XMAP215: A Tip Tracker that Really Moves

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DOI 10.1016/j.cell.2007.12.019

XMAP215 is a microtubule plus-end binding protein implicated in modulating microtubule dynamics. In this issue, Brouhard et al. (2008) propose a new mechanism to explain how XMAP215 promotes microtubule growth. They report that XMAP215 moves with the growing microtubule plus ends where it catalyzes the addition of tubulin subunits.

Cell growth and differentiation require dramatic rearrangements of the microtubule cytoskeleton. Microtubules are primarily remodeled by addition and loss of tubulin subunits at their ends, so it is no surprise that cells contain many microtubule-associated proteins that bind to and regulate microtubule tips (for example, see Akhmanova and Hoogenraad, 2005). Several large conserved families of tip-binding proteins have been identified, all of which are implicated in cell morphogenesis in a variety of contexts. However progress toward understanding how tip-binding proteins alter microtubule dynamics has been comparatively slow, partly due to a lack of techniques for observing them in action.

In this issue, Brouhard and coworkers (2008) report on the direct visualization of a tip-binding protein called XMAP215 interacting with individual dynamic microtubules. XMAP215 promotes microtubule assembly in *Xenopus* egg extracts (Gard and Kirschner, 1987), and its homologs are likely to modulate microtubule dynamics in a wide variety of organisms, including *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila*, and humans. The molecule contains five paddle-shaped TOG domains implicated in tubulin binding (Al-Bassam et al., 2006), which are connected by presumably flexible linkers to one another and to a C-terminal domain that may mediate attachment to the microtubule wall. XMAP215 is proposed to accelerate filament growth by acting as a template for the head-to-tail oligomerization of multiple tubulin dimers and enhancing delivery of these preassembled protofilaments to the microtubule tip (Gard and Kirschner, 1987; Kerssemakers et al., 2006; Slep and Vale, 2007) (Figure 1A). This templating model (called the tubulin shuttle model by Brouhard et al. [2008]) is consistent with the elongated configuration of purified XMAP215 (Cassimeris et al., 2001) and with the presence of multiple tubulin-binding TOG domains in the molecule. This model has also been supported by recent high-resolution recordings of filament assembly during XMAP215-stimulated growth (Kerssemakers et al., 2006), which show sudden jumps in filament length (∼60 nm) that are larger than the size of a single tubulin subunit (8 nm). However, the templating model seems inconsistent with the observations that some XMAP215 homologs, such as Stu2p from budding yeast, bind to only one tubulin heterodimer (Al-Bassam et al., 2006). The templating model also does not explain how the molecule promotes microtubule shortening under certain conditions (Vasquez et al., 1994; Shirasu-Hiza et al., 2003). By directly observing XMAP215 in action, Brouhard and co-workers (2008) find evidence for a nontemplating mechanism that can account for these observations.

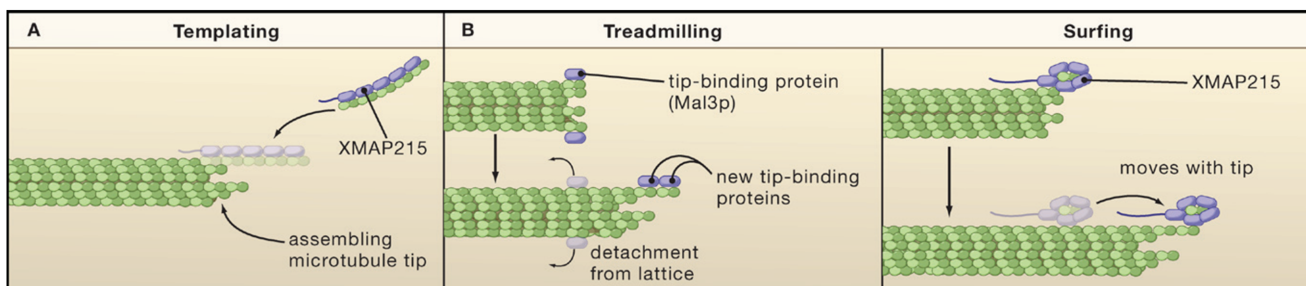

Figure 1. Accelerating Growth at Microtubule Plus Ends and Tip Tracking
(A) The templating model for XMAP215 function. In this model, XMAP215 accelerates filament growth by acting as a template for the head-to-tail oligomerization of multiple tubulin dimers and enhancing delivery of these preassembled protofilaments to the microtubule tip (Gard and Kirschner, 1987; Kerssemakers et al., 2006; Slep and Vale, 2007).
(B) Two classes of tip-tracking behavior: treadmilling and surfing. During treadmilling, individual tip-binding proteins continuously bind to newly added tubulin subunits at a growing tip and later dissociate from the lattice, without moving along the microtubule. In principle, treadmilling might also occur on disassembling tips, by continuous binding to tubulin subunits just before they detach from the filament, although this has not been observed. EB1 and its yeast homolog Mal3p appear to track growing tips by treadmilling (Tirnauer et al., 2002; Bieling et al., 2007). During surfing, the individual tip-binding proteins move with the assembling (or disassembling) tip. Brouhard and coworkers (2008) report that XMAP215 surfs on assembling and disassembling tips. In addition, they argue that XMAP215 accelerates microtubule growth not by a templating mechanism but rather by processively adding individual tubulin subunits to the ends of microtubules.

The authors modified an in vitro assay (Helenius et al., 2006) to record the movement of fluorophore-tagged proteins on microtubules undergoing dynamic instability that are not artificially stabilized by the addition of the microtubule stabilizing drug taxol. This new assay revealed that GFP-tagged XMAP215 dynamically labels the tips of growing and shortening filaments in vitro. In principle, such tip tracking might happen either by treadmilling (Figure 1B, left), where the individual tip-binding proteins do not actually move, or by surfing (Figure 1B, right), where the proteins are transported along with the tip. Brouhard et al. (2008) report that when moderate levels of XMAP215-GFP were present, disassembling tips captured and carried fluorescent particles, causing the tips to become brighter as shortening continued. This accumulation of fluorescence implies surfing on disassembling tips. To test for surfing on growing tips, unlabeled XMAP215 was mixed with a small amount of XMAP215-GFP to reveal individual molecules that remained bound to the tip and underwent assembly-coupled movement for several seconds. During this interval, the presence of XMAP215 caused the addition of ~330 tubulin dimers to the tip (~25 onto each of 13 protofilaments) based on the measured rate of filament growth. These observations show that XMAP215 moves processively with both assembling and disassembling microtubule tips.

Tip surfing by XMAP215 differs notably from the behavior of the canonical plus-end binding proteins (+TIPs), EB1 and CLIP-170. These three tip-binding proteins are often grouped together (Akhmanova and Hoogenraad, 2005; Slep and Vale, 2007), and it has been argued that they all share a common mechanism for altering microtubule dynamics (Slep and Vale, 2007). But EB1 and its fission yeast homolog Mal3p apparently track tips by treadmilling, not surfing (Tirnauer et al., 2002; Bieling et al., 2007), and they label only growing (not shortening) tips. Moreover, the fission yeast CLIP-170 homolog Tip1p does not track tips on its own, instead requiring Mal3p and a plus-end directed kinesin motor, Tea2p, for this property (Bieling et al., 2007). Thus, the autonomous tip surfing of XMAP215 suggests that it is mechanistically distinct from the classic +TIPs.

Brouhard et al. (2008) also observed several other interesting properties of XMAP215. First, it forms a 1:1 complex with tubulin, even in the presence of excess tubulin. This 1:1 stoichiometry is supported by gel filtration, analytical ultracentrifugation, and single-molecule fluorescence intensity measurements, and it matches the 1:1 binding of tubulin to the budding yeast XMAP215 homolog Stu2p (a dimer containing four TOG domains) (Al-Bassam et al., 2006). Second, like Stu2p, the elongated XMAP215 molecule undergoes a substantial compaction when it binds to a tubulin dimer, possibly wrapping around the dimer. Third, XMAP215 molecules undergo one-dimensional diffusion on the microtubule lattice—a property reminiscent of several other proteins (such as Kif1A, MCAK, the Dam1/DASH complex) and expected to increase their rate of targeting to microtubule ends (Helenius et al., 2006). Fourth, in the absence of free tubulin, XMAP215 depolymerizes microtubules. This reversibility suggests that XMAP215 acts like a traditional catalyst, capable of promoting either assembly or disassembly by stabilizing a high-energy transition state on the biochemical reaction pathway for the addition of tubulin dimers.

Based on their observations, Brouhard et al. (2008) argue that XMAP215 does not act as a template to promote the preassembly of tubulin protofilaments but rather acts as a processive polymerase, catalyzing the addition of $\geq$ 25 tubulin dimers as it moves with an assembling microtubule tip. The 1:1 stoichiometry of the XMAP215:tubulin complex is perhaps the best evidence against the template model. However, it is important to note that the stoichiometry of the XMAP215:tubulin complex was measured under conditions where microtubules do not assemble. The movement of tip-bound XMAP215 molecules with growing tips strongly suggests processive assembly catalysis. An additional argument for processive catalysis is based on a diffusion-based mass transfer calculation showing that end targeting of an XMAP215 molecule via lattice diffusion is too slow to account for the rate of tubulin addition at the tip. However, some XMAP215 appears to bind to the tip directly, without first diffusing on the lattice (see Figure 1B, right). Any population of XMAP215 molecules that binds directly to the tip is ignored in the mass transfer calculations but in principle could account for the discrepancy between growth rate and targeting via lattice diffusion. Nevertheless, the hypothesis of a processive assembly catalyst is compelling.

Many questions remain about how XMAP215 and its homologs function in cells and how they are regulated. The approach taken by Brouhard et al. (2008) of reconstitution from pure components followed by interrogation using single-molecule techniques will undoubtedly be crucial for uncovering in mechanistic detail how these and other tip-binding proteins work.

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