Poster Presentation

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Tubulin tyrosine ligase - structural and functional studies

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Microtubules are polymers essential for cell morphogenesis, cell division and intracellular transport. This polymer's basic building block is the α/β tubulin heterodimer, which associates head-to-tail and laterally to form the microtubule. Tubulin is subject to diverse, abundant and evolutionarily conserved post-translational modifications that mark subpopulations of microtubules. The highest density and variety of post-translational modifications are found in neurons or cilia. Not surprisingly, tubulin modification enzymes have been linked to human diseases including cancers and neurodegenerative disorders. We will present our recent work using a combination of X-ray crystallography, small angle X-ray scattering and functional assays to investigate the mechanism of tubulin tyrosine ligase (TTL). TTL catalyzes the ATP-dependent post-translational addition of a tyrosine to the C-terminal end of detyrosinated α-tubulin. The detyrosination/tyrosination cycle regulates recruitment of motors and proteins that track with the growing end of the microtubule. TTL function is essential for neuronal development and reduction in TTL levels is strongly associated with aggressive tumors resistant to chemotherapy. Our first X-ray crystal structure of TTL, defines the structural fold of the TTL-like family of tubulin-modifying enzymes. We show that TTL recognizes tubulin via a dual strategy: it engages the tubulin tail through low-affinity, high-specificity interactions through a conserved positively charged surface, and co-opts what is otherwise a homo-oligomerization interface in structurally related enzymes to form a tight hetero-oligomeric complex with tubulin. TTL forms an elongated complex with the tubulin dimer and prevents incorporation of the dimer into microtubules by capping the tubulin polymerization interface. Interestingly, TTL and stathmin, a ubiquitously expressed tubulin sequestering protein, compete for tubulin binding in vitro and stathmin inhibits tubulin tyrosination. These results suggest that TTL and stathmin have either a partially overlapping footprint on the tubulin dimer or that stathmin induces a tubulin conformation incompatible with stable TTL binding.

[1] *Garnham C.P., Roll-Mecak A., Cytoskeleton (2012) 69(7), 442-63,* ***[2]*** *Szyk A., Deaconescu A.M., Piszczek G., et al., Nat. Struct. Mol. Biol. (2011) 18(11), 1250-8,* ***[3]*** *Szyk A., Piszczek G., Roll-Mecak A., J. Mol. Biol. (2012) 425(14), 2412-14*

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