Behind the formation and protection of microtubules

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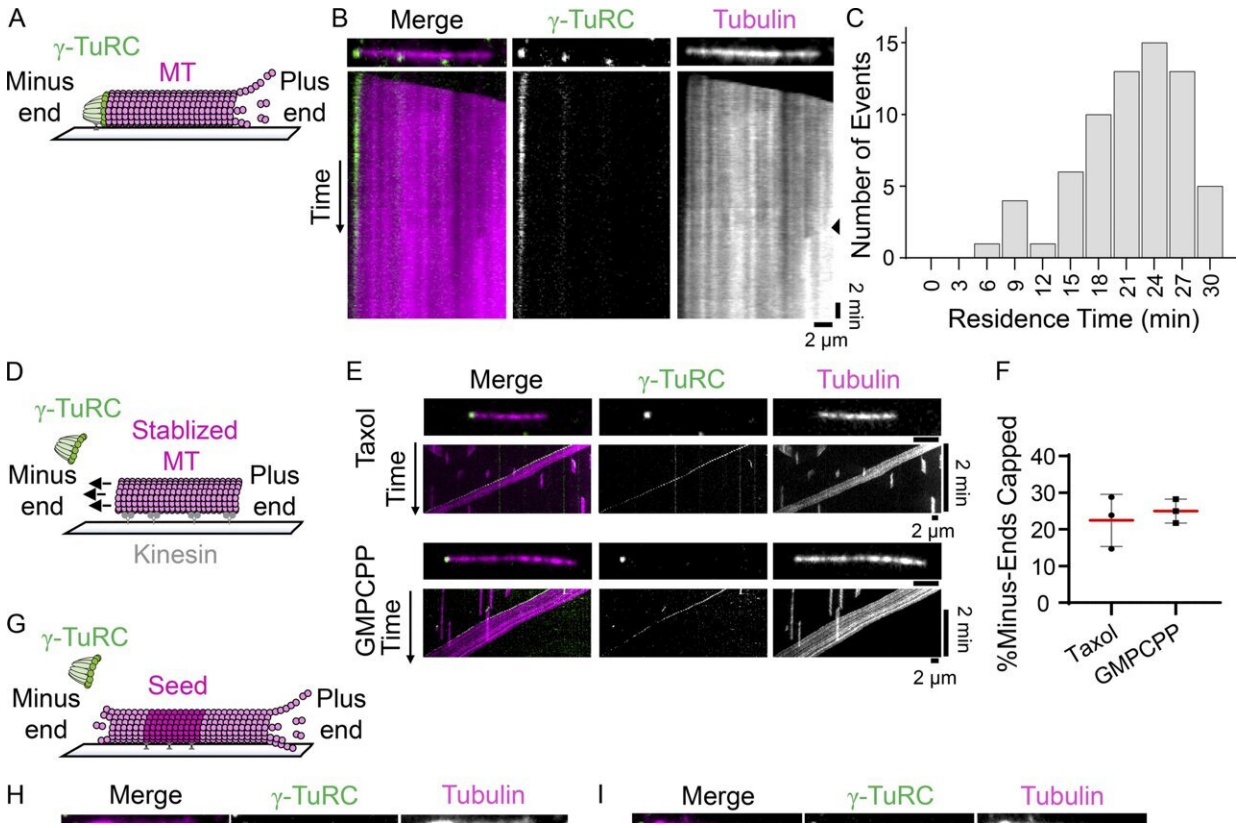

Recombinant γ-TuRC $^{\gamma\text{-Tub-WT}}$ caps nucleated microtubules and pre-formed minus-ends. (A) Schematic of the TIRF-based assay to analyze microtubules nucleated by recombinant γ-TuRC. Surface immobilized GFP-tagged γ-TuRC (green) and polymerized tubulin (pink) are shown. (B) Image and kymograph of a microtubule nucleation event from γ-TuRC$^{\gamma\text{-Tub-WT}}$. Two-color overlay of tubulin (magenta) and γ-TuRC$^{\gamma\text{-Tub–WT}}$ (green), and single-channel images are shown. Black triangle (right kymograph) marks signal from the appearance of another polymerizing microtubule nucleated nearby. (C) Frequency distribution

of the residence times of γ-TuRC$^{\gamma\text{-Tub-WT}}$ at microtubule minus-ends after a nucleation event. Bin size = 3 min, n = 67 events, N = 3 independent experiments. (D) Schematic of the assay to analyze GFP-tagged γ-TuRC (green) capping of stabilized microtubules (pink) bound to surface-immobilized kinesin motor domains (non-fluorescent). Arrows indicate the directional movement of microtubules in the presence of MgATP (100 µM). (E) Images and kymographs of γ-TuRC$^{\gamma\text{-Tub–WT}}$ capping taxol- or GMPCPP-stabilized microtubules. Two-color overlay of tubulin (magenta) and γ-TuRC$^{\gamma\text{-Tub–WT}}$ (green), and single channel images are shown. The images and kymographs are shown at different scales. (F) Percentage of taxol- or GMPCPP-stabilized microtubule minus-ends capped by γ-TuRC$^{\gamma\text{-Tub-WT}}$ at 3 min from the start of imaging. Mean (red line) and error (SD) are shown. Taxol: n = 1,770 total microtubules from N = 3 independent experiments. GMPCPP: n = 2,326 total microtubules from N = 3 independent experiments. (G) Schematic of the assay to analyze recombinant γ-TuRC capping dynamic microtubule minus-ends. Biotinylated “bright” GMPCPP seed (magenta, 12.5% X-rhodamine-tubulin), polymerizing “dim” (pink, 2.5% X-rhodamine-tubulin) minus- and plus-end extensions, and GFP-tagged γ-TuRCs (green) are shown. (H and I) Images and kymographs of γ-TuRC$^{\gamma\text{-Tub–WT}}$ capping events on dynamic microtubules. Two-color overlay of tubulin (magenta) and γ-TuRC$^{\gamma\text{-Tub–WT}}$ (green), and single-channel images are shown. (J and K) Cumulative frequency of the residence times of γ-TuRC$^{\gamma\text{-Tub-WT}}$ capping events where association and dissociation of the cap were observed from short (10 min; J) or long (30 min; K) duration experiments, fitted to a single exponential (red line) with indicated mean residence time, τ. Error = 95% C.I. J: n = 74 events (83% of total events), N = 3 independent experiments. K: n = 107 events (80% of total events) from N = 3 independent experiments. (L) Frequency distribution of γ-TuRC$^{\gamma\text{-Tub-WT}}$ residence times from longer duration experiments (30 min). Events where γ-TuRC$^{\gamma\text{-Tub-WT}}$ dissociation from minus-ends is observed (black bars) and where γ-TuRC$^{\gamma\text{-Tub-WT}}$ remained associated with minus-ends throughout the course of imaging (gray bars) are plotted. Bin size = 3 min. n = 134 total events from N = 2 independent experiments. Scale bars: distance (horizontal) = 2 µm, time (vertical) = 2 min. Credit: *Journal of Cell Biology* (2023). DOI: 10.1083/jcb.202204102

Cellular life hinges on a network of hollow cables called microtubules dynamically lengthening and shortening according to the needs of the moment. During cell division, for instance, these cables latch onto chromosomes and retract—yanking chromosomes to either end of the cell to ensure that each daughter cell receives an equitable share of genetic information. In addition to regulating the dynamics of microtubules, the cell also regulates the precise timing and location of microtubule formation. There's little room for error.

Now, a new study sheds light on how the formation of human microtubules drives cell division. The paper, published in the *Journal of Cell Biology*, describes the inner workings of the γ-Tubulin Ring Complex (γ-TuRC), an assembly of proteins responsible for nucleating and stabilizing microtubules. The findings clarify the γ-TuRC's mechanism, and may inform researchers studying γ-TuRC mutations and associated diseases.

"We were able to characterize the γ-TuRC's capping activity, and explore its role in cell division," says Adi Berman, a graduate research fellow in the laboratory of Tarun Kapoor at the Rockefeller University. "The more we learn about what this complex does and how it does it, the more answers we might be able to find about how the γ-TuRC relates to human diseases."

A seed and a cap

The lifecycle of a microtubule typically begins when protein dimers, composed of alpha and beta tubulin, interact to form long tubular polymers. But that process takes time that the cell cannot always spare. When cells need to build microtubules in a matter of seconds, they instead rely on a microtubule nucleation complex called the γ-TuRC. In human cells, γ-TuRCs are anchored at microtubule organizing centers such as centrosomes, where tubulin dimers can assemble onto the γ-

TuRC and rapidly polymerize into microtubules.

This is not, however, the only role for the γ-TuRC in microtubule formation. Studies have shown that the γ-TuRC also serves as a cap for microtubules, preventing the sudden addition or loss of tubulin dimers and ensuring that microtubules-in-action are localized to the right parts of the cell.

"Capping is another critical function of the γ-TuRC," Berman explains. "It stabilizes the microtubule, which protects it from depolymerization, and it also allows the microtubule to become anchored at specific sites, which ensures that microtubules are positioned correctly."

Kapoor, Berman, and colleagues wanted to study the γ-TuRC's capping activity in isolation, so they collaborated with the laboratory of Brian Chait to manufacture and characterize a crippled form of γ-TuRC. This mutant was incapable of nucleating microtubules but it remained to be determined how this mutation affected the γ-TuRC's capping activity.

A cap in isolation

To find out whether γ-TuRC would deliver on its capping potential—or whether its nucleation function was so closely linked to its capping function that, if one went offline, the other would follow—they conducted a series of experiments and used a variety of imaging techniques to visualize the mutant γ-TuRC interacting with microtubules in vitro and in human cells.

Their results suggest that the mutant γ-TuRC can still cap microtubules—demonstrating, for the first time, that the γ-TuRC's role in capping microtubules is independent of its role in nucleating them. The team also showed that the mutant γ-TuRC plays an important role in microtubule formation outside of the centrosome during mitosis,

suggesting that capping itself contributes to microtubule formation.

The findings may have long-term implications for researchers studying developmental diseases linked to γ-tubulin irregularities, such as microcephaly, and cancers including medulloblastoma, myelomas, non-small cell carcinoma, breast cancer, gliomas, and glioblastoma. The work may also fill in the blanks for scientists who have long contended with an incomplete understanding of the γ-TuRC.

For instance, Berman says, the findings are among the first to suggest that perhaps the cell can modulate between two states, choosing if the γ-TuRC should be nucleating or capping a microtubule in a context dependent manner.

"This work, which combines biochemistry, structural biology, and cell biology, is shedding light on fundamental mechanisms," Kapoor says. "In the long term, this may help us better understand the emergence of diseases related to this complex."

More information: Adi Y. Berman et al, A nucleotide binding–independent role for γ-tubulin in microtubule capping and cell division, *Journal of Cell Biology* (2023). DOI: 10.1083/jcb.202204102

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