ATP-driven remodelling of the linker domain in the dynein motor

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Introduction

Dynein ATPases are the largest known cytoskeletal motor proteins in eukaryotic cells and perform critical functions: carrying cargo along microtubules in the cytoplasm and powering flagellar beating (Figure 1). Dyneins are members of the AAA+ superfamily of ring-shaped enzymes, but how they harness this architecture to produce movement is poorly understood. The work described in this report was focused on the fundamental mechanism that underlies movement and force production along microtubules. Dynein motors are also the largest of the three families of molecular motors- each motor domain being ~380 kDa in size, located within a ~520 kDa heavy chain that typically forms a much larger motor complex comprising between one and three heavy chains and numerous smaller polypeptide chains.

Results

We have used cryo-electron microscopy and single-particle image processing to determine (*ab initio*) three-dimensional maps of a native (full-length) flagellar dynein (from the single-celled alga *Chlamydomonas*) and an engineered cytoplasmic dynein motor domain (from the slime mold *Dictyostelium*) lacking the cargo-binding tail domain, in different nucleotide states. The structures show key sites of conformational change within the AAA+ ring and a large rearrangement of the "linker" domain, involving a hinge near its middle. Analysis of a mutant in which the linker "undocks" from the ring indicates that linker remodeling requires energy that is supplied by interactions with the AAA+ modules.

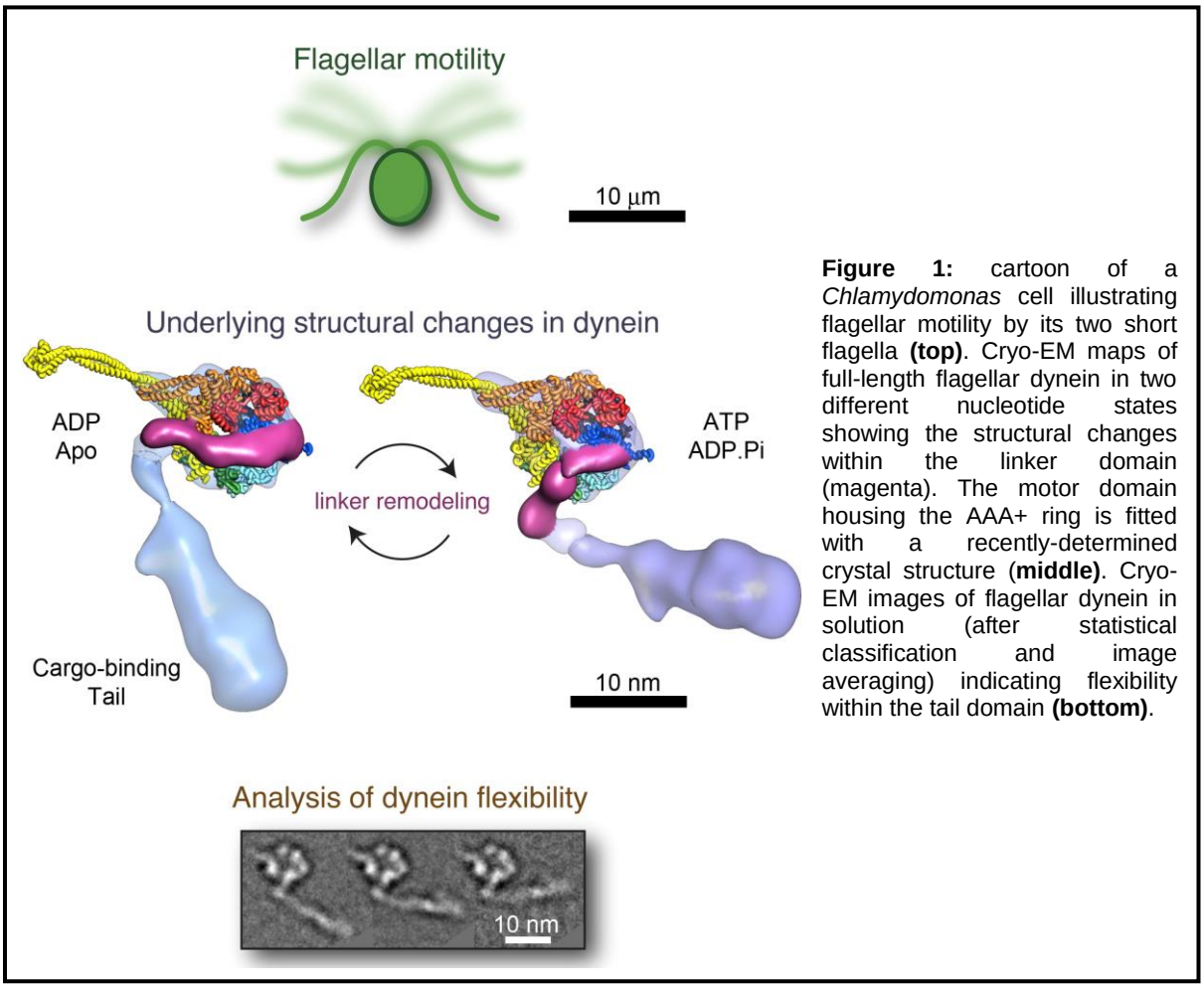

Figure 1: cartoon of a *Chlamydomonas* cell illustrating flagellar motility by its two short flagella **(top)**. Cryo-EM maps of full-length flagellar dynein in two different nucleotide states showing the structural changes within the linker domain (magenta). The motor domain housing the AAA+ ring is fitted with a recently-determined crystal structure (**middle)**. Cryo-EM images of flagellar dynein in solution (after statistical classification and image averaging) indicating flexibility within the tail domain **(bottom)**.

Remodelling of the linker is important for dynein because this domain connects directly to the cargo-binding tail domain. Analysis of individual cryo-EM images of full-length dynein shows that this tail domain is flexible in solution. Fitting our full-length dynein structures into lower resolution tomograms of whole flagella suggests how this mechanism could drive microtubule sliding that underlies the beating of cilia and flagella. The resulting configuration of dynein binding to microtubules also has implications for the mechanism of stepping by the dimeric cytoplasmic dynein that underlies cargo transport in many processes in eukaryotic cells essential for life.

Publications

Roberts, A., Malkova, B., Walker, M., Sakakibara, H., Numata, N., Kon, T., Ohkura, R., Edwards, T., Knight, P., Sutoh, K., Oiwa, K. & Burgess, S. (2012) ATP-driven remodeling of the linker domain in the dynein motor. *Structure* **20:** 1670-1680.

Funding

This work was supported by the BBSRC, the Wellcome Trust and the Human Frontiers Science Program Organization.

Collaborators

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