

Template-free 13-protofilament microtubule-MAP assembly visualised at 8 Å resolution

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Microtubules are long protein filaments that are part of the cell cytoskeleton and play an important part in processes such as mitosis and cell migration that are necessary for cells to grow, function and divide.

They are formed from many copies of a small protein called tubulin. The soluble form of this protein is a dimer of alpha- and beta- tubulin; these dimers polymerise head-to-tail, driven by GTP-GDP exchange, to form long fibrous protofilaments. These in turn associate into a cylindrical bundle or hollow tube of fibres that is termed a microtubule. Most microtubules *in vivo* are formed from thirteen protofilaments, arranged in a parallel, left-handed helix, although microtubules containing other numbers of filaments are not unknown.

Microtubules are stabilised - or, sometimes, destabilised - in different cellular contexts *in vivo* by association with different proteins, known generically as microtubule-associated proteins or MAPs. One of these is doublecortin, which is a stabilising MAP known to bind preferentially to 13- protofilament microtubules. Mutations in both tubulin and doublecortin that destabilise this association can cause devastating neuro-developmental disorders such as lissencephaly. Carolyn Moores, from the Department of Biological Sciences, Birkbeck, University of London and a core member of the Institute of Structural and Molecular Biology, working with colleagues in the ISMB and in two institutes in Paris, has now used electron microscopy to visualise the structure of doublecortin bound to the microtubule at 8 Ångstroms resolution [1]. This structure has yielded some important insights into the mechanisms of doublecortin binding and microtubule stabilisation *in vivo*.

When the protofilaments associate to form a microtubule, the side-to-side contacts are made between alpha and alpha and between beta and beta subunits. However, the geometry of the cylindrical microtubule requires it to have a single

discontinuity at which alpha and beta subunits make side-to-side contacts; this is termed the seam. Doublecortin is known to bind at the corner of four tubulin dimers [Figure 1].



Figure 1: Schematic diagram illustrating a DC domain of doublecortin (DC) bound at the corner of four tubulin dimers, in contact with two alpha and two beta subunits.

Moores and her co-workers used cryo-electron microscopy and single particle reconstruction to visualise the structure of doublecortin bound to microtubules which also had the motor domain of the protein kinesin-1 bound. This confirmed the findings from a previous, lower-resolution structure that the doublecortin molecules were wedged into the “valleys” between protofilaments, with a spacing of about 80 Ångstroms along the long axis of the microtubule [2]. The current higher resolution study revealed that doublecortin binds all lateral dimer-dimer interfaces in the microtubule except the seam, and the specific lattice geometry of the seam is preserved in the doublecortin-bound structure.

Averaging together the structures of all doublecortin binding sites yielded structures of the doublecortin-tubulin interface at 8 Ångstroms resolution, which is high enough for secondary structure elements and some protein loops to be clearly seen. The visible doublecortin structure

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corresponds to one of the two doublecortin (DC) domains in the full-length molecule. The electron density of doublecortin visible at the binding site matched the atomic-resolution structure of one DC domain [3], and electron density representing a chain of about five amino acids was also observed lying along the interface between tubulin dimers. The two DC domains are linked by a chain of 38 amino acids in the intact molecule, and from this reconstruction alone it is feasible but not proven that two domains from the same molecule bind at adjacent sites on the microtubule. It is equally possible from this data that only one domain from each doublecortin molecule binds the microtubule, with the rest of the molecule, including a second DC domain, unbound.

Atomic coordinates of the DC domain [3] and of the tubulin dimer [4] could be fitted clearly into the electron density of the complex structure, and this modelling has revealed some important structural features of the tubulin-doublecortin interface [Figure 2].

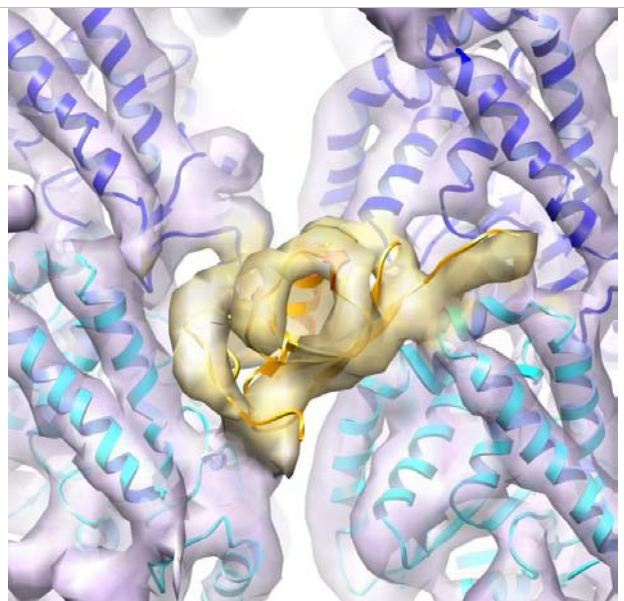


Figure 2: Electron density map of doublecortin (yellow) at the interface between four tubulin molecules (alpha subunits in blue and beta subunits in cyan). Ribbon representations of each protein's structure are docked into the electron density and key residues labelled.

Some tubulin residues that differ between alpha- and beta-tubulin are found close enough to the interface with doublecortin to explain why the DCX domain cannot bind to the seam with its different organisation of alpha- and beta-tubulin domains. Furthermore, mutations in both molecules that are

known to cause lissencephaly and other neuronal disorders were seen to occur at the interface between the proteins and to disrupt the stabilising interactions between doublecortin and the microtubule.

This structure also allowed the researchers to visualise details of the interface between tubulin dimers in adjacent proto-filaments in the microtubule. The tubulin molecules in 13-protofilament microtubules are in a straight conformation. Many of the close contacts between adjacent dimers occur through loops that are conserved in structure but divergent in sequence between the alpha- and beta-tubulin molecules. Lateral interactions between alpha and beta tubulin molecules occur only at the microtubule seam. The seam, which lacks bound doublecortin, is clearly visible in this structure, and its structure appears to indicate that it is stabilised largely by the rest of the microtubule lattice.

Moores and her co-workers speculate that the binding of doublecortin at the interface of two tubulin dimers, between two alpha- and two beta- subunits, could be the smallest structure that could act as a nucleus for microtubule formation. Since doublecortin only binds the most common, thirteen-protofilament microtubules, they suggest further that other microtubule-associated proteins will be found that bind to and stabilise rarer microtubule architectures.

References

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