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New TIPs for successfully growing microtubules

Two studies identify new proteins that promote microtubule dynamics.

Dynamic microtubules grow and shrink at their plus ends, and the balance between these two processes is regulated by a host of plus end–tracking proteins (+TIPs). van der Vaart et al. (1) and Li et al. (2) describe two new +TIPs that boost microtubule dynamics in mammalian and *Drosophila* cells.

The EB1 family of +TIPs are the master regulators of microtubule plus ends (3). "They can autonomously bind to the growing tips of microtubules," explains Gohta Goshima from Nagoya University in Japan. "They recruit many other +TIPs, but none of these other proteins can accumulate without EB1."

Anna Akhmanova, from the Erasmus Medical Center and Utrecht University in the Netherlands, and Michel Steinmetz, from the Paul Scherrer Institute in Switzerland, used a biochemical pull-down approach to look for additional EB1 binding partners in mammalian cells (1). They identified a strong interaction between EB1's C terminus and a largely uncharacterized protein called SLAIN2.

SLAIN2 localized to microtubule plus ends in cells, where it not only bound EB1 but several other +TIPs as well, including members of the CLASP and CLIP families. Akhmanova says that this network of interactions helps +TIPs stay associated with growing microtubule ends. "All the +TIPs compete with each other at plus ends, so it's good to have several different binding sites."

van der Vaart et al. were particularly interested in SLAIN2's interaction with ch-TOG, the mammalian homologue of the microtubule polymerase XMAP215. "ch-TOG is the only protein known to strongly accelerate microtubule growth," says Akhmanova.

When the interaction between SLAIN2 and ch-TOG was disrupted—either by depleting SLAIN2 or by expressing a dominant-negative version of the protein—ch-TOG was no longer recruited to microtubule plus ends. "Microtubules then have difficulty growing," Akhmanova says. "They only polymerize in very short bursts."

Focal Point

Two groups of researchers identify new microtubule plus end–tracking proteins (+TIPs) that regulate microtubule dynamics. (Left to right) Babet van der Vaart, Cristina Manatschal, Michel Steinmetz, Anna Akhmanova, and colleagues (not shown) describe SLAIN2, a mammalian protein that binds to several other +TIPs, including EB1 and the microtubule polymerase ch-TOG. In the absence of SLAIN2, ch-TOG isn't recruited to plus ends, and microtubule polymerization is slowed. Meanwhile, (second image from right) Wenjing Li, Gohta Goshima, and colleagues (not shown) identify a *Drosophila* +TIP called Sentin that also binds EB1 and is required for the recruitment of the ch-TOG/XMAP215 homologue Msps (mini spindles). Compared to a control cell (right image, top), Sentin RNAi (bottom) leads to the loss of Msps (green) from the plus ends of microtubules (red).

VAN DER VAART, MANATSCHAL, STEINMETZ, AND AKHMANOVA PHOTOS COURTESY OF THE AUTHORS; LI AND GOSHIMA PHOTO COURTESY OF MOMOKO NISHINA

This was somewhat surprising because, in vitro, XMAP215 is capable of binding and polymerizing microtubules by itself (4). "We think that SLAIN2 is needed in vivo because microtubule plus ends are a competitive environment," Akhmanova explains. "SLAIN2 is a linker that positions and concentrates ch-TOG at the tips." This activity is no longer required in mitosis when, the researchers found, SLAIN2 is highly phosphorylated, which blocks its interactions with EB1 and ch-TOG. "Microtubules behave differently during mitosis, so a lot of +TIP interactions are fine-tuned," Akhmanova says.

> "Microtubule plus ends are a competitive environment."

Meanwhile, Li et al. identified a *Drosophila* +TIP that may have a similar function to SLAIN2 (2). These researchers, led by Goshima, performed an RNAi screen for proteins whose depletion results in abnormally short mitotic spindles. Using this approach, Li et al. identified a previously uncharacterized protein—not conserved in vertebrates—that they named Sentin, based on the Japanese word *sentan*, meaning "tip."

Sentin bound directly to EB1, and, as long as EB1 was present, Sentin tracked microtubule plus ends in vivo. In the absence of Sentin, interphase microtubules were less dynamic, pausing for longer periods instead of actively growing and shrinking.

"EB1 depletion has a similar phenotype," Goshima says, which is significant because knocking down other EB1-interacting proteins has different, often milder, effects on the behavior of microtubules. "We think that Sentin is the most important EB1-binding protein for promoting microtubule dynamics," Goshima states. Indeed, expressing a version of Sentin fused to the microtubule-binding domain of EB1 was sufficient to rescue the microtubule dynamics of EB1-depleted cells.

Like SLAIN2, Sentin may promote microtubule dynamics by recruiting an XMAP215 homologue to the plus ends. Cells lacking Sentin lost XMAP215 from their plus tips, and XMAP215 knockdown has the same effect on microtubules as depleting Sentin or EB1. "We hope to figure out Sentin's function at microtubule tips using in vitro reconstitution," Goshima says.

Akhmanova meanwhile hopes to obtain a complete picture of mammalian microtubule plus ends. "We want to explore the +TIP proteome to get the full list of proteins that regulate microtubule dynamics," she says.

1. van der Vaart, B., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201012179.
2. Li, W., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201101108.
3. Akhmanova, A., and M.O. Steinmetz. 2008. *Nat. Rev. Mol. Cell Biol.* 9:309–322.
4. Kinoshita, K., et al. 2001. *Science*. 294:1340–1343.