- 3P140 **ダイニンの構造的最小単位の同定**
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Are six AAA+ modules sufficient to form the ring of dynein?
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Cytoplasmic dynein is a motor complex responsible for diverse cellular processes within eukaryotic cells, including mitosis, Golgi dynamics and the retrograde transport of various vesicles and organelles along microtubule filaments. The motor unit of dynein (heavy chain) belongs to the AAA+ family, and consists of three structurally distinct domains: a head domain, which includes six tandemly linked AAA+ modules, a cargo-carrying tail domain, and a microtubule-binding stalk domain. Since the head domain of dynein has an appearance of a ring ("AAA ring") of seven subdomains when observed under an electron microscope, it is supposedly formed by the six AAA+ modules plus either the N-terminal region upstream of the first AAA+ module or the C-terminal non-AAA region. However, neither of these models has been tested.

In an attempt to reveal the structural components of the AAA ring, we expressed and purified constructs devoid of the N-terminal region (linker) and/or the C-terminal non-AAA region of the Dictyostelium cytoplasmic dynein and determined their structures. Although none of these constructs retained microtubule-activated ATPase activity or microtubule-sliding activity, electron microscopy revealed that they all retained ring-like structures. This result indicates that the six AAA+ modules and possibly intervening regions between them are sufficient to form the AAA ring. We suggest that the N-terminal and C-terminal flanking sequences are not part of this ring but interact with it and enable it to function as a motor.

- 3P142 **細胞質ダイニンの2つのAAAリングの協調性**
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Behavior of two heads of cytoplasmic dynein
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Cytoplasmic dynein, a large (1.2MDa) molecular motor, contains two identical ring-shaped heads (AAA rings) and has been reported to take 8nm steps along microtubules. Unlike the two other cytoskeletal motor proteins (myosin and kinesin), the mechanism of dynein motility remains poorly understood. To clarify the dynein stepping mechanism, it is important to elucidate contribution of each AAA ring for the processive movement. Additively, because the diameter of the AAA ring is ~15nm, which is approximately twice of the step size, it is an interesting question how the huge dynein molecule takes such short steps. To answer these questions, a method for distinguishing the manner of one AAA ring from the other is required. Therefore, we have tried to generate a heterodimeric dynein construct in which each AAA ring is labeled with different fluorochromes. Here, we would like to report our data obtained from the experiments using this construct and discuss about the stepping behavior of dynein.

- 3P141 **Cys-light ダイニンの構築**
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Construction of Cys-light cytoplasmic dynein
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Cytoplasmic dynein is a motor protein that moves unidirectionally along a microtubule utilizing energy released by ATP hydrolysis. To elucidate the molecular mechanism of the force generation process of dynein on a microtubule, it is necessary to introduce probes such as fluorescent dyes that report structural changes and protein-protein interactions at particular locations. The most versatile approach for site-directed attachment of probes to dynein requires two steps of protein engineering: the removal of reactive Cys residues locating on the protein surface and the subsequent site-directed insertion of a reactive Cys residue to which many fluorescent dyes and other probes with maleimide group can readily react. The motor domain of Dictyostelium cytoplasmic dynein with full motor activities (the 380kDa fragment) contains 33 Cys residues. Among them, 12 residues are expected to be located on the surface and reactive to probe molecules with a maleimide group. Therefore, we are now constructing the Cys-light dynein by genetically replacing these reactive Cys residues with other residues. Once the Cys-light dynein which retains motor activities is available, we can insert a reactive Cys residue at any site on the dynein surface for a site to attach the reporter molecule. We will report the progress of the construction of Cys-light dynein.

- 3P143 **細胞質ダイニンの複数サイトにおけるヌクレオチド結合および加水分解の情報のやりとりと力発生との関連**
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Dissection of inter-modular communication among multiple nucleotide-binding/hydrolysis sites of cytoplasmic dynein
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Dynein heavy chain belonging to the AAA+ superfamily contains six AAA+ modules that form the ring-like structure characteristic of the AAA+ proteins. Among these AAA+ modules, each of the four (AAA1-AAA4) has a putative nucleotide binding/hydrolysis site. We have previously shown that the AAA1 site is likely the primary ATPase site directly responsible for force generation on a microtubule, while the others (AAA2, 3, and 4) would indirectly participate in the process by modulating the ATPase cycle at the primary site. To further elucidate the functional roles of the multiple nucleotide binding/hydrolysis sites, we introduced mutations expected to block ATP hydrolysis at the four sites and assessed effects of these mutations on the dynein-tail swing by pre-steady-state measurements of GFP-based FRET changes. Our results suggest that (1) the AAA1, 3, and 4 sites hydrolyze ATP; (2) the hydrolysis cycle at the AAA3 or AAA4 site is tightly coupled with that at the primary AAA1 site; (3) the AAA2 site, which is expected to bind nucleotide without hydrolyzing it, may selectively bind ADP to modulate the ATPase cycle at the AAA1 site.