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SPOTLIGHT

Specialist α-tubulins for pluralist microtubules

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α- and β-tubulins are encoded by multigene families, but the role of tubulin diversity for microtubule function has been a longstanding mystery. A new study (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202010155) shows that the two budding yeast α-tubulins have distinct roles during mitotic spindle positioning.

By 1976, the discovery of tubulin was only a few years old, and a provocative new idea was proposed to explain how αβ-tubulin building blocks could form diverse types of microtubule networks in cells. Fulton and Simpson's "multi-tubulin hypothesis" stated that organisms possess multiple genes encoding functionally distinct α- and β-tubulins, and use these to build microtubule structures with different architectures and functions (1). This hypothesis was based on experiments in *Naegleria gruberi* that showed that tubulin isolated from the outer microtubule doublets of flagella exhibit unique immunochemistry and are expressed at different stages of the *Naegleria* life cycle, compared with the majority of tubulin in the cytoplasm or the tubulin expressed during the amoeba stage. The multi-tubulin hypothesis introduced the idea that differences in αβ-tubulin heterodimers can guide higher-order functions of microtubule networks; but a test of this hypothesis, and the basis for the biochemical differences between αβ-tubulins, was still years away.

The subsequent discovery of multigene families that encode different α- and β-tubulin "isotypes" provided a potential molecular explanation for the origins of multiple tubulins. Four distinct α- and β-tubulin mRNAs were initially discovered in chicken embryos (2). Six distinct α-tubulin mRNAs and five β-tubulin mRNAs were discovered in mice and shown to exhibit different expression patterns during development and across tissues (3, 4). Tubulin gene families in humans are now thought to include 8–9 α-tubulins and 9 β-tubulins, and exhibit cell and development-specific expression patterns. In general, α- or β-tubulin isotypes exhibit a high degree of amino acid sequence identity but are divergent in the ∼15 amino acids at the carboxy-terminus. These differences are conserved in tubulins across species, suggesting that isotypes represent conserved classes of tubulins.

During the period of isotype discovery in vertebrates, Raff and colleagues demonstrated that the β2-tubulin isotype in *Drosophila melanogaster* is specifically expressed in testis and required for spermatogenesis (5). This provided the first evidence that isotypes may be necessary for tissue-specific functions. More recently, mutations in human α- and β-tubulin isotypes are linked to a variety of tissue-specific developmental disorders, further supporting this notion (6). While tubulin isotypes are consistent with the original multi-tubulin hypothesis, they also invite an alternative version of the hypothesis—multiple tubulins are used as expression-control modules for meeting a cell's cytoskeletal demands. These two versions of the multi-tubulin hypothesis are not mutually exclusive, but it is challenging to distinguish between them since experiments that ablate a particular tubulin isotype would be expected to eliminate both protein-specific functionality and gene-specific expression.

A new study by Nsamba and colleagues takes on this challenge using the budding yeast model (7). Budding yeast possess two α-tubulin isotypes, known as Tub1 and Tub3, that were created from the yeast whole genome duplication, and a single β-tubulin isotype, Tub2. The second β-tubulin isotype was lost at some point after the genome duplication. Classic genetic experiments from the Botstein and Solomon laboratories showed that loss of *TUB3* causes modest phenotypes. Loss of *TUB1* is lethal but can be rescued by increasing the expression of *TUB3* (8). These results are consistent with the hypothesis that tubulin isotypes are expression control modules and can therefore be functionally replaced by a sufficient supply of an alternative isotype.

Nsamba et al. reexamine this conclusion using genome editing to replace either *TUB1* or *TUB3* ORF with the alternative isotype at the native chromosomal locus. This elegant approach creates yeast cells that express homogenous αβ-tubulin heterodimers containing only Tub1 or Tub3, while maintaining normal α-tubulin expression at the mRNA and protein levels. Finally, differences in isotype expression can be uncoupled from differences in protein sequence.

Having created isotypically pure cells, Nsamba et al. further leverage their yeast model by conducting a genetic interaction screen to define the functional impact of losing isotype diversity. The screen reveals overlapping sets of genes where null mutants exhibit additive fitness defects in Tub1-only or Tub3-only cells. This indicates

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that either isotype alone cannot match the level of α-tubulin function provided by the WT blend of Tub1 and Tub3, and points to pathways that may require a specific isotype. In particular, the screen with Tub3-only cells exhibits genetic interactions that are similar to mutants known to disrupt dynein. This suggests that the Tub3 may support a lower level of dynein activity than Tub1.

The authors test this through a series of experiments comparing microtubule function in Tub1-only versus Tub3-only cells (7). They find that Tub3-only cells exhibit decreased accumulation of dynein and its regulators at astral microtubule plus ends and fewer characteristic microtubule-sliding events where dynein pulls astral microtubules along the cell cortex to move the mitotic spindle. In contrast, Tub1-only cells exhibit greater dynein accumulation at plus ends and more frequent and longer-lived sliding events. Interestingly, they find the opposite effect on a separate pathway that acts earlier in the cell cycle to orient astral microtubules toward the newly formed bud. Here, the Tub1-only cells exhibit defective astral microtubule orientation compared with Tub3-only cells and WT controls. The authors demonstrate that this effect is caused by insufficient recruitment of the end binding (EB) family homologue Bim1 to the ends of Tub1-only microtubules. Based on these data, Nsamba et al. conclude that the Tub1 and Tub3 have evolved to support differential levels of activity for dynein and EB/Bim1 and their related pathways. This specialization could allow WT cells to build microtubules that are an alloy of Tub1 and Tub3 heterodimers and simultaneously support sets of microtubule-associated proteins with distinct binding modes or impacts on tubulin structure.

Specialization for spindle positioning pathways may be only the beginning of the story for Tub1 and Tub3. An important next step will be to define the biochemical differences between Tub1 and Tub3 that lead to increased dynein or EB/Bim1 recruitment. Tub1 and Tub3 share 91% amino acid sequence identity, leaving a short list of candidate regions. Figuring this out could inform predictions on the specialization of mammalian tubulin isotypes. In addition, it is likely that specialization of Tub1 and Tub3 extends beyond spindle positioning. Neither dynein nor EB/Bim1 are essential in budding yeast, so spindle positioning roles do not explain why loss of Tub1 is lethal while loss of Tub3 is tolerated. However, the genetic screen identified more interactions with mitotic spindle genes in Tub3-only cells than in Tub1-only cells, hinting at specialized roles for Tub1 in spindle assembly and chromosome segregation. The details of these roles, including the possibility of cell cycle-specific expression, await discovery.

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