Tools of the trade

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Probing cytoskeletal remodelling by cutting and marking filaments

Many cytoskeletal structures remodel themselves on the seconds-to-minutes timescale. For example, the network of microtubules that segregates chromosomes during cell division, known as the spindle, is remarkably dynamic. Notably, spindles can rebuild their architecture when they attain an incorrect shape or when they incorrectly attach to chromosomes. How does the spindle remodel itself? In principle, probing spindle dynamics during local remodelling events can uncover local mechanisms of spindle self-organization. However, locally inducing these remodelling events while measuring spindle dynamics is challenging as it requires the ability to simultaneously perturb and mark the system. Historically, targeted lasers have been used either to damage spindle structures and induce remodelling, such as severing microtubule bundles, or to track microtubule end dynamics by photomarking a microtubule region. Here, I describe a method to probe mechanisms of cytoskeletal network re-organization that uses a targeted laser to trigger network remodelling and track network dynamics during remodelling.

To study the dynamics of spindle microtubules in response to perturbation, we used a nanosecond-pulsed dye laser capable of both severing microtubule bundles and creating local photomarks on microtubules. To shorten microtubules and test their length-regulating mechanisms, we used high laser power to cut a microtubule bundle. We verified damage by observing depolymerization of severed microtubules, recoil of kinetochores no longer under tension, and movement of microtubule bundles towards poles as they grew back during repair. Then, to visualize the resulting spindle remodelling, we used lower laser power to create a bleach mark on the severed microtubule bundle. Importantly, for both steps we chose a laser dye whose emitted wavelength was close to the peak excitation wavelength of the fluorophore labelling the microtubules to optimize bleaching. Ablation and bleaching can be performed in either order to test how dynamics change before, during and after remodelling is induced.

With this tool, we found that microtubule bundles in the mammalian spindle locally regulate their own length. They can increase or decrease their end dynamics after being shortened by laser ablation, which drives their length back to their steady-state length. More broadly, this tool can be applied to any self-organizing cytoskeletal structure to probe the dynamics, turnover and movement of its building blocks as it remodels.

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Competing interests

The author declares no competing interests.

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