Structural studies of the motor proteins dynein and myosin

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Introduction/Background

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.

My lab is interested in discovering the mechanism of action of the motor domain of dynein. We have shown by electron microscopy (EM) that dynein has a stalk-head-tail structure. 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.

In collaboration with Prof. Kazuo Sutoh's group (University of Tokyo) we mapped the locations of key sites within the motor domain using GFP-labeled fusion proteins and truncated motor domain constructs. We showed that the N-terminal sequence defines an elongated lever which undergoes a nucleotide-driven swinging action. We also showed that sliding of the two a-helices in the stalk governs microtubule-binding and ATP hydrolysis by dynein.

Main body

Studies in my lab are focused on understanding the structure and mechanisms of the molecular motor dynein alongside continuing studies of myosin motors in collaboration with Profs Peter Knight and John Trinick within the Astbury Centre.

My lab is pursuing the 3D structures of dynein by cryo-EM (in collaboration with Prof. Kazuhiro Oiwa's group, KARC, Kobe, Japan) and recombinant cytoplasmic dyneins (in collaboration with Prof. Sutoh and Dr. Kon, University of Tokyo), funded by BBSRC. In collaboration with Dr Andrerw Carter (LMB-MRC) we are examining by EM recombinant dynein's from fungi.

A new project funded by the Human Frontiers Science Program (HFSP) is to investigate the biochemical and biophysical properties of dimeric cytoplasmic dynein bound to microtubules as well as their structure(s) by cryo-EM. The collaborators in my team are Dr. Takahide Kon and Prof. Hideo Higuchi (University of Tokyo) and Dr. Andrej Vilfan (Ljubljana, Slovenia).

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

Kon, T., Imamula, K., Roberts, A., Ohkura, R., Knight, P., Gibbons, I., Burgess, S. and Sutoh, K. (2009) Helix sliding in the stalk of dynein modulates ATPase and microtubule binding. *Nat Struct Mol Biol* **16**:325-333.

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Oke, O., Burgess, S., Forgacs, E., Knight, P., Sakamoto, T., Sellers, J., White, H. and Trinick J. (2010) Influence of lever structure on myosin 5a walking. *Proc Natl Acad Sci USA* **107**:2509-2514.

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Collaborators
Prof. Kazuhiro Oiwa and Dr Hitoshi Sakakibara, KARC, Kobe, Japan
Prof. Kazuo Sutoh and Dr. Kon, University of Tokyo, Japan
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