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Spotlight

Strengthening Microtubules by Cuts that Heal

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Microtubule-severing enzymes, which can remove tubulin dimers from microtubule lattices, participate in cytoskeletal remodeling in various contexts. A recent study showed that partially damaged microtubule shafts and new microtubule ends generated by these enzymes can incorporate GTP-tubulin and serve as sites of microtubule rescue and re-growth, explaining how severing enzymes can amplify microtubule arrays.

Microtubules are dynamic polymeric tubes, which control numerous aspects of cell architecture and dynamics, such as cell morphology, polarity, intracellular transport, and chromosome segregation during cell division. Microtubules are built and broken down through the addition and removal of tubulin dimers at their ends. Growing microtubule ends can switch to shortening, whereas shrinking microtubule ends can be "rescued" and start elongating again. This dynamic behavior depends on GTP hydrolysis by tubulin: freshly incorporated GTP-bound tubulin subunits stabilize a microtubule, but when after some delay GTP is hydrolyzed to GDP, tubulin subunits undergo a conformational change that leads to microtubule destabilization. The transitions between microtubule growth and shrinkage are strongly regulated by diverse cellular factors, which together determine the geometry and density of microtubule arrays.

Whereas the regulation of tubulin turnover at microtubule ends has received a lot of attention, tubulin exchange in already polymerized microtubule lattices has traditionally not been regarded as a physiologically relevant process. However, recent *in vitro* work showed that microtubule lattice defects induced, for example, by mechanical stress or by laser light can be repaired by incorporation of GTP-bound tubulin (Aumeier et al., 2016; Schaedel et al., 2015). Since GTP-bound tubulin stabilizes microtubule lattices, "islands" of GTP tubulin can slow down microtubule disassembly and promote rescues (Aumeier et al., 2016; Dimitrov et al., 2008; Schaedel et al., 2015; Tropini et al., 2012). Experiments in cells suggested that the sites of microtubule repair, which appear to be more frequent at microtubule crossings, can coincide with the sites of microtubule rescue (Aumeier et al., 2016; de Forges et al., 2016).

An important emerging question is how microtubule damage and repair are controlled in cells. Very interesting candidates for this process are microtubule-severing enzymes, such as spastin and katanin. These hexameric ATPases can use the energy of ATP hydrolysis to extract tubulin subunits from the microtubule lattice and cause microtubule fragmentation (Roll-Mecak and McNally, 2010; Sharp and Ross, 2012). The overall outcome of the activity of these enzymes depends on the destiny of the resulting microtubule pieces. Since a severing enzyme can remove the stabilizing cap from a freshly formed microtubule end, the microtubule can be expected to depolymerize, and the microtubule density would decrease. Enhanced activity of severing enzymes can indeed cause microtubule disassembly, and this property is used by cells, for example, to reorganize microtubule networks during cell division or to disassemble flagella (Roll-Mecak and McNally, 2010; Sharp and Ross, 2012). However, if the newly generated microtubule fragments persist and grow, microtubule severing can amplify microtubule arrays without the need for *de novo* microtubule nucleation. Consistent with this view, loss of severing enzymes leads to a reduction in microtubule density in diverse systems ranging from fly and fish neurons to plant cells and meiotic spindles (Roll-Mecak and McNally, 2010; Sharp and Ross, 2012).

A beautiful *in vitro* study recently published in *Science* now shows that severing enzymes can autonomously amplify dynamic microtubules (Vemu et al., 2018). The authors reconstituted the activity of fly spastin and worm katanin on dynamic microtubules and used electron microscopy to show that the first step in the action of these enzymes is to "bite out" small parts of microtubule shafts (Figure 1). Subsequent light and electron microscopy experiments convincingly demonstrated that in the presence of free tubulin, these nanodamage sites are actively repaired. Severing enzymes can thus "nibble" on microtubules in an ATP-dependent manner, but the damaged microtubule lattices rapidly "heal" by incorporating GTP-tubulin. The authors further showed that the transient presence of "islands" of GTP-tubulin generated by severing enzymes promotes rescue and thus increases the overall microtubule stability. Finally, even if microtubules were eventually severed, the newly generated ends were often stable and could quickly re-grow. This effect depended on the ATPase activity of the severing enzymes, indicating that in the conditions used, these proteins did not passively stabilize microtubule ends but rather generated microtubule fragments bearing protective GTP caps that could resist depolymerization. The combination of enhanced microtubule rescue with the generation of stabilized microtubule "seeds" explains how spastin and katanin can increase microtubule numbers.

These *in vitro* findings raise several interesting questions. First, how big is the contribution of severing enzymes to the induction of microtubule rescue in cells? Microtubules in many cell types are decorated by microtubule-associated proteins that have a stabilizing influence; these can autonomously induce rescue. The relative importance of different rescue pathways for microtubule stabilization will thus need to be determined.

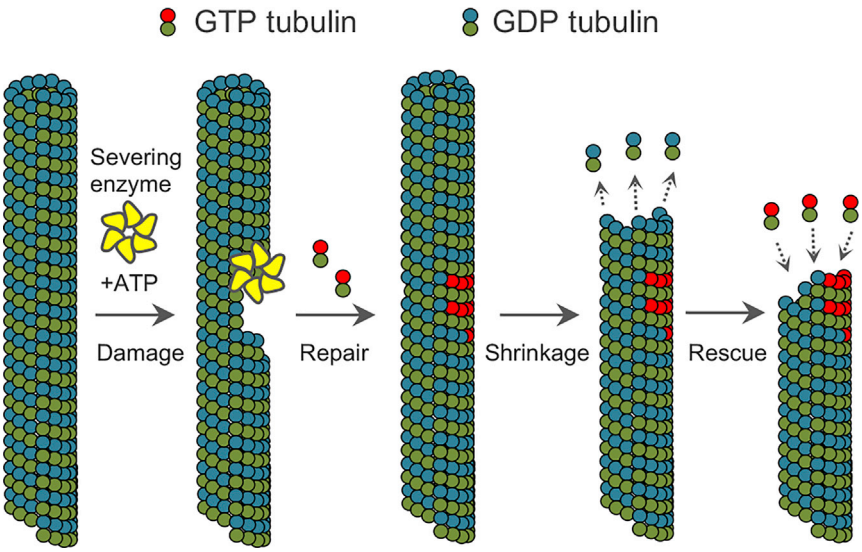

Figure 1. Microtubule Damage by Severing Enzymes Promotes Rescue
In the presence of ATP, a severing enzyme can remove a part of microtubule shaft. The damaged site within the GDP-bound microtubule lattice (blue) can be repaired by incorporation of GTP-tubulin (red). The resulting "GTP island" can arrest microtubule depolymerization and induce rescue.

Another question concerns the frequency of microtubule repair events and the size of repair sites in cells. A way of detecting such sites seemed to be offered by a monoclonal antibody selected to recognize tubulin bound to the non-hydrolysable GTP analog GTPγS (Dimitrov et al., 2008). This antibody highlights discrete spots on microtubules, and it was shown that these spots often coincide with sites of microtubule rescue (Dimitrov et al., 2008). However, subsequent careful *in vitro* experiments showed that most of the regions of microtubule elongation and repair were not labeled by this antibody, which instead appeared to have a preference for microtubule lattice defects (de Forges et al., 2016). The nature of the epitope and the microtubule regions recognized by this interesting antibody thus remain to be further determined.

Freshly incorporated tubulin dimers can also be detected using photoconversion of fluorescently tagged tubulin (Aumeier et al., 2016), but such assays are technically challenging because of the sparse labeling and the difficulties in distinguishing between microtubule repair and end polymerization in dense, rapidly growing cellular microtubule arrays. End binding (EB) proteins, such as EB1 and EB3, provide another potential tool to address this question, as they bind to the stabilizing cap at microtubule ends (Maurer et al., 2012). EBs indeed decorate the sites of microtubule repair *in vitro*, nicely confirming the transient presence of GTP-tubulin in the microtubule regions undergoing repair (Aumeier et al., 2016; Vemu et al., 2018). However, in cells, EB proteins have not been observed at distinct spots along microtubule shafts, possibly due to the high background or the small size of the repair sites, and additional tools will need to be developed to reliably visualize microtubule repair *in vivo*.

Furthermore, the whole cellular arsenal of biochemical regulators that interact with microtubule ends, including microtubule polymerases, depolymerases, and end-stabilizing proteins, will likely recognize microtubule damage sites due to the presence of discontinuous protofilaments. These factors could either promote or prevent microtubule "healing" and thus modulate the microtubule-amplifying activity of severing enzymes. In this respect, it is interesting that katanin strongly interacts with two microtubule minus-end binding protein families, ASPM and CAMSAPs/Patronin, which recognize free microtubule minus ends (Jiang et al., 2017) and which could participate in regulating the destiny of microtubule damage sites.

Finally, the induction of microtubule damage and repair by severing enzymes is likely to be related to the properties of the microtubule arrays on which they act, for example, the abundance of microtubule intersections or post-translational modifications, such as polyglutamylation, which can regulate the localization and activity of these enzymes (Valenstein and Roll-Mecak, 2016). The challenge for future research is to understand how severing enzymes participate in feedback mechanisms connecting microtubule organization and longevity and support the emergence of elaborate and diversified microtubule arrays required for complex cellular functions.

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