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細胞質ダイニンの微小管相互作用と尾部スイングの協調
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The coordination of cyclic microtubule association/dissociation and tail swing of cytoplasmic dynein
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The dynein motor domain is composed of a tail, head, and stalk, and is thought to generate a force to microtubules by swinging the tail against the head during its ATPase cycle. This force generation mechanism (power stroke) would require the tight coordination of the tail swing and microtubule association/dissociation. However, although recent studies have provided a detailed picture of the former process, there have been few studies regarding the latter process. Here, using the single-headed recombinant motor domain of Dictyostelium cytoplasmic dynein, we address the questions of how the interaction of the motor domain with a microtubule is modulated by ATPase steps, how the two mechanical cycles (the microtubule association/dissociation and tail swing) are coordinated, and which ATPase site among the multiple sites in the motor domain regulates the coordination. Based on steady-state and pre-steady-state measurements, we demonstrate the following: (1) at most of the intermediate states, the MT affinity change is synchronized with the tail swing: the motor domain in the post-stroke state binds strongly to the microtubule with a Kd of about 0.2 micro M, while most of the motor domains in the pre-stroke state bind weakly to the microtubule with a Kd of more than 10 micro M. (2) the timings of the MT affinity change and tail swing are staggered at the recovery stroke step. (3) The ATPase site in the AAA1 module of the motor domain is responsible for the coordination of the two mechanical processes.

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単頭ダイニンによって駆動されるビーズの運動のモデル解析
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Model analysis of the motion generated by single-headed cytoplasmic dyneins attached to a bead
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Cytoplasmic dynein is a double-headed motor protein which moves processively along a microtubule. It has a complex and fundamentally different structures from other biological molecular motors, and the mechanism of its motility has not been understood well compared to myosin and kinesin. The recent experiments by Higuchi's group on (double-headed) single dynein molecules [PNAS, **103**, 5741 (2006)] and on single-headed dyneins (presentation at the EABS & BSJ 2006 meeting) seem to provide important information on the mechanism of dynein motility.

The purpose of the present work is to carry out computer simulations of the motion of a bead generated by two or more single-headed dyneins bound on it, by using a "power stroke" model. In this model, the motion is caused by the conformational change of a head (when it is bound to the microtubule) and the thermal fluctuation of the head (when it is detached from the microtubule). The distributions of the step size and the dwell time between consecutive steps, and the stall force are analyzed and will be compared with the experiments.

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ダイニーストーク部位のアイソフォームに依存した撓やかさ
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flexibility change of dynein stalk depend on their isoforms
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Dynein is categorized as moter protein family as well as kinesin and myosin.However, the microtuble binding domain of dynein is separated far from the AAA core of the motor by the stalk domain. This is different from that of myosin and kinesin.Thus,the informartion between the active site and microtible body must be communicated through the stalk, which has been predicted to consist of an anti-parallel coiled coil by second structure predicting tools. We showed that, by molecular dynamics method, the stability of the dynein stalk is lower than that of typical coiled-coil and the stalk is a highly flexible coiled coil. The stability, in addition, depends on the isoform.Interestingly, the bending of the stalk as a coiled coil at the specific regions was also observed, especcially, the innerarm dynein c has the specific bending site.We considered these phenomena result from two arguments.The first possibility is that the stalk of dynein works as a transmission device between mechanical and chemical reactions for sliding motion.The other is that dynein is architected to fit to a work place, distance between dynein and microtuble, spacing between neiboring isoforms and so on.Even in each case, our result may cast aside a conventional model such that stalk is a simple rod and give us a new vision.

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フォトクロミック ATP アナログの合成及びモータータンパク質との相互作用
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Synthesis of photochromic ATP analoues and interaction with motor proteins
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Previously we have demonstrated that microtubules dependent ATPase activity of the kinesin modified by spiropyran and azobenzene derivatives was regulated by UV-VIS light irradiation. Moreover, we have also succeeded to control the binding of calmodulin to the target peptide using azobenzene derivatives. However, it was not so easy to incorporate the photochromic molecules into the functional site of enzymes without altering the native enzymatic properties. In the present study, we have designed ATP analogues consist of photochromic molecules in order to photo-regulate ATPases without their chemical modification. We have synthesized two kinds of non-nucleotide ATP analogues: spirobenzopyran-ethyl-triphosphate (SPETP) and azobenzene-triphosphate (ABTP). Both of SPETP and ABTP showed reversible UV/VIS absorpotion spectral change by UV-VIS light irradiation. ABTP and SPETP were hydrolyzed by skeletal muscle myosin and conventional kinesin. Moreover, SPETP induced dissociation of acto-myosin. After complete hydrolysis of SPETP to SPEDP, rebinding of myosin to actin was observed. For kinesin, ABTP showed alteration of ABTPase between UV and VIS light irradiation. However, both of the photochromic ATP analogues did not induced motility in actin-myosin system and kinesin-microtubule system.