ment of the various species of dynein proteins, which generate power using energy released upon ATP hydrolysis, plays important roles in the flagellum beating.

Recently, great progress has been made in understanding of construction of a *Chlamydomonas* flagellum by the use of cryo-electron microscopic tomography. Dyneins are arranged in 96-nm periodic manner for a direction of a longitudinal axis of a flagellum. 8 species of dyneins are called as "inner-arm dyneins". In 96-nm period, they make specific 4 pairs and are arranged in equally-spaced 4 places.

Making use of *in vitro* motility assay, we can evaluate activities of dyneins as "microtubule sliding". Dynein "c" slides microtubules relatively fast among the 8 inner-arms, and is well-studied. It has shown that dynein c pairs with dynein "e" in a flagellum. However, properties of dynein e are not known much. One reason for the difficulty is that anion exchange chromatography, a major purification method for inner arm dyneins, cannot separate dynein e and dynein d well.

We obtained highly purified dynein e and c using two steps of anion exchange chromatography. We will present properties of dynein e and of a mixture of dynein e and c.

3P177 Analysis of the motility characteristics of myosin VI constructs with mutations in the base region of the converter domain

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Myosin VI moves processively on actin filaments towards the P-end with 30-36 nm steps, which are too large to be explained simply by the lever arm mechanism, because the lever arm of myosin VI contains only two CaM-binding sites. Structural studies suggested that the lever arm swing greater than 140 degrees, compared to the 70 degree swing in myosin II, and the conformational rearrangement in the converter both contribute to the large step of myosin VI. In other myosin structures, however, the converter itself is thought to rotate as a rigid body, rectifying and amplifying the structural changes within the motor domain. To investigate whether the rearrangement of the converter structure is required for the large step, we constructed human myosin VI mutants, in which the converter rotation was restricted by the amino acid substitutions at Gly703-Gly704-Tyr705 that directly connects SH1 helix to the converter, and examined the ATPase activities and motile properties of the mutants. Surprisingly, alanine mutation of Tyr-705 (F705A) had no effect on the lever arm swing, in contrast to myosins II and V, in which the corresponding mutations completely abolished the ability to generate force and rotate the lever arm. Furthermore, ATPase activities and sliding velocities of myosin VI mutants, in which Gly703 was replaced with bulky residues such as arginine, partly retained their ability to translocate actin filaments. Here, we report the properties of these myosin VI mutants in detail.

3P178 リバースジャイレースの温度依存性 3B1220 Temperature dependence of positive supercoiling by reverse gyrase

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Reverse gyrase is a unique type I topoisomerase that can introduce positive supercoils into DNA. Its reaction is ATP-dependent, and it is a hyperthermophile-specific protein. This positive supercoiling is thought to be involved in protecting DNA from denaturation by increasing its torsional stiffness and in stabilizing the B-form DNA duplex even at high temperatures. In spite of extensive biochemical studies, the mechanism of reverse gyrase reaction is yet unclear.

Here, we have investigated the rate of its reaction under a temperature-controlled optical microscope: a bead attached to an end of a double-stranded DNA segment tethered to a surface rotated for many turns in one direction only at temperatures above 50 °C. The rotary speed was highly dependent on the temperature. When a single-stranded bubble was introduced in the DNA, the rate of rotation increased at all temperatures examined, and the DNA with a bubble rotated even at low temperatures (40-50 °C) where fully complementary DNA showed only Brownian fluctuation. The number of rotating beads per field of view was found to vary with the enzyme to DNA ratio, but the rotary speeds appeared constant at the same temperature, irrespective of the enzyme/DNA ratio. Apparently, a single molecule of reverse gyrase drives the observed rotation, binding to a region of DNA that has a greater tendency to melt. But other interpretations are possible, and we are making effort at reaching a consistent view of how reverse gyrase rotates DNA.

3P179 細菌べん毛モーター高速回転時のステップ検出 Discrete steps in fast bacterial flagellar rotation detected by back-scattering microscopy

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The bacterial flagellar motor is a rotary molecular machine driven by ion flux across the cytoplasmic membrane. It can rotate at several hundreds Hz (revolutions per second). The free energy gained from passing ions through the ion channel of the stator is converted to the mechanical torque by interaction between a stator and a rotor. Coupling ion is either protons or sodium ions, depending on the bacterial species.

We previously reported the discrete angular steps of the flagellar rotation of sodium-driven chimeric motor up to 10 Hz under very low sodium-motive force. To analyse their statistical properties and dependence on free energy, it is required to detect the steps with a wide range of input energy, in other words, under conditions of faster flagellar rotation. Here we report discrete steps of the motor in ~100 Hz spinning measured by back-scattering laser microscopy. Our microcopy has better than 1 nm spatial resolution at 110 kHz sampling rate by imaging back-scattering light from gold nanoparticles. To reduce damping factor in the system, a 100 nm gold particle, which is close to zero load on the motor, is attached to the hook of the motor as a marker. Detected steps are essentially consistent with our previous result, 26 steps per revolution. Further analysis is in progress.

3P180 Mechanical properties of MCAK, the microtubule-depolymerizing kinesin 3B1235

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During cell division the chromosomes must be correctly segregated to the daughter cells. The attachment of kinetochores to microtubules, which emanate from the opposing spindle poles, generates tension to drive the separation of chromosomes at anaphase. It has been proposed that tension is modulated by the microtubule-based motor proteins, especially microtubule-depolymerizing kinesins, such as mitotic centromere-associated kinesin (MCAK), which is essential for pulling the chromosomes. MCAK, which is a member of the kinesin-13 family, can rapidly depolymerize both ends of microtubules. Although the force production by the motile motors, such as kinesin-5 and dynein, has been characterized well, little is known about whether MCAK is able to produce force by its depolymerization activity. To address this, we observed the MCAK-microtubule interaction under the microscope equipped with the optical tweezers. Here we show the behavior of the MCAK-bound bead interacting with a single microtubule and the force production by the microtubule-depolymerizing activity of MCAK.

3P181 好熱菌由来 F₀F₁-ATP 合成酵素の ATP 合成における ΔpH と 3B1250 Δψ の寄与 Kinetic equivalence of Δψ and ΔpH in ATP synthesis by *Bacillus* BS3 F₀F₁-ATP synthase

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F₀F₁-ATP synthase produces ATP, coupled with proton flow driven by proton motive force (pmf). Pmf consists of transmembrane difference of electrical potential (Δψ) and proton concentration (ΔpH). They are thermodynamically equivalent but their kinetic equivalence (contribution to ATP synthesis rate) has been controversial. Here, using thermophilic *Bacillus* PS3 F₀F₁-ATP synthase (TF₀F₁) with a mutation lacking inhibitory effect of ε subunit, we have developed highly reproducible, simple procedures for preparation of active proteoliposomes and for kinetic analysis of ATP synthesis, which was driven by acid-base transition and K⁺-diffusion potential. TF₀F₁ showed maximum rates of ATP synthesis of 18 s⁻¹ at 30 deg with K_ms for ADP and Pi, 19 and 500 μM, respectively. Then, rates of ATP synthesis were determined under several combinations of Δψ and ΔpH. The rates were highly correlated to the pmf value calculated from Δψ and ΔpH, although small deviation still remains. Importantly, as [K⁺]_{in} was decreased below 5 mM with fixed [K⁺]_{out}, ATP synthesis rate became constant as if effective Δψ was saturated. Taking these observations into account, we conclude that rates of ATP synthesis are solely dependent on the magnitude of pmf under the conditions tested. We are now setting up a system for direct observation of the C-ring rotation during ATP synthesis.