Emmanuel Nsamba Ph.D. Defense Seminar

Major: Genetics and Genomics; **Major Professor:** Mohan Gupta

Tuesday, July 13, 2021 • 12-1 p.m.

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Molecular and Functional Characterization of Tubulin Isotypes in Budding Yeast (*Saccharomyces cerevisiae*)

Abstract: Microtubules (MTs) are intrinsically dynamic polymers comprised of tubulin protein, which is a heterodimer of α-and β-subunits. They are found in all eukaryotic cells and underlie diverse biological processes, including cell division, intracellular transport, and axon formation. There is no question that utilizing multiple tubulin isotypes is a common strategy underlying the diverse roles of the MT cytoskeleton. Indeed, most eukaryotes express multiple variants, or isotypes, of α- and β-tubulin that copolymerize to form individual MTs. Yet, how each isotype contributes to MT-dependent functions is extremely limited in any organism. The need for this understanding is highlighted by the expanding set of mutations in specific human tubulin isotypes known to cause a range of fertility and neurological disorders, collectively referred to as tubulinopathies.

This question is highly tractable in simple model systems because, relative to humans, they encode fewer α-and β-tubulin variants, use less tubulin post-translational modifications, and are compatible with a range of genome-editing techniques. Budding yeast (*Saccharomyces cerevisiae*) is an advantageous model for tubulin isotype study because it uses just one β-tubulin (*TUB2*) and two α-tubulin isotypes (*TUB1* and *TUB3*), without PTMs, to build MTs for distinct tasks. Additionally, proteins regulating the yeast MT cytoskeleton are conserved in higher organisms, including humans. Consequently, yeast has been an integral system for elucidating the molecular basis of tubulinopathies.

In this dissertation, I developed otherwise isogenic strains expressing only one of the α-tubulin isotypes, termed Tub1-only and Tub3-only cells. Many studies have established the two primary functions of the microtubule cytoskeleton during yeast mitosis – spindle positioning and chromosome segregation. We utilized an interdisciplinary approach, including genetics, live-cell imaging, and quantitative microscopy, to define the role of each isotype in distinct MT-mediated processes. During mitosis, yeast cells assemble a mitotic spindle comprised of three different MT subpopulations that must be properly coordinated to ensure effective chromosome segregation. Using functional assays, we show that tubulin isotypes influence the properties and function of specific MT classes during spindle positioning, spindle assembly, and spindle elongation at distinct stages of the cell cycle. We demonstrate that one key mechanism underlying specialized isotype function is the differential localization of MT-associated proteins (MAPs) and regulatory proteins to microtubules.

Previous studies suggested that the difference between the isotypes is simply quantitative, resulting from higher mRNA levels of *TUB1* than *TUB3*. In contrast, our genetic and molecular characterization demonstrates that both isotypes normally contribute equal amounts to total transcripts and tubulin protein levels in wild-type cells. Additionally, we provide the first evidence for their unique contribution to spindle positioning, spindle function, and chromosome segregation. Overall, the results of this study provide the most comprehensive molecular and functional characterization of tubulin isotypes in any system.

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