energy landscape than those of few atomic systems as is shown by D. A. Evans et al. (D. A. Evans et al., J. Chem. Phys. 120, 148-(2004)) and has 102 internal degree of freedom. In this talk, we will present several results of our analysis of this system in terms of classical molecular dynamics simulation to uncover the underlying mechanism that makes the mode selectivity survive in such a highly complicated system and identify under which condition that kind of mode selectivity exist.

3P-126 ヘテロ 2 量体であるクラミドモナス鞭毛内腕ダイニン亜種 f のそれぞれの重鎖は異なる特性を持つ

Distinct Roles of 1α or 1β Heavy Chains of the Inner Arm Dynein II of *Chlamydomonas* Flagella

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Inner arm dynein II of *Chlamydomonas* flagella, also known as dynein f, is a protein complex composed of two distinct heavy chains, three intermediate chains and a number of light chains. Dynein II translocates microtubules at the comparatively low velocity, and is supposed to be a processive motor (Kotani et al., 2007). In studies of other processive motors, a mechanism has been proposed in which two identical motor domains alternately swing forward at each step while one motor domain remains attached on the track. In the dynein II, however, its heterogeneous motor domains do not need to behave the same. To determine the role of each motor domain in processive movement, we took advantage of mutants lacking either the 1α or 1β motor domains (Myster et al., 1999; Perrone et al., 2000). Gel electrophoresis and immunoblot analysis confirmed that the mutant molecule was intact except lacking one head domain. In negative-staining electron microscopy, both 1α and 1β showed the one-headed configuration. We performed conventional *in vitro* motility assays showing that the 1α and the 1β translocate microtubules smoothly on glass surface at 1.1 $\mu\text{m}/\text{sec}$ and 3.0 $\mu\text{m}/\text{sec}$ at 1 mM MgATP, respectively. 1β has also higher basal and microtubule stimulated ATPase activity than 1α and 1α . Thus, we concluded that each dynein head of II distinct motile and enzymatic properties. A certain coordination mechanism may exist between each motor domain of an intact dynein II complex, such that one head assists power stroke of the other.

3P-127 免疫電子顕微鏡法による軸系外腕ダイニン - 微小管複合体の構造解析

Immuno-electron Microscopic Observation of Outer Arm Dynein Molecules Bound to Microtubules

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Dynein consists of 1-3 heavy chains (HCs) with several intermediate chains (ICs) and light chains (LCs). Functions of ICs and LCs are not fully known, and even the localization of them within a molecule is not known in detail, especially when dynein is bound to microtubules (MTs). To understand the mechanism of regulation by ICs and LCs, we are studying the localization of these subunits both in isolated dynein molecules and in the dynein-MT complex. We have previously used outer arm dynein purified from sea urchin sperm to make the dynein-MT complex, in which dynein cross-bridged two MTs periodically. However, purified sea urchin dynein did not contain the docking complex, which is thought to be responsible for periodical binding of dynein in *Chlamydomonas* axoneme [K. Wakabayashi et al. (2001)]. To make the dynein-MT complex including these subunits, we used outer arm dynein isolated from *Ciona intestinalis* sperm axoneme. When mixed with MTs, we saw some MTs cross-bridged by dynein, but most of the dynein molecules bound irregularly to the MTs. We also tried polymerizing tubulin in the presence of dynein, because this method is known to produce the *Chlamydomonas* dynein-MT complex in which dynein molecules regularly cross-bridge two MTs [L. Haimo et al. (1979)]. With *Ciona* dynein, this method produced some regularly cross-bridged MTs, but the population of periodical binding was low. Search for a condition to increase regular binding and labeling of dynein molecules using antibodies against ICs and LCs are in progress.

3P-128 微小管は 2 つの異なる領域を介してキネシンと相互作用する

Microtubules interact with kinesin via 2 independent regions

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Kinesin can move along a microtubule (MT) several microns in length and generate a force of approximately 8 pN using the chemical energy produced by ATP hydrolysis. High-resolution cryo-electron microscopy of the kinesin-MT complex has revealed a conformational change in the nucleotide-binding pocket between the open and closed configurations, which was not detected using x-ray crystal structural analysis of kinesin. This conformational change might support the results of biochemical studies showing that the ATPase activity of kinesin is stimulated by the MT. The kinesin-MT interaction might function as a "switch" to induce a conformational change in the nucleotide-binding pocket of kinesin.

To examine this hypothesis, we conducted mutational analysis of tubulin combined with single-molecule motility assay and MT-activated ATPase

measurement. The results revealed that 2 separate regions on the microtubule are involved in its interaction with kinesin: the helix11-12 loop of α -tubulin and helix12 of β -tubulin. Unbinding force measurements showed that while the former region is involved in both strong (AMP-PNP and nucleotide-free) and weak (ADP) binding states, the latter is involved only in the strong binding state. Moreover, the measurement of the stall force revealed that the generation of force by kinesin is dependent on the binding stability in the nucleotide-free state. In this meeting, we will discuss the mechanisms of kinesin motility and force-generation, focusing on the distinct roles of the 2 kinesin-interaction regions on MT.

3P-129 1 分子回転観察における V_1 -ATPase の不活性化の解析

Analysis of inactive V_1 -ATPase during rotation by single molecule measurement

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V_1 -ATPase (V_1) is the water-soluble part of the vacuolar-type ATPase (V_0V_1 -ATPase). V_1 from *Thermus thermophilus* is composed of a rotary shaft and a cylindrical stator ring which comprise DF or A_3B_3 subunit complex, respectively. The DF rotor shaft makes a 120-degree step rotation upon one ATP hydrolysis. It is known that V_1 molecules gradually lapse into the ADP-inhibited form when measured in a bulk assay solution. In this study, we observed the V_1 rotation at a single molecule level for the study of the ADP-inhibited form of V_1 . When observed at an ATP saturating condition, V_1 shows two pausing states; one is a transient state of a few second. V_1 readily regains from the pausing state to resume the active rotation. The other one is an irreversible pausing state which is so stable that V_1 molecules cannot revive from this inactive state in the observation time (more than 1 hour). Both types of pauses occur at the same three angular positions, 120-degree apart from each other. These pausing states cannot be attributed to the ATP-binding dwell because the time constant of ATP binding in the experimental condition should be less than 10ms. The dwell time analysis of these pausing states suggests that the transient pausing state is a newly found inactive state while the persistent one corresponds to the ADP-inhibited form observed in the bulk ATPase measurement.

3P-130 FRAP 実験による機能的べん毛モーター回転子構成タンパク質の交換の可視化

FRAP analysis for visualization of exchange of rotor component in functional bacterial flagellar motor

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Bacterial flagellum is a supramolecular complex and consists of a basal body, a helical filament, and a hook. A basal body embedded in cell membrane functions as a rotary motor driven by electrochemical potential of specific ion, and rotates flagellar filament like as screw. The rotor consists of MS-ring (FlhF) and C-ring (FlhG, FlhM, and FlhN). In some recent study, the dissociation and re-association of stator proteins was shown. In this study, we constructed fusion proteins of rotor components and GFP (GFP-FlhF, GFP-FlhG, FlhM-GFP, and GFP-FlhN), and investigated whether rotor components are exchanged in a functional motor by Fluorescent Recovery After Photobleaching (FRAP) analysis for a single motor labeled with GFP. Using tethered cell method, we observed each GFP fusion was localized at the rotational center. Each GFP fusion might be incorporated into flagellar motor as a rotor component. To observe the exchange of rotor components, we carried out FRAP analysis using evanescent illumination. When fluorescent spot of FlhM-GFP or GFP-FlhN localized at rotational center in the tethered cell was photobleached, the recovery of fluorescence at the rotational center was observed with time. On the other hand, the recovery of fluorescence was not observed in the cell producing GFP-FlhG. These results suggest that some rotor components, FlhN and FlhM at least, assemble to motor even after functional motor is constructed. We would like to clarify turnover rate of rotor component until the annual meeting.

3P-131 ATP 駆動の回転において V -ATPase は特徴的なステップ状回転を示す

Distinctive rotational steps of V -ATPase in ATP-driven rotation

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Vacuole-type ATPase (V -ATPase) of *Thermus thermophilus* is capable of ATP synthesis or proton transport across the membrane. Like the F_1F_0 -ATP synthase, it consists of two rotary motors, soluble V_1 domain and membrane-embedded V_0 domain. The V_1 domain contains a cylinder of A_3B_3 subunits surrounding a putative rotor D subunit, which is connected via other subunits to the terminal rotor in V_0 of a ring of c_{12} subunits. ATP hydrolysis/synthesis in the catalytic sites in A is functionally coupled with proton pumping/permeation in V_0 . Here we have observed ATP-driven rotation of single V -ATPase molecules using laser dark-field illumination and high-speed imaging (1k-8k frames per seconds). 40-nm beads attached to the D subunit in a V_1 construct rotated at ~ 60 revolutions per second (rps) at 2 mM ATP with clear 120° steps. When the beads were attached to an A subunit in an upside-down V_0V_1 , the rotary rate was reduced to 1-20 rps and we observed dwelling positions separated by $\sim 30^\circ$. The twelve positions were not