1P168 クライオ電子顕微鏡法による車軸藻ミオシンの高速移動分子メカニズムの解明
Elucidation of the molecule mechanism of Chara myosin's fast movement by electron cryomicroscopy

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Chara corallina class XI myosin is a motor protein, which is involved in cytoplasmic streaming of *Chara corallina*. The myosin has the fastest actin sliding velocity ($\sim 70\mu\text{m/s}$) among all myosin classes known so far. To unravel the mechanism how Chara myosin moves so fast, the kinetic properties of the myosin have been analysed. These studies elucidated that Chara myosin has high ATPase activity. In addition, in its ATPase cycle, the ATP binding rate is extremely fast and the ADP release rate is dramatically accelerated by binding to actin(1000-fold). The sequence analysis and electron microscopy show that Chara myosin consists of a motor domain (motor domain : MD) that belongs to class XI, a neck region with six tandem IQ motifs that serve as binding sites for six light chains, an α -helical coiled-coil domain for dimerization, and a globular tail domain to bind to cargo. Nonetheless, its detailed structural features are not clear enough to understand the molecular mechanism of its kinetics.

Thus, we have analyzed the structure of Chara myosin by electron cryomicroscopy and 3D reconstruction. Then we obtained 3D maps of the myosin with/without ATP to find the nucleotide-binding cleft is open without nucleotide in binding actin filaments. We are now trying to acquire high resolution 3D-structures of Chara myosin MD without nucleotides and Chara myosin tightly bound to actin. We will here report the recent progress to clarify the relationship between structure and kinetic feature of Chara myosin.

1P169 タンパク質標識に用いる金ナノ粒子の作製法の開発
Development of a new method to synthesize functionalized gold nanoparticles for labeling a protein.

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Analysis of the detailed structure of protein complexes is very important to understand their functions. Especially, to identify a position of a sub-unit in a protein complex is desired. A local labeling in a protein using gold nanoparticles (Au NPs) is attractive because it is applicable to structural analysis by using transmission electron microscopy (TEM). The purpose of this study is to develop a new simple method to functionalize an Au NP surface with nickel-nitrilotriacetic acid (Ni-NTA) that selectively binds a polyhistidine-tag (His-tag). In this method, the Ni-NTA Au NPs were made by only the three steps. Firstly, Au NPs were synthesized by means of reduction of HAuCl₄ using trisodium citrate and tannin acid. Secondly, the Au NPs were functionalized with NTA group by simply mixing with C2-NTA that binds specifically to the Au NP surface with a reactive disulfide group. Finally, the mixed solution was passed through an ion-exchange column that had been previously charged with nickel ion to form Ni-NTA Au NPs. The Au NPs were highly homogeneous. The mean diameter was $4.7\pm 0.5\text{nm}$. The Au NPs mixed with kinesins that have the His-tag were observed with TEM to estimate affinity between them. TEM images show that the Au NPs are surrounded by the kinesins, which indicates that the Ni-NTA Au NPs preferentially bind the His-tag in the protein. In conclusion, the Ni-NTA Au NPs synthesized by our new method are useful for elucidating the position of the sub-unit containing the His-tag in a protein complex by using TEM.

1P170 金ナノ粒子を用いた細胞質ダイニン複合体の電子顕微鏡観察
Electron Microscopic observation of cytoplasmic dynein using gold nanoparticles.

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Cytoplasmic dynein walks along microtubule toward its minus end utilizing the energy from ATP hydrolysis, and has many basic and important roles in eukaryotic cells including mitosis, vesicle transport and cell migration. Cytoplasmic dynein is a huge protein complex having two domains, the head domain, and the tail domain. So far, many studies have investigated about the head domain, because it is a motor domain consisting of six AAA modules and a stalk, and the conformational change depending on the nucleotide

binding states was elucidated. On the other hand, the tail domain consists of N-terminal third of heavy chains, intermediate chains (IC), light intermediate chains (LIC), and light chains (LC), and is implicated in cargo binding. However, the detailed molecular structure of the tail domain remains to be unveiled. In this study, we used nitrilotriacetic acid (NTA) conjugated gold nanoparticles to label polyhistidine tag (His-tag) of recombinant proteins and observed them in a electron microscopy (EM) by negative staining. We identified the C-terminal positions of the IC and LIC in the tail domain. Based on our observations, we will propose a new model of the architecture of the dynein tail domain and discuss about its functions.

1P171 多機能 GFP タグで見えてきた哺乳類細胞におけるダイニン-ダイナクチンのダイナミックな動態
Dynamic behavior of dynein-dynactin in living mammalian cells visualized by multifunctional GFP tag

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Cytoplasmic dynein is a minus-directed microtubule (MT) molecular motor and plays an important role in various cellular functions. We have recently demonstrated cell cycle-dependent dynamic behavior of dynein in living mammalian cells, by visualization with a novel multifunctional GFP (mfGFP) tag (Kobayashi & Murayama, 2009). Dynactin is a large multisubunit protein complex that is essential for most types of dynein-mediated transport. However, it remains unclear how dynein-dynactin interaction is regulated. To investigate spatiotemporal patterns of dynein-dynactin interaction, we visualized dynactin in mammalian cells and compared its behavior with that of dynein. Dynactin subunit p50 was fused with mfGFP and stably expressed in HeLa cells. Isolation of p50-mfGFP co-purified with other dynactin subunits. In combination with nanogold labeling technology, location of mfGFP was mapped on the dynactin structure under negative staining electron microscopy. Thus, p50-mfGFP was successfully incorporated into dynactin complex. On live-cell imaging of interphase cells, dynactin was observed as discrete foci in the cytoplasm which were moving along with MTs. In mitosis, dynactin was localized at spindle MTs where it was frequently moving toward MT plus ends. It also existed at kinetochores and near cell cortex. Dual color imaging demonstrated that dynactin was colocalized with dynein in most cases. These findings suggest that dynein-dynactin interaction may stably occur throughout the cell cycle.

1P172 ミオシンフィラメント双極トラックに沿ったアクチンフィラメント滑り運動の活性化エネルギー
Thermal activation energy for bidirectional movement of actin filament along bipolar tracks of myosin filaments

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In vitro motility assays using bipolar myosin thick filaments demonstrated that actin filaments were capable of moving in both directions along the myosin filament tracks. The movements, however, were slower in the direction away from the central bare zone than towards the zone. To understand the mechanism underlying these different motile activities depending on the direction of the movements, we have examined the effects of temperature on the velocities of the movements in the both directions along the reconstituted myosin filaments. Activation energies of the movements were determined by the Arrhenius plot at high and low concentrations of ATP. As a result, the thermal activation energy of the movement away from the central bare zone was significantly higher than that of the movement toward the zone. Given that the backward movement in the direction away from the central zone would cause the myosin heads to be constrained and increase the stiffness of the cross-bridge, these results may provide evidence that elastic energy required for the cross-bridge transition is supplied by thermal fluctuations.

1P173 1分子イメージングによるトランス-トランスレーションにおけるSmpBのC末端の機能解析
Functional analysis of the C-terminus region of SmpB in trans-translation by single-molecule imaging

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In Eubacteria, the stalled ribosomes are rescued by trans-translation mecha-