CYTOSKELETAL PROTEINS

Blades of microtubule scissors

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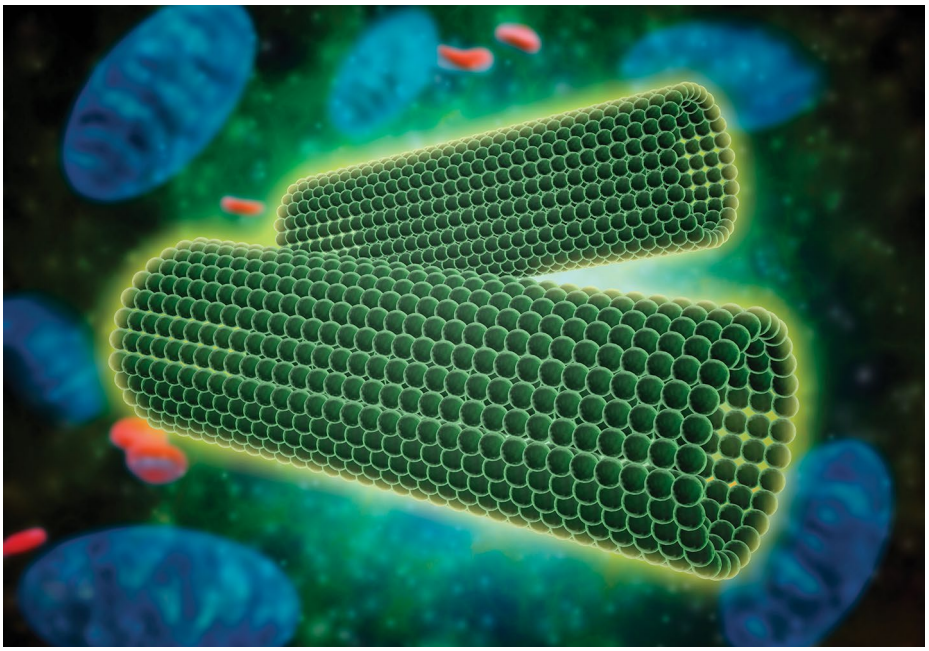

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Microtubules are highly conserved cytoskeleton polymers that are composed of tubulin dimers. They play diverse functions in all types of eukaryotic cells, such as involvement in cell division, intracellular trafficking, cell mobility and cell shape maintenance. For example, in plant cells, the cortical microtubules are essential to guide the correct patterning of cellulose microfibrils, thus controlling anisotropic cell growth. Functional microtubules require active dynamics, including the events of nucleation, assembly, disassembly and severing, in which there are various microtubule-associated proteins (MAPs) involved. One of the best-studied MAPs is katanin, an AAA ATPase that possesses microtubule severing activity to work as microtubule scissors. Recently, Antonina Roll-Mecak's group at the National Institute of Neurological Disorders and Stroke, United States, used single particle cryo-electron microscopy (cryo-EM) to determine high-resolution protein structures of the full-length katanin catalytic subunit in complex with a substrate so that detailed structural features and motions of the scissor blades were demonstrated.

Before resolving the protein structure, Zehr and Szyk et al. tested which tubulin domain is the major binding substrate of katanin. Using multiple in vitro and in vivo assays, they found that the polyglutamate C-terminal tail of β-tubulin is necessary and sufficient for katanin recruiting and severing. Therefore, they determined the cryo-EM structure of the *Caenorhabditis elegans* katanin catalytic subunit p60 (with a mutation that prevents ATP hydrolysis) in complex with a polyglutamate peptide that mimics the tubulin tails. Two reconstructions at high resolution (3.5 Å and 4.2 Å) revealed a spiral and a ring conformation of the katanin hexamer with the tubulin tail engaged with different residues in the central pore. This indicates that the tubulin tail may translocate through the central pore by alternating the binding sites with katanin, which experiences conformational changes during ATP hydrolysis. They also conducted site mutagenesis on p60 and/or the tubulin tail to investigate the residue and electric charge requirements for the binding and severing activity. Moreover, the structure-based models and further mutagenesis analysis are employed to show that two of the pore loop spirals play the main role in recognizing the tubulin β-tail through multivalent charged interactions. Additionally, this recognition mechanism is likely shared with another microtubule severing enzyme, spastin.

Katanin has similar functions in animals and plants. Although there is substantial sequence divergence between the animal and plant p60s, the mechanisms of core substrate recognition and catalysis can be highly conserved.

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