
Article:
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Mending microtubules enhances cell polarity

Abstract

Microtubule repair has recently emerged as a mechanism capable of enhancing the longevity of microtubules. In this issue of Developmental Cell, Andreu-Carbó et al. (2021) show that the microtubule motor kinesin-1 can create a cycle of microtubule damage and repair sufficient to bring about changes in cell polarity.

Main text

Microtubules are dynamic complex polymers, as well as the highways that support polarised long distance transport inside cells. The microtubule lattice is knitted together through rapid polymerisation of tubulin monomers onto the “plus-end” of the filament. The dynamics of this plus-end, which also experiences periods of rapid unravelling or catastrophe, has previously dominated ideas about how microtubules are built and recycled. More recently it has been discovered that tubulin monomers along the length of the microtubule shaft can also be lost, and that the holes created are repaired with fresh tubulin. Not only that, but repair sites go on to halt the rapid catastrophe of microtubules and rescue polymerisation of the filament. Andreu-Carbó et al. ask whether the forces exerted on microtubules by walking microtubule motor proteins are sufficient to damage the microtubule shaft and initiate a mend. In the process they show that the action of motor proteins can cause a positive feedback loop of damage and repair, to the extent that the increased microtubule mass caused by walking motors can accentuate cell polarity.

Microtubule repair along the shaft has been demonstrated through a series of *in vitro* reconstitution studies using purified proteins in microfluidic chambers. Microtubules repeatedly bent within these controlled environments decrease in stiffness, but can recover when free tubulin is present (Schaedel et al., 2015). Similarly, motor proteins attached to a glass surface whilst sliding microtubules, can remove individual tubulins from the lattice through molecular wear and tear (Dumont et al., 2015). The microtubules used most frequently for *in vitro* experiments are chemically stabilised, masking this intrinsic propensity for working microtubules to become damaged. Consequently, unless protected by the addition of extra free tubulin to the system, microtubules not chemically stabilised can be destroyed quickly in these assays (Triclin et al., 2021). In fact the turnover of tubulin within minimally stabilised microtubules has also been observed even without external mechanical forces (Schaedel et al., 2019), indicating how widespread this phenomenon might be.

One critical feature of microtubule repair sites is the nucleotide state of the tubulin forming the patch. Tubulin heterodimers are formed of α and β tubulin, where the GTP bound to β tubulin is exchangeable and hydrolysable. GTP bound heterodimers are incorporated into the polymerising plus-end of the microtubule. However, the GTP is quickly hydrolysed to GDP even as more tubulins are added to the end, creating a ‘GTP-cap’ at the plus end of the microtubule. The GTP-cap is thought to protect the growing microtubule end from unravelling, a rapid depolymerisation also referred to as microtubule catastrophe. Critically, when the microtubule shaft needs repairing it is GTP tubulin used for the mend. This creates GTP-tubulin patches in the GDP-shaft, with the new ability to act as rescue sites during microtubule catastrophe (Aumeier et al., 2016). Strikingly, repeated laser damage to microtubules in the periphery of cells can promote

microtubule growth (Aumeier et al., 2016). As stressful as life inside a body can be, it's not often subject to highly focused laser insults to damage microtubules. In this new work, Andreu-Carbó et al. establish that microtubule motors in cells are capable of producing a cycle of microtubule damage and repair that accentuates cell polarity, by increasing the density of the microtubule network.

Given the spontaneous exchange of tubulin in the microtubule lattice that has recently been observed (Schaedel et al., 2019), Andreu-Carbó and colleagues set out to establish that the microtubule motor kinesin-1 can cause additional lattice damage and repair even at very low concentrations. Using *in vitro* reconstitution they observed microtubule dynamics with low concentrations of kinesin-1 and found microtubule rescue frequency could be doubled in these conditions. Increases in microtubule rescue frequency only occurred when kinesin was walking, as the same experiments performed with the non-hydrolysable ATP analogue, AMP.PNP, did not produce the same effect. Taken together with previous work, there is a highly reproducible ability for microtubule mend sites to behave as rescue sites during catastrophe.

Andreu-Carbó and colleagues estimate that tubulin incorporation into the microtubule shaft happens once every ~11k kinesin-1 steps; a rare event that is non the less capable of causing substantial change in microtubule rescue frequency. Using simulations they found that microtubule length increased with the number of motor proteins moving along the microtubule. Confirming this observation in their *in vitro* experimental system, they discovered that above a critical concentration of kinesin-1, microtubule growth became unlimited as a result of increased rescue frequency. Having established this basic principle of rare kinesin induced lattice repair being sufficient to modulate microtubule properties, they moved on to the complicated cellular environment.

The results from *in vitro* work to date indicate that a positive feedback loop, between the frequency a microtubule is used by motor proteins and the stability of that microtubule, is possible. Andreu-Carbó et al., manipulated kinesin-1 expression levels and activity to establish that positive feedback is also possible in cells. Firstly, by RNAi knockdown to reduce microtubule rescue frequency. Secondly, by overexpression of a constitutively active kinesin truncation mutant, K560, increasing microtubule rescue frequency. Finally, by acute application of kinesore, a small molecule designed to promote kinesin-1 activation (Randall et al., 2017), also increasing microtubule rescue frequency and lifetime. Increased kinesin activity does seem to create a more stable pool of microtubules in cells. However, some of the most striking findings presented are those on cell polarity (Andreu-Carbó et al., 2021). Overexpression of constitutively active kinesin-1 causes cells to become elongated, before this is accentuated still further into an almost neuron-like star shape with long processes. Cell shape changes are not seen when the same kinesin incorporates a point mutation that prevents kinesin walking along microtubules.

In summary, Andreu-Carbó and colleagues outline how motor proteins running on microtubules not only cause damage to the shaft, but also template repair (Figure 1). Holes in the microtubule shaft can be mended by filling them with fresh tubulin monomers, subtly changing microtubule properties by enhancing the ability to recover from catastrophe events. Eventually the repair process increases microtubule mass by increasing rescue events. At sufficient scale inside cells, this appears to be able to instigate cell polarisation through a positive feedback loop. There are indications that rather than relying on free tubulin in the cytosol to instigate microtubule mending, cells make use of proteins already known for suppressing catastrophe (Aher et al., 2020). Do cells regulate the ability to repair their microtubules and how does this process fit in with other microtubule modifications? And do all microtubule motors have similar positive feedback on microtubule longevity for their preferred roads inside cells? It's also intriguing to contemplate what

effect microtubule mending has on the cell biology of microtubule function in post mitotic cells such as neurons, where microtubules need to be extremely long lived. The further study of microtubule repair undoubtedly has important implications for our understanding of the establishment and maintenance of cellular polarity.

Figure Legend

A. The damage to the microtubule lattice caused by motor protein walking creates patches of GTP bound tubulin that can stop microtubule catastrophes, promoting repolymerisation and rescue.

B. In cells where a subset of microtubules are mended with relatively high frequency, microtubule growth is biassed through increased rescue events to cause cell elongation.

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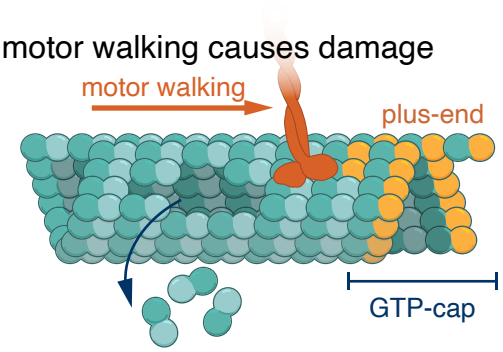
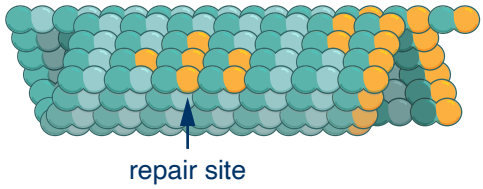
A.

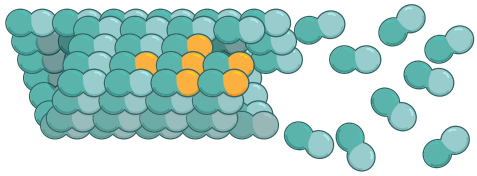
motor walking causes damage



damage repaired by GTP-tubulin

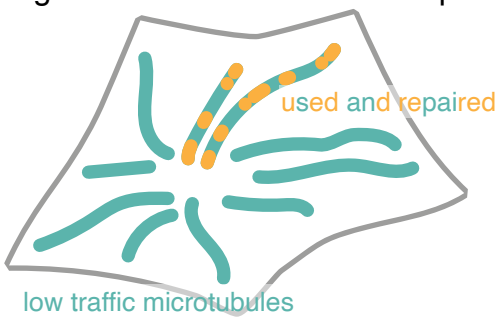


microtubule catatrophe halted by repair



B.

high traffic microtubules are repaired



repair sites bias microtubule growth