Molecular Systems Biology 5; Article number 251; doi:10.1038/msb.2009.7
Citation: *Molecular Systems Biology* 5:251

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molecular systems biology

NEWS AND VIEWS

Toward a comprehensive and quantitative understanding of intracellular microtubule organization

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Molecular Systems Biology 17 March 2009; doi:10.1038/msb.2009.7

The proposition that cell architecture is complex needs no justification. Moreover, intracellular organization changes with time in some cells yet persists in others. These properties are determined in part by microtubule cytoskeleton, which can provide both the consistency, e.g. by maintaining cell shape, and variability in response to changing intracellular tasks and environment. Microtubule behavior *in vitro* is described by four kinetic parameters (rates for growth and shrinkage and frequencies for the stochastic transitions between these states), and is also affected by the boundaries of a confined environment. In cells, there are multiple layers of additional regulation. Microtubule dynamics are affected globally by a large number of associated proteins, and locally through spatially distributed biochemical cues. Deciphering the interplay between microtubules, cell boundaries and their numerous regulators and components is therefore a daunting task. Two complementary articles published recently in *Molecular Systems Biology* provide a refreshing and inspiring example of how systematic, quantitative approaches that combine observations and mathematical modeling can help to break such complex problem into a set of logically appealing and manageable parts.

Tischer *et al* (2009) and Foethke *et al* (2009) have analyzed the spatial regulation of microtubule dynamics and its role in intracellular organization, using the unicellular eukaryote *Schizosaccharomyces pombe*. Its cytoskeleton has been studied intensely, facilitated by this cell's simple shape (Hayles and Nurse, 2001). Several overlapping microtubule bundles attach to a centrally positioned nucleus and run in opposite directions (Figure 1A). The distal microtubule tips, however, rarely curl around the cortex (Hagan, 1998; Drummond and Cross, 2000), prompting a question about the mechanisms that terminate microtubule growth at cell ends. Thus, the cell's shape affects the cytoskeleton, but the reverse is also true: normal microtubule organization is required to maintain the cylindrical shape and to center the nucleus (Mata and Nurse, 1997; Tran *et al*, 2001). According to Tischer, Foethke and colleagues, this conundrum can be explained by the spatial regulation of microtubule dynamics. Two key mechanisms appear to be at play: microtubule growth is terminated at the cell cortex, due to arising compressive forces, and there is also a long-range cytoplasmic modulation that enhances microtubule catastrophe (switch to depolymerization) in longer microtubules.

The compression-dependent regulation of microtubule growth is beautifully demonstrated by experiments of Tischer *et al*. This group has previously described quantitative interactions between polymerizing microtubules and a barrier *in vitro* (Dogterom and Yurke, 1997; Dogterom *et al*, 2005). Compressive forces generated by a growing tip reduce the rate of tubulin assembly, increasing the probability of catastrophe. It was natural for this group to suggest that similar mechanism restricts microtubule growth at rigid cell boundary, but it is one thing to speculate what *might* contribute—and another to actually *test this hypothesis* with hundreds of spatially resolved measurements using custom-written automated software. This analysis not only confirmed previous findings of frequent catastrophes at the cell ends (Figure 1B), but the authors also examined how these rates depend both on contact angles between microtubules and cell wall, and on connected microtubule bundles that touch the opposite cell pole. Both approaches provide convincing support for a mechanical component in cortex-dependent regulation, although biochemical cues localized at cell poles may also contribute.

There is also a smaller but statistically convincing contribution from a cortex-independent spatial regulation, whereby longer microtubules undergo more catastrophes than shorter ones, so the authors go on to investigate whether kinesin-8 might be responsible. This choice was driven in part by the *in vitro* observation that budding yeast kinesin-8, Kip3 depolymerizes longer microtubules faster than shorter (Varga *et al*, 2006). Intriguingly, fission yeast kinesin-8s, Klp5 and 6, are enriched at the ends of longer interphase microtubules

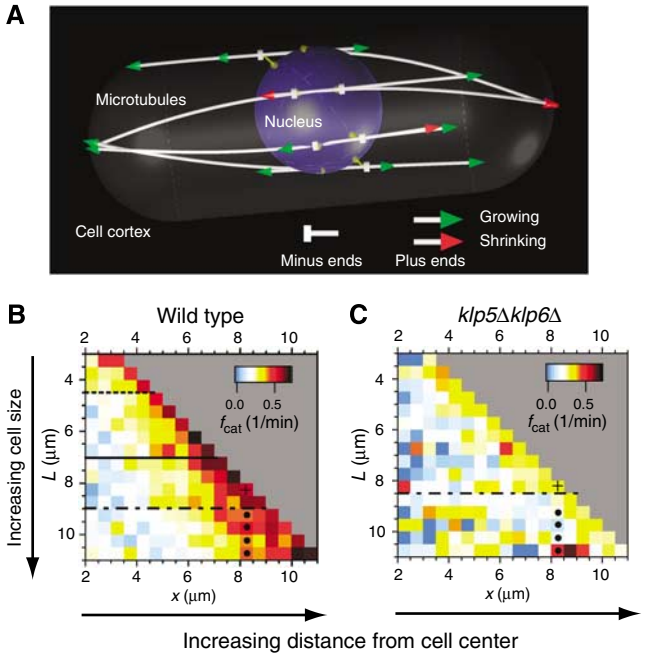

Figure 1 3D organization and spatial regulation of microtubule cytoskeleton in fission yeast. (**A**) Intracellular architecture of *S. pombe* cell, as viewed with 3D simulation (reproduced from Foethke *et al*, 2009). (**B**, **C**) Spatially resolved measurements of catastrophe frequency in wild type and *klp5*Δ*klp6*Δ cells as a function of distance to the cell center (*x*) in cells with different half lengths *L* (reproduced from Tischer *et al*, 2009). Darker red colors correspond to more frequent catastrophes. In wild-type cells with various lengths, microtubules inevitably undergo catastrophe after contacting the cell end (the diagonal darker area on (B)), but in elongated cells longer microtubules are destabilized even when their tips are $\geqslant 1$ μm away from cell end (red-colored area at the right lower corner of the graph). Deletion of Klp5/6 motors (C) leads to a loss of this long-range regulation and also reduces the strength of a cortex-dependent effects.

(Tischer *et al*, 2009). If these motors accelerate depolymerization, the longer polymers with more tip-associated Klp5/6 might be more destabilized, explaining the observed spatial regulation. Indeed, deletion of Klp5/6 stabilizes microtubules, they elongate and curl around the cell ends (West *et al*, 2001), and the long-range regulation is abolished (Figure 1C). Surprisingly, the cortex-dependent regulation is also greatly diminished, revealing once again a complex cross-talk between motors, microtubules and the cell cortex. Nonetheless, these findings are nicely consistent with a hypothesis that kinesin-8 is a length-dependent microtubule regulator. Unlike Kip3, however, heterodimeric Klp5/6 is a plus-end directed motor that does not depolymerize stabilized microtubules *in vitro* (Grissom *et al*, 2008), but rather induces catastrophes (Unsworth *et al*, 2008; Tischer *et al*, 2009). Is kinesin-8 the only basis for the long-range spatial regulation of microtubule dynamics? Answering this difficult question must await future studies, and currently one cannot rule out possible contributions from other spatial cues or factors that affect density of microtubule bundling, which normally diminishes toward the cell tips.

These experimental findings set up the stage for Foethke *et al* to use mathematical approaches and ask whether two levels of spatial regulation could explain *quantitatively* the self-organization of interphase microtubules and attached nucleus. They condense the large and complex set of relevant experimental observations into 10 quantitative 'traits' and analyze them using a detailed model of the microtubule cytoskeleton. The compression-dependent regulation can explain eight traits but fails with two, which describe re-establishment of intracellular organization in cells the architecture of which was disrupted by centrifugation. However, an additional assumption of length-dependent regulation is sufficient to match all 10 traits. This analysis suggests that two major components of the complex interplay that defines microtubule architecture and shape in fission yeast cells are now probably grasped. There are undoubtedly other regulatory layers that underlie these interactions, and their relative contributions will have to be determined. At this point, however, we seem to be out of new non-overlapping traits, so it is tricky to rule out or confirm unambiguously any new regulatory link. But one thing is certain: in the future, more complete understanding will be gained through a quantitative experiment going hand-by-hand with a predictive modeling.

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