Cryo-EM of cytoplasmic dynein motors during movement on microtubules

Hiroshi Imai, Stan Burgess and Peter Knight

Introduction

Cytoplasmic dynein uses ATP to fuel the transport of diverse cargoes along microtubules (MTs) from their plus to minus ends, which generally means cargoes are carried from the periphery of the cell towards the nucleus. Viruses can hi-jack this system in vertebrate neurones to be carried from a peripheral site of infection deep into the spinal cord. This dynein is a large complex of two massive dynein heavy chains, each ~500 kDa, associated with various smaller chains and with other macromolecular complexes that together form the functional transporter. Each heavy chain is the ATPase (a member of the AAA^+ family) that contains six concatenated AAA^+ modules that form a ring. The six modules have evolved distinct functions: AAA1 is the primary ATPase, AAA4 includes an 11-nm coiled-coil extension at the tip of which is the stalkhead that reversibly binds to the MT, AAA5 & 6 cannot bind ATP and may play a structural role as transmitters of allosteric information between the ATPase site and the MT binding site ~25 nm away. Extending across the ring from the N-terminus of AAA1 is the rod-like linker that is thought to switch from bent to straight to generate the powerstroke of the motor, and continues as the tail that dimerises dynein and forms larger complexes, including with cargo.

The structural basis for the movement of dynein along MTs is unknown because the molecule has not been visualised during stepping. We have worked with a minimal dynein motor construct in which dimeric glutathione S-transferase (GST) replaces the tail, and used flash-freezing, cryo-EM and image processing to see the dimeric motor during stepping along MTs at a physiological ATP concentration.

Results

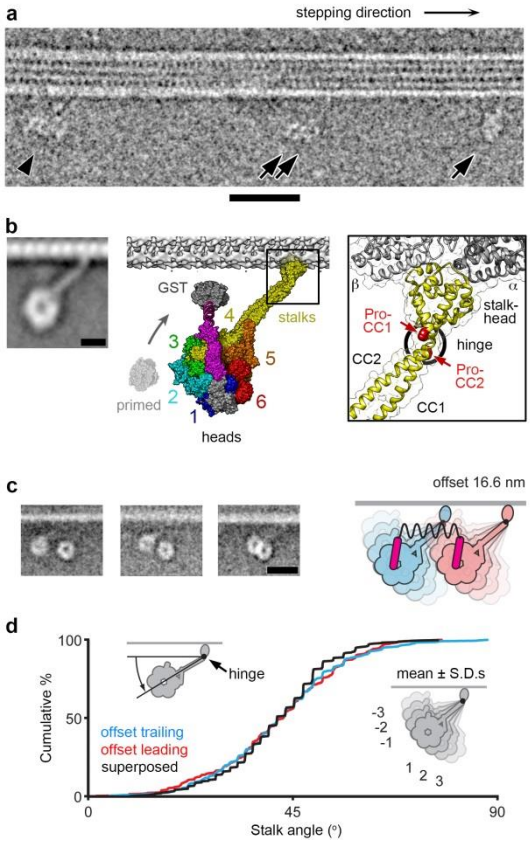

Figure 1: Dynein dimers stepping along MTs have diverse structures. (**a)** Raw cryo electron micrograph shows superposed motors (single arrow), offset motors (two arrows) and multiple dimers (arrowhead); scale bar 40 nm. (**b)** superposed dimers. Left panel is an averaged image of dimers attached to the edge of MT; scale bar 8 nm. Centre panel is an atomic model based on high resolution structures of dynein, GST and MT. The AAA modules are numbered. The location expected for GST in the 'primed', pre-powerstroke state is also shown. Right panel shows detail of the boxed region of the model, highlighting the proline residues at the hinge. (**c)** Offset dimers. Left panels are averaged images showing diversity in separation between the two rings and in their distance from the MT surface; scale bar 20 nm. Right panel diagram illustrates the swinging of the two motors about their hinges, and the spring-like connection between them (black coil spring). (**d)** quantitative analysis of flexibility at the stalk-stalkhead hinge shown as a percentage cumulative frequency (pcf) plot for the three classes of motors. Insets show the stalk angle measured and the magnitude of the Normally-distributed flexibility.

We find two distinct appearances of the dimeric motor: one in which the two rings are variably offset along the MT axis and another, equally abundant, in which the two rings are closely superposed (Fig. 1a). The abundance of superposed motors is unexpected and it suggests that the two motors interact. The superposed stalks are strongly angled to the MT pointing in the direction of movement of dynein along the MT, and the stalkhead can be seen binding at the interface of the α–β tubulin dimers in the MT. The GST dimerisation domain is visible between the superposed rings and MT, indicating that the linker is in the straight, unprimed state in both motors, which further indicates that both stalkheads are bound to the MT, on adjacent protofilaments (Fig. 1b). We find marked flexibility in the superposed dimer with a hinge at the stalk-stalkhead junction. The structural origin of this hinge is probably a pair of conserved proline residues that locally upset the α–helical structure of the stalk. It provides a basis for the highly variable step lengths found in dynein.

By contrast, offset dimers are highly variable in appearance with each ring flexing quasi-independently of its partner, with a wide range of separations between them. This means that each dimer is different in structure, posing challenges for obtaining detailed structures by image averaging (Fig.1c). Nevertheless we can deduce the stalk angles of each motor, and they show a similar wide spread as in superposed dimers (Fig. 1d). We can also make deductions about the pN forces experienced within the dimer, as we find that when rings are further apart, the connection between them acts as a spring, pulling the trailing stalk to a higher angle, and the leading stalk to a lower angle. These first images of stepping dynein open the way to understanding the structural mechanism of this remarkable motor protein.

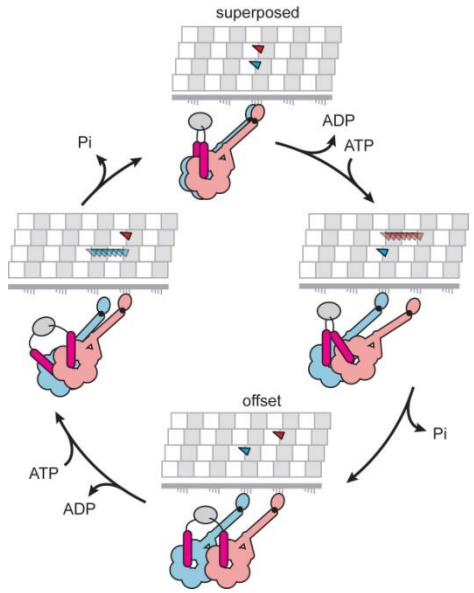

Figure 2: A model for a stepping cycle of dynein on the MT. It shows an example of a 2-step advance along the MT, tentatively correlated with consumption of two molecules of ATP, and showing the stalkhead binding sequence both as viewed from the rings looking at the MT surface (upper diagrams) and in the view seen in our data. The mobile part of the linker is illustrated (magenta) switching to the primed conformation during stalkhead detachment, as well as a possible trajectory of each detached stalkhead along the MT protofilament. In this example, 8-nm stepping occurs along two adjacent protofilaments and superposed and offset dimers alternate during stepping, illustrated here as an inchworm progression. The great flexibility at the stalk-stalkhead junction allows the detached motor explore the surface of the MT and take longer steps.

Publications

Imai H., Shima T., Sutoh K., Walker M.L., Knight P.J., Kon T. & Burgess S.A. (2015) Direct observation shows superposition and large scale flexibility within cytoplasmic dynein motors moving along microtubules. *Nat. Commun.* **6**: 8179.

Funding

Funded by the BBSRC and the Human Frontiers Science Programme Organization.

Collaborators

External: T. Shima (RIKEN, Japan); K. Sutoh (Waseda University, Japan); M. Walker (Launceston, UK); T. Kon (Osaka University, Japan).