

Title of Project	Structure and function of microtubule nucleation proteins
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Summary of project
<p>The project will combine structural biochemistry analysis of microtubule nucleation proteins in vitro with functional analysis in vivo using yeast genetic methods and imaging. Microtubules (MTs) are present in all eukaryotic cells and have critical roles in cell division, intracellular transport, cell motility, and neuronal function. In vivo, MTs are nucleated by a megadalton-sized multiprotein complex called the gamma-tubulin complex (γ-TuC). Our group identified the Mto1/2 complex as a critical regulator of the γ-TuC in fission yeast <i>S. pombe</i>. Mto1/2 is composed of multiple copies of the proteins Mto1 and Mto2, and mutations in the human homolog of Mto1 cause microcephaly. Recently we reconstituted MT nucleation in vitro using a "holocomplex" of purified recombinant γ-TuC and Mto1/2, thereby defining a minimal set of proteins sufficient for nucleation (six different polypeptides, each present in multiple copies). The project will involve purification, biochemical characterisation, crystallization and X-ray structural analysis of components of γ-TuC and Mto1/2, individually and/or in combination. Structural information gained from this research will then be used to formulate hypotheses about the functional importance of specific amino acids and protein-protein interactions within the Mto1/2- γ-TuC holocomplex. To test hypotheses, we will generate mutations in the relevant genes in fission yeast, and analyse how this affects MT nucleation in vivo. We will also be able to assay function by reconstitution of mutant holocomplex in vitro.</p>