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

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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

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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

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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

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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).
- 4) N. Koder, D. Yamamoto et al., Nature 468, 72-76 (2010).

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