# 3-2 Mechanisms of Dynein Functions as Information Processing Devices

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We study mechanisms of motor-protein functions to find sources of new technology of information processing devices. I introduce here our recent results of the functions of flagellar dynein, which generate the force of flagellar beating of eukaryote and function as oscillator. We succeeded to measure the motility of single molecules of inner-arm dynein subspecies c (dynein c) and found that dynein c is a single headed processive motor, which is capable to move on protein filament without detachment in multiple steps. We also revealed new structure details of dynein c by using negative staining electron microscopy and single particle image analysis. In the work, we compared the structures of pre- and post- power stroke of dynein c and indicated aspects of dynein power-stroke. These evidences are very important to clarify the mechanisms of dynein functions.

***Keywords***
Dynein, Microtubule, *In vitro* motility assay, Negative-staining electron microscopy, Single-particle image analysis

## 1 Introduction

Nowadays, studies in the field of nanotechnology are in favor. Yet several billion years ago, long before human beings became interested in nanomachines, living creatures acquired nanomachines, and have been using them ever since. These nanomachines are protein molecules. Living creatures have flexible, sophisticated functions that computers and artificial machines cannot follow. These functions include autonomy, self-assembly, self-recovery, autoreproduction, learning, and creativity. The fundamental elements of these functions are biopolymer assemblies known as "supramolecules," and proteins are the primal members of such supramolecules. Although supramolecules are in the nanometer scale, they have sophisticated information processing capabilities. These supramolecules are autonomously constructed and function in living cells, providing information processing functions uniquely typical in a living body, including information reception and storage, adaptability, and communication.

We, the members of the Protein Biophysics Group, study the principle of operation of supramolecules, particularly proteins, to develop new techniques for manipulating and constructing supramolecules. This research field is expected to lead to the development of intelligent information processing devices with flexible functions similar to those observed in living creatures, and to the evolution of new network algorithms.

We focus on the proteins called motor proteins. This article reports the results of our studies on dyneins, motor proteins with particularly characteristic movements.

## 2 Dyneins

### 2.1 Flagellar dyneins

Dyneins are motor proteins which were discovered as the movement-generating proteins of eukaryotic flagella. They also play

important roles in movement in living cells other than flagellar movement, such as the nuclear movement of cells, the axonal transport, etc. The dyneins related to movement inside a cell are called cytoplasmic dyneins, and those that generate flagellar movement are called flagellar dyneins.

Figure 1 shows a cross-section electron micrograph of a flagellum. Flagella and cilia are basically the same—those present in a large number on a cell are referred to as cilia. The structure is common whether it is the flagellum of *Euglena*, the cilia of a human trachea cell, or the flagellum of a sperm. The structure of a flagellum consists of a pair of microtubules in the center and 9 doublet microtubules (a completely cylindrical and a semi-columnar microtubule, together) surround the central microtubules. This structure is called the 9+2 structure. Flagellar dyneins are aligned on the A-tubules of the outer doublet microtubules. The dyneins on the A-tubule interact with the adjacent B-tubule, moving on it to cause the sliding movement between the two outer microtubules. The sliding movement is regulated in time and space and converted into the regular beating movement of the flagellum. A recent study demonstrated that dyneins have the capability to generate oscillation per se [1]. There are several dynein subspecies on a flagellum and each has the capability to cause the microtubule to slide. Their properties—for example, sliding velocity—differ among different dynein subspecies. On the flagellum of *Chlamydomonas*, a monad green alga, there is a single species of outer-arm dynein and 7 subspecies of inner-arm dyneins [2]. We focus on inner-arm dynein subspecies c and f among them.

### 2.2 Dynein c

Dynein subspecies c (hereafter referred to as dynein c) molecules induce the fastest sliding velocity to the microtubule among the subspecies [2]. Dynein c is a protein complex consisting of a heavy chain and 2 types of light chains. It is a large protein with a molecular weight exceeding 600k. Two protrusions

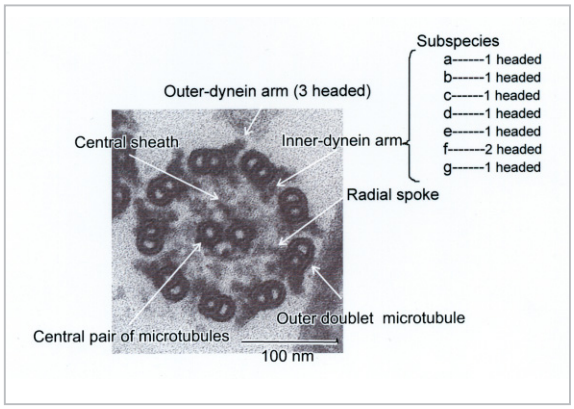


**Fig.1** *A cross-section electron micrograph of a flagellar axoneme of* Chlamydomonas

appear on the ring-shaped head, which is 12 nm in diameter (Fig.2). Of the two, the thin protrusion approximately 13 nm long is called the stalk. The structure of the head and the stalk together is mostly common among the different species of dyneins, and this structure has the capabilities of a motor. ATP is hydrolyzed in the head. On the other hand, the dynein molecule binds the microtubule at the tip of the stalk [3][4]. When a dynein molecule is not bound to ATP, the tip of the stalk strongly binds the microtubule. When an ATP molecule is bound to the ring-shaped head, the stalk dissociates from the microtubule. Thus, the binding of ATP and the binding of the stalk to the microtubule occur antagonistically. Some information must be transferred between the two regions, which are a long distance apart.

The tail and the base differ significantly among different species of dynein. The base dictates the site of function and the substance to transport in each species of dynein.

## 3 Sliding movement of microtubules caused by a single dynein c molecule

### 3.1 In vitro motility assay analyses

There are motor proteins a single molecule of which can move on a protein filament, such as actin or a microtubule, in multiple steps without detachment. We call such motor proteins processive motors. The requirement for

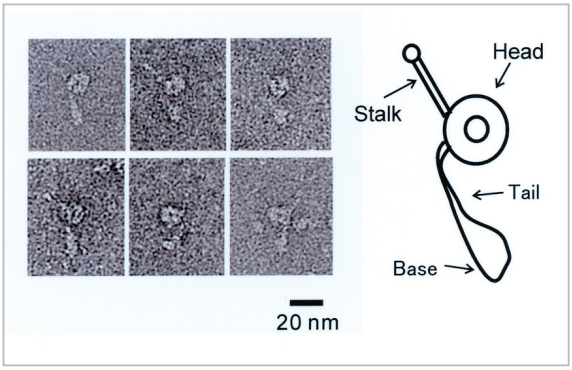


**Fig.2** *Structure of a dynein c molecule*

On the left are electron micrographs of negatively-stained dynein c, taken by the author, and on the right is its schematic diagram. The scale is 20 nm.

a motor protein to be a processive motor is that the motor protein stays attached to the protein filament throughout the motor cycle. If the motor molecule and the filament dissociate, thermal fluctuation separates them to a distance over which no interaction reaches within a millisecond. We examined properties of dynein c by in vitro motility assays and demonstrated that dynein c is a processive motor [4].

In our in vitro motility assay system, we observed microtubule movement on a glass surface coated with dynein c. The relationship between the number of microtubules, which appear to attach and then slide on the glass surface per unit time per unit area, and the density of dynein c on the glass surface enabled us to estimate the number of dynein c molecules required to bind a microtubule and cause the sliding movement [5]. It was estimated that a single dynein c molecule is sufficient to bind a microtubule and cause the sliding movement (Fig. 3). The sliding movement of the microtubules was also observed under a low dynein density of 0.1 molecules/$\mu$m$^2$ or less. Here, the microtubule was supported at a fixed point on the glass surface and swung its ends right and left around the point, while still progressing (Fig. 4). Probably, a single dynein c molecule was present at the point where the microtubule was supported, and slid the microtubule continuously. As discussed earlier, a dynein c molecule has only a single head that provides the motor activity. In the past, it has been believed that motor protein movement required at least 2 heads to alternate association and dissociation with the filament while keeping the attachment. That is not true of dynein c. There must be a new mechanism for keeping the attachment to the filament (microtubule). The microtubule-sliding distance over a single dynein c molecule was distributed exponentially, with a distance constant of 3.6 $\mu$m.

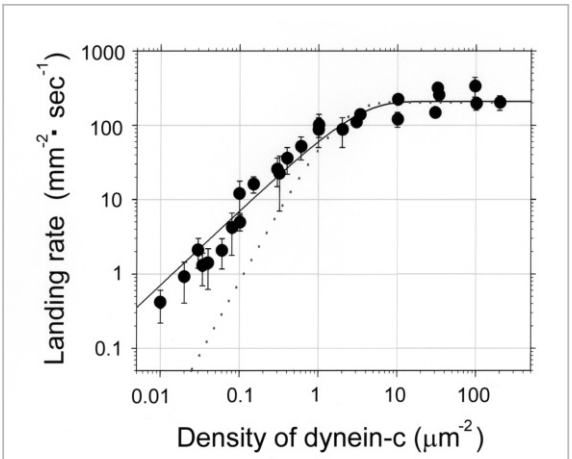


**Fig.3** *Relationship between the dynein c surface density and the number of observations (landing rate) of a microtubule attaching and sliding on the glass surface*

The graph is a log-log plot of the number of microtubules observed per unit time and unit area to adsorb and then slide on the glass surface vs. the surface density of dynein c. The solid curve is derived from a model assuming that a single dynein molecule is sufficient to slide the microtubule. The gradient of the curve approaches 1 in the region where the surface density of the dynein is low. The broken curve is derived from a model assuming that at least 2 dynein molecules are required to slide the microtubule. In this model, the gradient of the curve approaches 2 in the region where the surface density of the dynein is low.

### 3.2 Duty ratio

In the past, a high duty ratio (the ratio of time spent in a strong binding state with actin or a microtubule: (strong binding time)/(strong binding time + other time [total time])) was thought to be important for a single motor

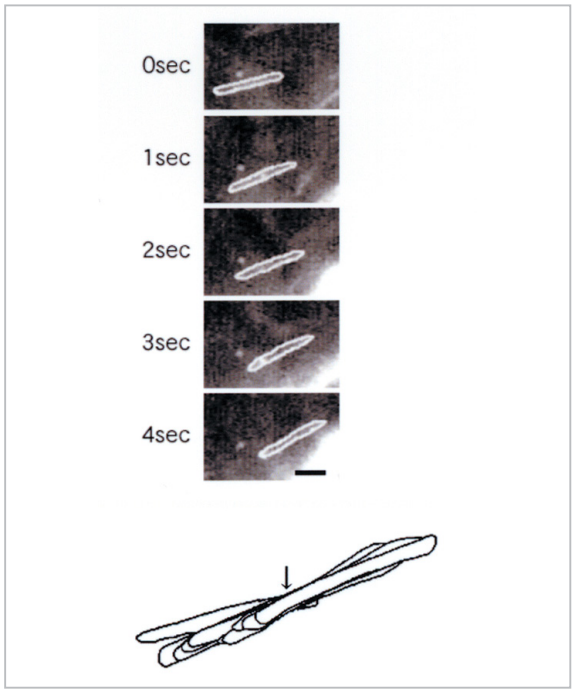


**Fig.4** *Sliding movement of the microtubule on a single dynein c molecule*

The upper figure is a series of dark-field microscope images. The edge of the microtubule dark field image is traced. The lower figure shows superposed traces of the microtubule images. The microtubule is supported at a fixed point (indicated by an arrow) and translocates in a single direction while whirling the edges right and left. The scale is 2.5 µm. The average surface density of dynein is 0.05 molecules per 1µm$^2$.

protein molecule to move continuously in multiple steps without dissociating from actin or microtubules. Kinesin, a representative processive motor, has 2 heads, one of which is always bound strongly to the microtubule during movement. However, dynein c is estimated to have a low duty ratio. This result indicates that dynein c stays attached to the microtubule with a strategy other than increasing the duty ratio.

The duty ratio can be calculated from the relationship between the dynein c density on the glass substrate surface and the sliding velocity of the microtubules [6]. It is believed that a motor protein must be strongly bound to the filament to transmit forces during force generation. If a dynein c molecule advances a constant distance d in a single association-dissociation cycle, with a large enough ATP concentration the average movement generated by a singe dynein c molecule is d/(strong binding time + other time [total time]). When microtubules on a large number of dynein c molecules move, the microtubules are always bound to one or more dynein c molecules. The velocity induced by multiple dynein c molecules is d/(strong binding time). Thus, the duty ratio is calculated as (velocity for a single molecule)/(velocity for multiple molecules). The sliding velocity of the microtubules induced by dynein c largely depends on the dynein c density on the glass substrate (Fig. 5). Under a low dynein c density condition where each microtubule moves supported at a point, the sliding velocity of the microtubules averages 0.7 $\mu$m/s. On the other hand, as dynein c density increases, the sliding velocity of the microtubules saturates at 5.1 $\mu$m/s. Thus, the duty ratio is estimated to be approximately 14%. During movement, dynein c must preserve its bond with the entire microtubule even when it does not have a one-to-one strong binding with the tubulin molecule in the microtubule. This attachment to the microtubule is considered to be weak so that the dynein c molecules do not inhibit each other's movement.

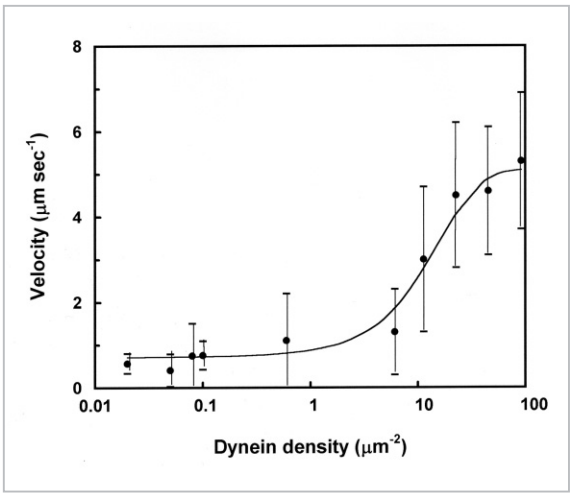


**Fig.5** *Relationship between the sliding velocity of the microtubule and the surface density of dynein c*

The sliding velocity of the microtubule increases as the surface density of dynein c increases. The solid curve is the approximation by an equation obtained based on the model proposed by Uyeda et al. [6].

### 3.3 Mechanical measurement of a single dynein c molecule

The mechanical measurement of a single dynein c molecule was performed using the laser trap technique (Fig. 6). For the measurement, polystyrene beads adsorbing an average of less than 1 active dynein c molecules per single polystyrene bead (1 $\mu$m in diameter) were used. Since the movement of the beads under laser trap is restricted, they do not slide on the microtubule. Nevertheless, continuous movement was apparent across approximately 100 nm (several-folds of a dynein c molecule). (The stiffness of the laser trap was 0.02 pN/nm.) These data also support that dynein c is a processive motor. The force that a single dynein c molecule generated was 1 to 2 pN. A detailed analysis of the bead displacements showed stepwise displacements of 8 nm, which corresponds to the structural period of the tubulin dimer, the protein that constitutes the microtubule. This result indicates that a dynein c molecule interacts with each tubulin molecule when it moves on a microtubule. It was also observed that a dynein c molecule often moved backward by several steps when moving on a microtubule under high load. The frequency of occurrence of the backward movement increases as the load increases. This backward movement according to the load is considered to be due to the capability of dynein c molecules to adjust their output power autonomously using data input into the molecule. These results of in vitro motility assays and mechanical measurement were published in Nature, a famous international scientific journal [4].

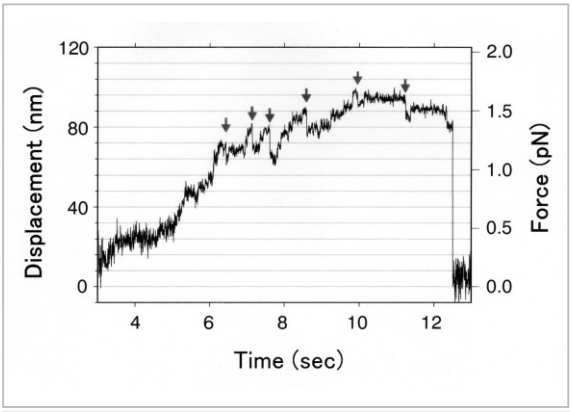


**Fig.6** *Movement of a single dynein c molecule, and power generation.*

When a dynein c molecule interacts with the microtubule, it moves continuously over several steps. The step of the movement is 8 nm, and additional lines are drawn to point out the steps clearly. The arrows indicate backward movement.

## 4 Structure of dynein c and its power stroke

The images of dynein c molecules were analyzed with negative-staining electron microscopy and single-particle image analysis. The results revealed structural detail of dynein c at a resolution of 2 nm. We further performed a comparison of the structures of dynein molecules in different states in the dynein motor cycle, and clarified that a dynein molecule undergoes a structural change of as much as 15 nm throughout the entire molecule during the "power stroke," in which the dynein interacts with the microtubule to generate the sliding force [7].

When dynein c is negatively stained under optimum conditions and observed by electron microscopy, we can clearly see the ring-shaped head, the stalk, and the tail in its molecular image, as shown in Fig. 7. Nevertheless, the dynein c molecules are adsorbed on the carbon film on the grid for transmission electron microscopy in various orientations and in various forms, including straightened and bent structures, producing various images. Thus, image resolution was improved by classifying and averaging images based on the single-particle image analysis. First, over 10,000 dynein molecular images recognizable as single particles were extracted from the electron micrographs of dynein c. The molecular images were re-positioned by translation and rotation so that these could be overlaid on one another. The positioned images were classified into over 200 classes, and then superposed and averaged in each class. The single-particle analysis showed a detailed molecular structure unrecognizable in the original dynein

images (Fig. 8). It is the first observation indicating that the head of a dynein molecule does not have a simple symmetric ring structure but is complicated with different surface structures on each side. It also clearly shows that the head consists of 7 sub-domains (Fig. 8A g).

Next, to examine the structural change during the force generation of dynein, dynein c molecules corresponding to before and after the power stroke were prepared, and structural analysis was performed on them. When vanadate (Vi) interacts with dynein under the presence of ATP, the vanadate is known to bind to the dynein, replacing the phosphate (Pi) to produce a stable complex corresponding to the intermediate state (dynein-ADP-Pi) before the power stroke, based on kinetic analyses [8]. Using vanadate, the dynein c corresponding to the state before the power stroke (dynein-ADP-Vi), and the dynein c with released ADP and phosphate corresponding to the state after the power stroke (apo-dynein) were prepared, and a single-particle image analysis of the electron micrographs was performed with them. The results indicate that several differences exist in the structure of the head before and after the power stroke. The head of the ADP-Vi-dynein is, as shown in Fig. 8A c and d, relatively symmetric and compact, but the gap in the center of the head is unclear. On the other hand, the head of the apo-dynein is, as shown in Fig. 8A g and h, asymmetric, and the gap in the center is clear. The positions at which the stalk and the tail protrude from the head are also different: the positions are significantly separated on the head for the ADP-Vi-dynein while close to each other for the apo-dynein (Fig. 8B). We selected a conspicuous structure in the head, examined the protruding positions, and found that the position of the stalk does not change but the position of the tail moves on the head. A characteristic molecular structure was observed in a small portion, 6%, of the molecules in the sample. The tails of these molecules are approximately 10 nm longer than those observed in the majority of the other molecular images and the protruding position of the tail is at 180 degrees opposite the position observed for the apo-dynein. The extended portion of the tail is on the head in normal molecules. As this portion connects the tail body and the head domain, we call this structure the "linker." During the power stroke, the head rotates by an average of approximately 25 degrees around the base of the linker. Superposing the average class images for the ADP-Vi-dynein and the apo-dynein at the position of the tail has clarified that the section that binds the microtubule (the tip of the stalk) moves nearly 15 nm. The microtubule is believed to move with this stroke. The step size measured in single molecule measurement is 8 nm. However, the stroke size measured in single-particle image analysis corresponds to the unloaded state. Loading may decrease the step size.

Based on the existence of the linker and the comparison between the molecular structures corresponding to the two nucleotide states (Fig. 8B), we have proposed a model for the dynein power stroke. Of the 7 sub-domains in the head of the dynein c, the ATP hydrolysis related to the movement is considered to occur in sub-domain 1, the closest to the tail. When a microtubule binds the tip of the stalk, the structure between head sub-domains 4 and 5 changes. This structure change is transmitted to domains 3, 2, and then 1, promoting the release of ADP+Pi bound to domain 1. As a result, domain 1 changes its structure and the angle at which the linker is connected to domain 1 changes. After this angle change, the linker starts docking along the head ring. The docking of the linker to the head ring rotates the head and, as a result, the stalk tip bound to the microtubule strokes by nearly 15 nm, causing the sliding movement of the microtubule. The power stroke of the dynein would not function without the information transfer from the stalk tip to the head and the collaborative operation of the head sub-domains. The structure change of the head between the ADP-Vi bound state and non-bound state is considered to be due to the cooperative structural change of the head

sub-domains. The detailed structure analysis and the study on the power stroke were published in Nature, a famous international scientific journal [7].

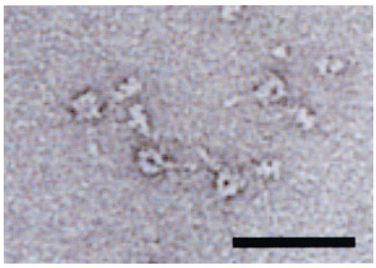

**Fig.7** *Negative-staining electron micrograph of dynein c used in the single-particle image analysis*

The figure clearly shows the microstructures, including the tail and the stalk. The scale is 50 nm.

## 5 Functions of dynein as an information device

Motor proteins have all the key technologies necessary for nano-technology, particularly for constructing nanomachines. For example, motor proteins can construct fibrous assemblies. In a living body, the length and thickness of fibers are precisely controlled. This is an important key technology related to the capabilities of nano-particles for self-organization and self-assembly. Motor proteins are also an ultra-compact actuator that converts chemical energy into mechanical energy. It functions under normal temperatures in aqueous solutions. Despite its input level being only approximately 20 times the thermal noise, its energy conversion efficiency reaches 30% to 100%. The mechanism for this highly efficient energy conversion is knowledge that should be obtained in order to construct nano-meter scale signal processing systems. The research on motor proteins is not only biologically and biophysically important but is also regarded as fundamental research that can provide valuable engineering knowledge.

Dynein is one of the motor proteins that provides the largest amount of fundamental information for nano-technology. A flagellum, a micro-actuator powered by dynein, is autonomously constructed in the sophisticated 9+2 structure by 200 or more proteins (See Fig. 1). Each dynein molecule moves autonomously, organizing and integrating power generation to create a sinusoidal flagellar wave. The present study has found out that dynein c moves several steps backwards according to the load applied. This behavior is believed to occur because dynein c molecules have the capability to calculate data inputs within the molecule to adjust the output power autonomously. In other words, dynein itself has a built-in tension sensor and uses feedback from the output status of other molecules in a living body to reflect and organize the movement of the entire system. Such an autonomous distribution mechanism is considered a key technology in constructing nanomachines. Detailed structure analysis of the dynein before and after the power stroke also indicates that the head sub-domains cooperatively change their structures and that information is transferred between the stalk and the head ring. It is believed that understanding this mechanism will provide information leading to key technologies for inter-dynein communication and activity control in constructing sub-domains in nanomachines.

## 6 Conclusions

The movement of a single molecule of dynein was measured using “dynein c”, a single-headed flagellar dynein. The results show that dynein c is a processive motor and that it interacts with each tubulin molecule during movement on the microtubule. The details of the head structure of dynein were clarified using negative-staining electron microscopy and single-particle image analysis techniques. The head shows a relatively symmetric structure before the power stroke when ADP and Pi are bound to the head, whereas it changes to an asymmetric structure after releasing ADP

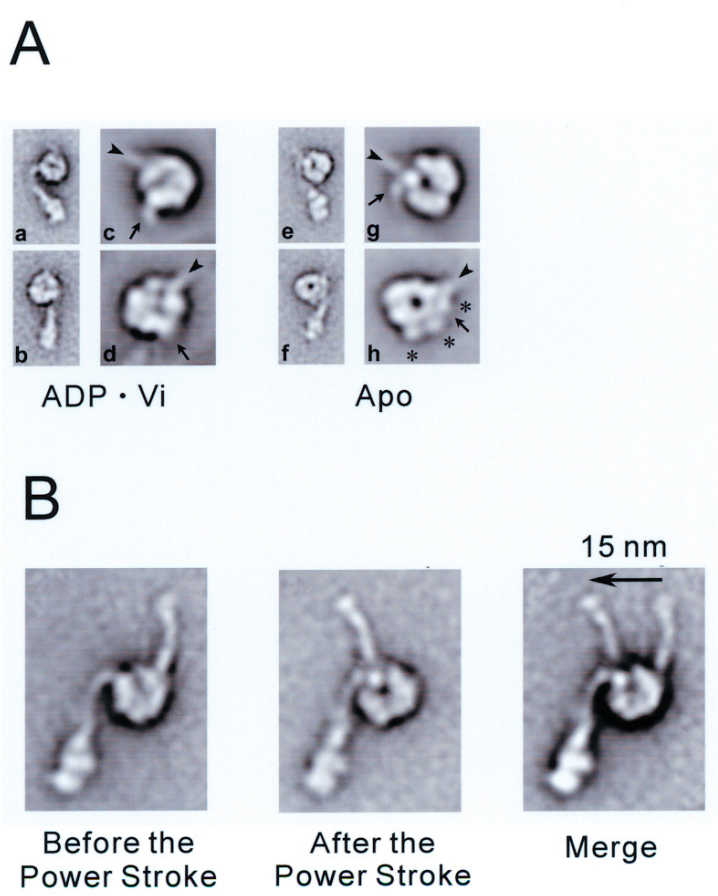


**Fig.8** *Structural change of dynein induced by nucleotides, and its power stroke*

A: Detailed molecular structures of dynein c molecules clarified by single-particle image analysis. According to the adsorption orientation to the supporting film, the molecules face different directions, either left (upper column) or right (lower column). Figures a–d show dynein c in the ADP-Vi state. Figures e–h show dynein c after releasing the hydrolysates. Figures c, d, e, and h are averaged only at the head. The figures clearly show the inner structure of the head. The dynein c in the apo-state has clearer channels in the head and greater asymmetry than the dynein c in the ADP-Vi state. Fig. h shows the sub-domains (*). The arrowheads indicate the base of the stalk, and the arrows indicate the base of the tail. After the power stroke, the bases of the stalk and the tail change positions, moving closer to each other.

B: Structural change of the entire dynein c molecule from before to after the power stroke. As a result of the structural change in the head, the binding site for the microtubule at the tip of the stalk moves by approximately 15 nm.

and Pi. A linker exists on the head to connect the tail, and the entire head rotates during the power stroke.

To elucidate the mechanism of dynein operation, other issues still need to be clarified, including the nature of the weak interaction with which dynein retains the microtubule, the identity of the information transfer between the binding site of the microtubule and that of ATP on dynein, and the interaction between the linker and the head ring.

Dr. Kazuhiro Oiwa (Group Leader), Dr. Hiroaki Kojima, and Ms. Yukako Sakai of the Protein Biophysics Group were the main contributors to the present study. It has been making significant advances via collaboration with Dr. Eisaku Katayama of the Institute of Medical Science at the University of Tokyo and Dr. Stanley Burgess and Dr. Peter Knight of the Structural Molecular Biology group at University of Leeds.

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*The Biophysics of Protein Motors*