Structural studies of the motor protein dynein

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Introduction

Dynein is a family of minus-end directed microtubule motors that function in a wide diversity of cellular processes in eukaryotes including the trafficking of numerous cargoes (e.g. vesicles, mRNA, mitochondria), the positioning of the nucleus, Golgi apparatus and the mitotic spindle as well as driving the propagated-bending waves of cilia and flagella. Dynein is one of three different families of molecular motors, the others being kinesin and the actin-based motor myosin, and by far the least well understood. Dynein is large (~ 520 kDa), with a motor domain ~ten times larger than that of the other microtubule-based motor kinesin and has an evolutionary origin within the AAA+ superfamily of mechanoenzymes, unlike kinesin and myosin.

Little is known about dynein's structure and mechanism. We showed previously by negative stain electron microscopy (EM) that dynein has a stalk-head-tail structure (Fig. 1A). The head is ring-like and contains six AAA+ domains. ATP hydrolysis primarily in AAA1 drives the conformational changes associated with the power stroke and those governing its binding to and release from, the microtubule track via a small domain at the end of the ~12nm long anti-parallel coiled coil of the stalk.

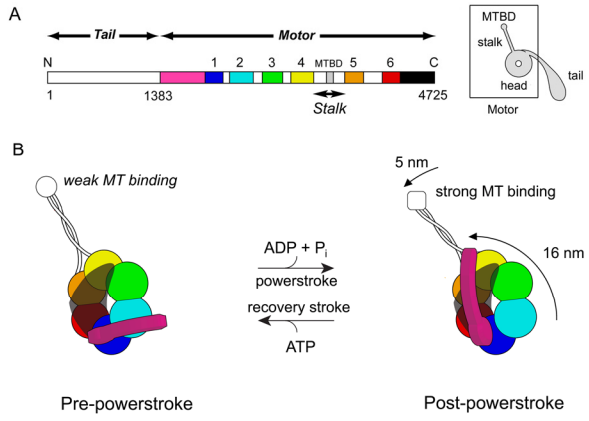

Figure 1
Structure and powerstroke mechanism of dynein. (**A**) Heavy chain sequence showing the subdomain organization including AAA+ modules (1-6), microtubule-binding domain (MTBD) and flanking N- (magenta) and C-terminal (black) sequences essential for motor activity. Inset: cartoon of dynein heavy chain structure. (**B**) New model of dynein motor subdomain organization and power stroke mechanism (unpublished). ATPase activity in AAA1 (blue) drives long-range conformational changes associated with the powerstroke- stalk tilt and linker swing.

Current Research

Structural studies in my lab are focused on understanding the structure and mechanisms of the molecular motors dynein and myosin, with recent emphasis on dynein and its subdomain organization three dimensional (3D) structure.

In collaboration with Prof. Kazuo Sutoh's group (University of Tokyo) we have mapped the locations of key sites within the motor domain using GFP-labeled fusion proteins and truncated motor domain constructs. We have identified three of the AAA+ modules within the ring, as well as the locations of flanking N- and C-terminal sequences (Fig. 1B). We show that the N-terminal sequence defines an elongated lever which swings this lever swings by > 90° during the powerstroke and the stalk tilts by 15° (Fig. 1B).

In collaboration with Prof. Kazuhiro Oiwa's group (KARC, Kobe, Japan) we are also pursuing the 3D structure of the motor domain by cryo-EM. We have obtained 3D density maps of the motor in both pre- and post-powerstroke conformations for the first time. These studies have led us to propose a new model for the structure and mechanism of dynein motors (Fig. 1B).

Finally, in collaboration with Dr. Tom Edwards (University of Leeds) and Dr. Dan Mulvihill (University of Kent), we are pursuing atomic resolution structures of subdomains of the motor. Expression trials of various subdomains are currently underway.

Publications

Kotani, N., Sakakibara, H., Burgess, S.A., Kojima, H. & Oiwa, K. (2007) Mechanical properties of inner-arm Dynein-F (Dynein I1) studied with *in vitro* motility assays. *Biophys. J.* **93**, 886–894.

Burgess, S. A., Yu, S., Walker, M. L., Hawkins, R. J., Chalovich, J. M. & Knight, P. J. (2007). Structures of smooth muscle myosin and heavy meromyosin in the folded, shutdown state. *J. Mol. Biol*. **372**, 1165-1178.

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