

Distinct roles of doublecortin modulating the microtubule cytoskeleton.

The following commentary was written by Dr Clare Sansom and Dr Carolyn Moores. The original article was published in the October 2006 issue of EMBO Journal [1]: Moores CA, Perderiset M, Kappeler C, Kain S, Drummond D, Perkins SJ, Chelly J, Cross R, Houdusse A and Francis F. Distinct roles of doublecortin modulating the microtubule cytoskeleton. EMBO J. 2006, 25, 4448-4457

Microtubules are long protein filaments that form an essential part of the cytoskeleton and are involved in cellular processes including mitosis and cell migration. In living cells, they continually grow and shrink, and the balance between these processes is crucial for normal cell function. Many proteins including tau, which has been linked to Alzheimer's disease, are known to bind to microtubules and to be involved in controlling this dynamic balance.

Doublecortin [2] is a microtubule-associated protein that is expressed in neurons within the central nervous system and is known to be vital for normal brain development. Mutations in the doublecortin gene are associated with a type of brain disease called lissencephaly. This developmental disorder is characterised by a malformation in which the brain surface appears smooth, rather than convoluted, and it causes epilepsy and severe mental retardation. How this protein influences the stability and dynamic balance of microtubules, and therefore brain development, has until now been uncertain. Carolyn Moores from Birkbeck College London, and a core member of the Institute of Structural Molecular Biology (ISMB), with fellow ISMB member Steve Perkins from University College London's Department of Biochemistry and Molecular Biology and collaborators from the Marie Curie Research Institute, Oxted, and Paris, has now solved part of this mystery. Moores and co-workers have examined the ways doublecortin interacts with microtubules and have also showed that microtubules that are stabilised by doublecortin can act as "tracks" for the molecular motors, kinesins. These discoveries shed light on the unique function of this protein in regulating microtubule dynamics and function, and may illustrate why doublecortin mutations

have such a devastating effect on brain development.

Microtubules are, as their name implies, tube-like structures about 24 nm (2.4×10^{-5} mm) in diameter. The "building block" from which they are formed is a protein called tubulin, which occurs in solution as a dimer of two similar subunits known as alpha- and beta-tubulin. These dimers polymerise end to end to form protofilaments, and these associate together to form the hollow tubes that are the microtubules. Microtubules in cells are made up of 13 protofilaments arranged in an approximately helical formation, although microtubules with other numbers of protofilaments can occur (Figure 1).

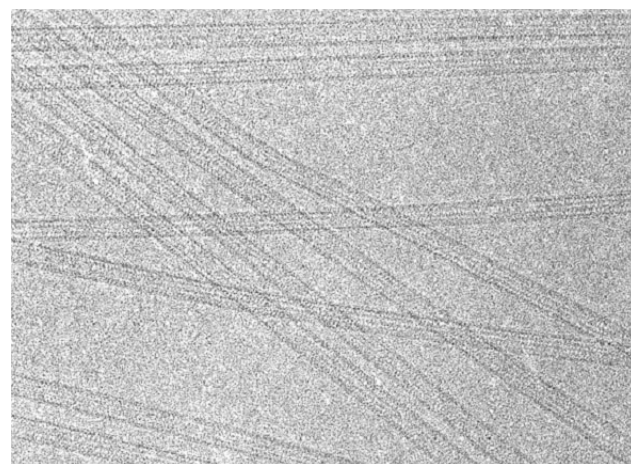


Figure 1: Cryo-electron micrograph of microtubules. These microtubules were grown in vitro and have a variety of protofilament architectures, reflected in differences in their diameters.

Doublecortin binds to microtubules between the protofilaments (*Figure 2*) and is known to preferentially stabilise those containing 13 such protofilaments [3]. Mutations that are known to cause lissencephaly cluster in the domains that bind to the microtubules, which underlines further the importance of doublecortin binding for normal microtubule function [4].

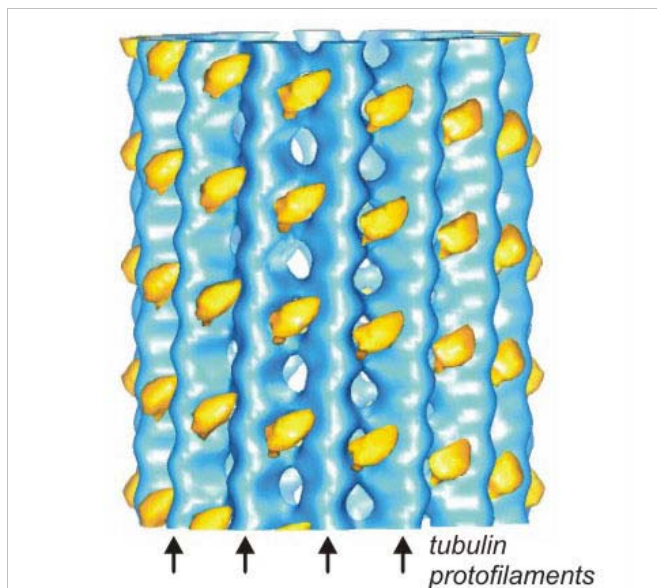


Figure 2: Low resolution 3D reconstruction of a doublecortin-bound microtubule calculated using cryo-electron microscopy images [3]. The microtubule is blue and doublecortin is yellow.

Moore and her co-workers used microscopy to examine microtubule structure and function in the presence and absence of doublecortin. Firstly, they examined the microtubule system using light microscopy in order to probe the effect of doublecortin on microtubule growth. They found that the microtubule growth rate in the presence of doublecortin was the same as for pure isolated microtubules. However, increasing the concentration of doublecortin inhibited microtubule shrinkage, clearly indicating that doublecortin stabilises microtubules.

One of the principal functions of microtubules is as “molecular tracks” along which kinesins and related proteins “walk” in

order to transport other cellular components around the cell. Migrating neuronal growth cones

require a steady stream of such components. Electron micrographs of doublecortin bound to microtubules have suggested that the doublecortin might protrude from the grooves between protofilaments and that it might therefore impede kinesin movement along the microtubules. Moore and co-workers therefore examined kinesin motion along doublecortin-stabilised tracks and found, surprisingly, that doublecortin binding did not prevent walking by kinesin motors. This means that cellular transport can occur along doublecortin microtubules and, significantly, that cellular transport will probably be disrupted when doublecortin is missing.

Cells organise their microtubule cytoskeleton using several mechanisms and contain a microtubule organising centre (MTOC) from which new microtubules are grown. In neurons, however, microtubules must also be formed in the growth cones, which are located at some distance (at the cellular scale) from the MTOC. The mechanism of microtubule formation in the growth cone is not yet understood. Knowing that doublecortin has been shown to nucleate microtubules *in vitro* [3], and that it is concentrated in the extremities of migrating neurons [5], Moore hypothesised that it was implicated in this mechanism. She first showed that microtubule nucleation occurred when tubulin was incubated with doublecortin at 37°C.

Electron micrographs of microtubules forming in the presence of doublecortin (*Figure 3*) showed, in the early stages, many small sheets of protofilaments; later, the only structures visible were complete microtubules comprised of exactly 13 of them. Together with the knowledge that doublecortin binds between protofilaments [3], this suggests strongly that one function of doublecortin is to stabilise lateral contacts between protofilaments as the microtubule walls form.

These results are all consistent with the theory that doublecortin can nucleate and stabilise neuronal microtubules both independently of, and remote from, the microtubule organising centre. Moore further proposed the intriguing theory that the doublecortin-rich zones located

at the distal end of migrating neurons [5] could be “factories for stable microtubules” that would modify the cell cytoskeleton in response to external stimuli and so promote neuronal migration.

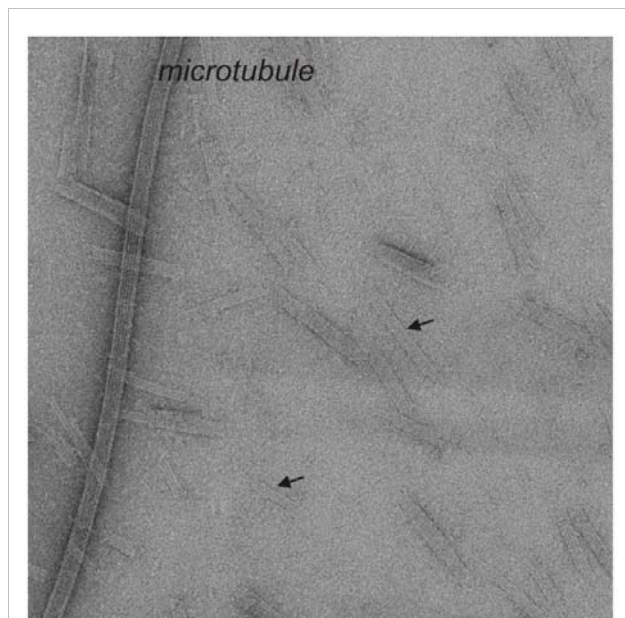


Figure 3: Electron micrograph, prepared by negative stain, of the early stages of microtubule growth in the presence of doublecortin. A single, completely polymerised microtubule is present along with many small protofilament sheets (examples of which are indicated by arrows) that ultimately anneal to form 13 protofilament microtubules [1].

It is clear that mutations in doublecortin give rise to errors in the formation and regulation of the cytoskeleton that have very serious consequences for human health; insights like these into the normal mechanism of its regulation may, eventually, lead to potential treatments for one of the more devastating of genetic diseases.

References

- [1] Moores CA, Perderiset M, Kappeler C, Kain S, Drummond D, Perkins SJ, Chelly J, Cross R, Houdusse A and Francis F. Distinct roles of doublecortin modulating the microtubule cytoskeleton. *EMBO J.* 2006, **25**, 4448-4457

 - [2] des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrie A, Gelot A, Dupuis E, Motte J, Berwald-Netter Y, Catala M, Kahn A, Beldjord C, & Chelly J. A novel CNS gene required for neuronal migration and involved in X-linked subcortical laminar heterotopia and lissencephaly syndrome. *Cell* 1998, **92**, 51-61

 - [3] Moores CA, Perderiset M, Francis F, Chelly J, Houdusse A, & Milligan RA. Mechanism of microtubule stabilisation by doublecortin. *Molecular Cell* 2004, **18**, 833-839.

 - [4] Kim MH, Cierpicki T, Derewenda U, Krowarsch D, Feng Y, Devedjiev Y, Dauter Z, Walsh CA, Otlewski J, Bushweller JH, & Derewenda ZS. The DCX-domain tandems of doublecortin and doublecortin-like kinase. *Nature Structural Biology* 2003, **10**, 324-33

 - [5] Schaar BT, Kinoshita K & McConnell S. Doublecortin microtubule affinity is regulated by a balance of kinase and phosphatase activity at the leading edge of migrating neurons. *Neuron* 2004, **41**, 203-213
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