# Researchers unlock secrets of cell division, define role for protein elevated in cancer

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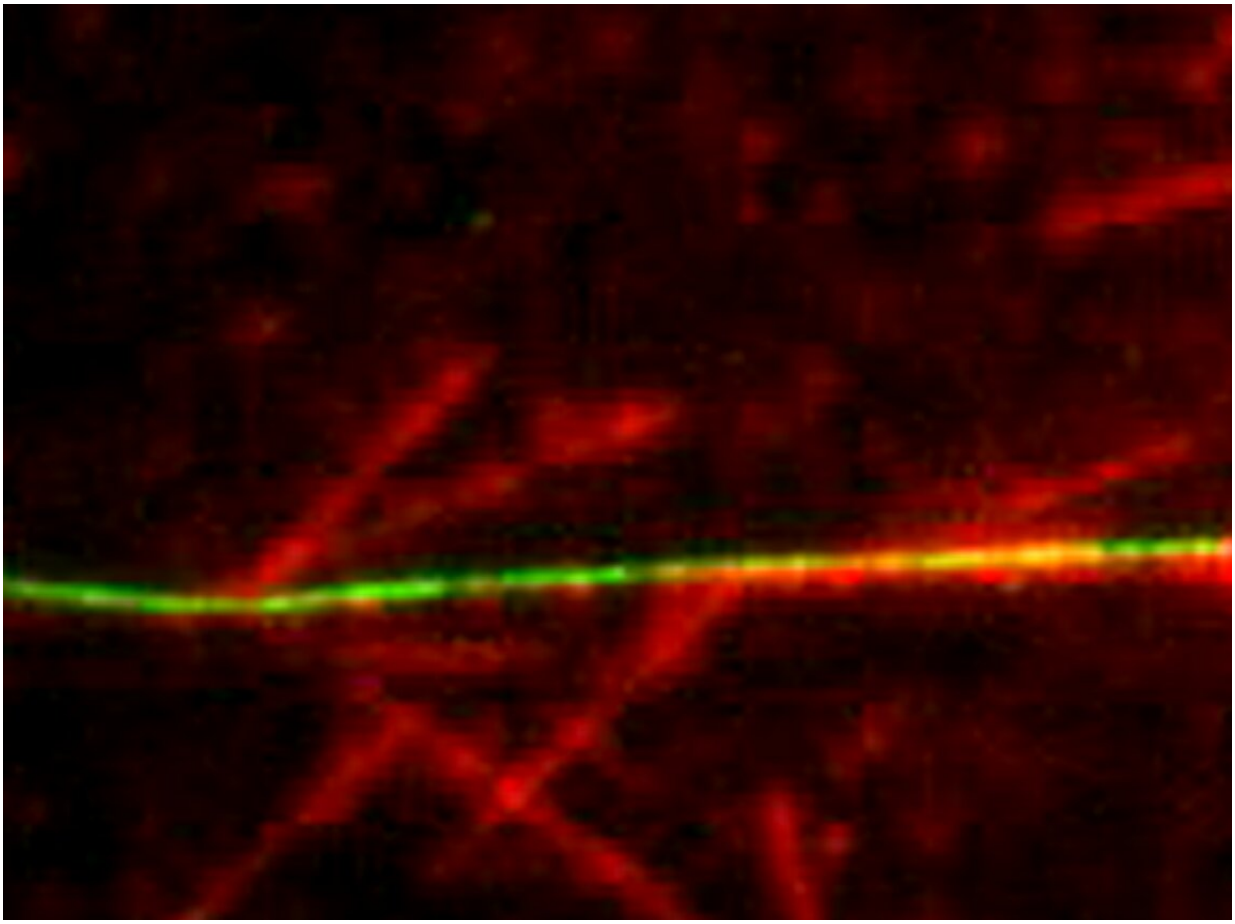

Microtubules (red) branch off from a template microtubule (green) Credit: Raymundo Alfaro-Aco, Sabine Petry group, Princeton University

Researchers at Princeton University have successfully recreated a key

process involved in cell division in a test tube, uncovering the vital role played by a protein that is elevated in over 25% of all cancers. The researchers' findings, described in a pair of papers published in the journals *eLife* and *Nature Communications*, are a key step toward recreating the entire cell division machinery and could lead to new therapies aimed at preventing the growth of cancer cells.

When cells divide, a spindle-like structure composed of thousands of filaments called microtubules attaches to chromosomes and pulls equal numbers of them into each newly forming cell. Each microtubule is assembled from individual tubulin molecules and, because errors in chromosome segregation can lead to cancer, it is vital that they assemble into microtubules at the right time and place to form a functional spindle apparatus. Branching microtubule nucleation, in which a new microtubule forms from the side of an existing one, is crucial to this process because it allows the cell to form large numbers of microtubules that all point toward chromosomes, enabling their capture by the spindle.

Branching microtubule nucleation depends on several pieces of molecular machinery. One piece, called the gamma-tubulin ring complex (γ-TuRC), initiates the assembly of tubulin molecules into microtubules, while another, known as the augmin complex, recruits γ-TuRC to the side of existing microtubules. A protein called TPX2, whose levels are elevated in over 25% of all cancers, is also involved in branching microtubule nucleation. Elevated TPX2 levels lead to both aberrant microtubule assembly in cells and poor outcomes in cancer patients. But how TPX2 works with augmin and γ-TuRC to mediate branching microtubule nucleation and spindle assembly has remained unknown.

"To better understand the mechanism of branching microtubule nucleation, we set out to reconstitute the process outside of the cell using purified proteins," said Sabine Petry, assistant professor of molecular biology at Princeton.

In the *eLife* study, graduate students Raymundo Alfaro-Aco and Akanksha Thawani describe how they recreated branching microtubule nucleation in a test tube. One key finding from the study is that, like augmin, TPX2 can bind to microtubules and recruit γ-TuRC to initiate branching microtubule nucleation. Another surprising finding was that TPX2 also helps recruit augmin to microtubules, further enhancing the recruitment of γ-TuRC.

"Branching microtubule nucleation therefore occurs most efficiently when augmin, TPX2, and γ-TuRC are all present," Alfaro-Aco said. "Surprisingly, TPX2 lies at the very heart of controlling this reaction, despite being only a single protein amongst the large multi-subunit complexes augmin and γ-TuRC."

In the *Nature Communications* paper, Petry and her former graduate student Matthew King further reveal that TPX2 behaves like a liquid in promoting branching microtubule nucleation. Specifically, TPX2 forms a liquid layer on the surface of existing microtubules that beads up into tubulin-containing droplets, much like morning dew on spider webs. The researchers found that TPX2 and tubulin can condense together to form liquid-like droplets through a phase-separation mechanism identical to the one that causes oil droplets to form in water. New microtubules can form from these TPX2-tubulin droplets and, because the droplets condense on the surface of existing microtubules, this results in the formation of branched microtubule structures.

"The study suggests that the co-condensation of TPX2 and tubulin creates a local reservoir of tubulin on a pre-existing microtubule that may be necessary to efficiently promote branching microtubule nucleation," King said.

Together, both studies reveal that TPX2, somewhat overlooked before, is the lynchpin of branching microtubule nucleation. It travels to

microtubules first to assemble all of the other components that ultimately give rise to a branching microtubule, and it does this while behaving like a liquid. Petry and colleagues think that cellular signals may regulate TPX2 condensation to ensure that it only occurs when a cell is dividing and needs to form a spindle. "Our findings on the behavior of TPX2 may guide future therapeutic efforts aimed at modulating cell division," Petry said. "In addition, our reconstitution of branching microtubule nucleation is an important step toward reconstituting the entire spindle apparatus, as well as other cellular structures that depend on this microtubule assembly pathway."

**More information:** Matthew R. King et al, Phase separation of TPX2 enhances and spatially coordinates microtubule nucleation, *Nature Communications* (2020). DOI: 10.1038/s41467-019-14087-0

Raymundo Alfaro-Aco et al. Biochemical reconstitution of branching microtubule nucleation, *eLife* (2020). DOI: 10.7554/eLife.49797

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