連結部の3次元再構成

The structure of the flagellar cap-junction complex by electron cryomicroscopy

Fumiaki Makino, Takayuki Kato, Tomoko Miyata, Keiichi Namba (*Frontier Bioscience*)

The bacterial flagellum is a biological nanomachine for the locomotion of bacteria. The cap at the growing end of the bacterial flagellum is essential for its growth. Flagellar axial proteins travel through the central channel of the flagellum to the distal end where self-assembly occurs. After flagellar hook has reached its mature length, the two junction proteins FlgK and FlgL assemble to form the hook-filament junction, and then the filament cap protein FliD forms a cap structure at the distal end of the junction to form the cap-junction complex. When FliC molecules are exported to the distal end, the FliD pentamer works as the filament cap for filament growth. It has been revealed from the cap-filament structure that five leg-like anchor domains of the pentameric cap flexibly adjust their conformations to keep just one FliC binding site open at a time. This has suggested a cap rotation mechanism to promote FliC self-assembly. However, the boundary between the cap and the filament end has remained unclear. In this study, we report 3D structure of the cap-junction complex, in which we