New and Notable

Aging Gracefully: A New Model of Microtubule Growth and Catastrophe

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Although dynamic instability has been studied for 30 years, the structural details and molecular mechanisms that underlie microtubule growth and catastrophe are still hotly debated (1). Microtubules play defining roles in cell shape and stiffness, they are targets of anticancer drugs, and they can generate mechanical forces in both their growing and shrinking phases. Thus, in addition to being a fascinating system for experimental biophysics and computational modeling, the molecular understanding is relevant to both fundamental cell biology questions and clinical applications. It is known that microtubule dynamics are controlled by a huge number of regulatory proteins in cells, but it is difficult to pinpoint precisely how these proteins carry out their regulation without first nailing down the specific mechanisms governing microtubule growth and catastrophe in more simplified systems.

The textbook model used to describe microtubule dynamic instability is the GTP cap paradigm, whereby straight GTP tubulin dimers are incorporated into the growing microtubule and converted to GDP tubulin, which prefers a bent conformation. As long as the rate of GTP tubulin addition outpaces the rate of GTP hydrolysis in the lattice, the GTP cap is maintained; but if hydrolysis outraces subunit addition, GDP subunits are exposed at the end, leading to breaking of lateral contacts, protofilament splaying, and catastrophe.

Work over the last decade has added significant complexity to this picture. The observation that microtubule plus-ends rapidly fluctuate over lengths of multiple tubulin subunits ruled out models in which the GTP cap is only a few subunits in length (2), and led to a model in which tubulin on- and off-rates are very fast and the growth rate represents only the net rate of tubulin addition (3). One attractive feature of this model is that subtle changes in tubulin on- and off-rates by regulatory proteins can have large effects on polymerization dynamics. Other work found that the catastrophe frequency went up over time, which is inconsistent with a single rare-event-triggering catastrophe (4,5). This nonexponential lifetime distribution was interpreted by a multi-hit model in which defects in the lattice accumulate over time and each subsequent defect increases the probability of catastrophe (5). While a three-hit model could quantitatively explain the data, the results could also be explained by a model in which growing microtubule ends become more tapered over time, and these slowly evolving tip structures lead to increased catastrophes (6).

In this issue of the *Biophysical Journal*, Zakharov et al. (7) present a detailed computational study of microtubule growth dynamics that puts forward a new interpretation of the age-dependent catastrophe rate and provides a framework for interpreting how regulatory proteins and drugs alter microtubule dynamics. Their molecular-mechanical model starts with polymerized tubulin having a straight GTP state and a bent GDP state, chooses lateral and longitudinal bond energies by matching depolymerization rates to experimental values, and chooses a tubulin on-rate by matching to experimental growth rates. A significant advance is that the model incorporates thermally driven bending of protofilaments and tubulin-tubulin bond fluctuations; thus, protofilament peeling and reannealing are simulated in time and space. To start, this level of complexity generates spectacular movies of growing and shrinking microtubules (see Movies S1 and S2 in Zakharov et al. (7) and Fig. 1). More importantly, however, incorporating these Langevin dynamics enables the microtubule tip to access a vast number of mechanochemical states, allowing detailed analysis of whether specific structural states (like splayed filaments or loss of the GTP cap) lead to catastrophes.

In addition to matching experimental results for tubulin-dependent growth and shrinkage rates, the model reproduces the tip fluctuations observed experimentally (2), generates ram's-horn curls during depolymerization that match structures seen by cryo-electron microscopy (8), and simulates proper EB1 comet lengths (9). One feature that the model did not generate was long tapers at growing ends, which were previously observed in both experiments and models (3); however, the authors point out that other experiments fail to detect significant plus-end tapering under normal conditions (9,10), so this will continue to be an ongoing debate. The richest information came from an analysis of events preceding catastrophe. The simulations are computationally intensive, so to achieve a sufficient number of catastrophes for statistical analysis, the authors increased the GTP hydrolysis rate in the lattice and justified this by showing that the catastrophe frequency, the size of the GTP cap, the time it takes to achieve a steady-state cap size, and the frequency of observing different numbers of curved protofilaments at the tip all scaled in predictable ways with the hydrolysis rate. Importantly, the model recapitulated experimental growth lifetime distributions, allowing direct comparison

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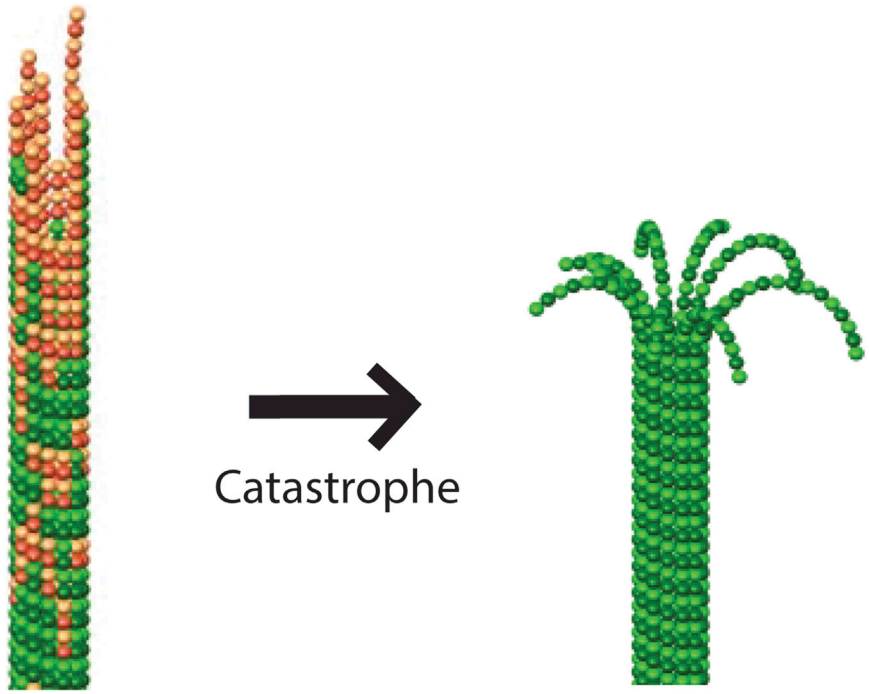

FIGURE 1 Simulation of a growing and a shrinking microtubule. (*Orange*) GTP tubulin subunits; (*green*) GDP subunits. Images are from a computational simulation of microtubule growth and catastrophe by Zakharov et al. (7) in this issue. Reprinted with permission.

to previous models. Contrary to the multihit idea (5), there was no evidence of permanent defects in the lattice, and contrary to the idea that increased end-tapering over time leads to larger catastrophe rates (6), the ends appeared ragged rather than tapered and they achieved their end structures within a few seconds.

To study the catastrophe mechanism, the authors started simulations from defined end structures (blunt end, splayed ends, etc.), and recorded the probability of catastrophe ensuing. Interestingly, blunt microtubules containing no GTP tubulin generally grew rather than shrank, demonstrating that for the chosen set of parameters, the cooperative interactions that hold together the lattice provide stability even without a GTP cap. In contrast, ends having combinations of splayed protofilaments and laterally stabilized straight protofilaments tended to progress to catastrophe because protofilaments with only one lateral stabilizing partner splayed and then shrank. Along the same lines, the authors monitored the number of splayed protofilaments and the size of the GTP cap at times just preceding catastrophe and found that there was no change in the GTP cap size, but there was an increase in splayed protofilaments just before catastrophe. The model also enabled the study of alternate histories by rewinding to a time before catastrophe, restarting simulations from there, and determining the inevitability of catastrophe. If the movie was rewound 11 s, the resulting lifetimes from that point matched the steady-state lifetimes, giving an indication of the relatively short history and lack of memory embedded in the lattice and tip structures in this framework.

Instead of permanent lattice defects or specific tapered end-structures, microtubule aging in the Zakharov model is driven by the slow evolution of events that take place continuously at the growing MT end. The idea is that in a stochastic system having many possible states, even if the transition rates are relatively fast and reversible, the system evolves slowly over time, which results in an age-dependent catastrophe rate. In applied probability, this is called a phase-type density, and it is commonly used in queuing theory and reliability modeling. The idea dates back to pioneering queuing work by Erlang (11) who, in 1909, studied traffic patterns in the Cophenhagen telephone system. Zakharov et al. (7) provide a simple stochastic model to demonstrate the idea, and they show that identical lifetime distributions can be achieved with a three-state model with irreversible transitions (i.e., hits) that occur roughly every 2 min, a 13-state model having reversible transitions with 5 s lifetimes, or a 50-state model with half-second lifetimes. While the system is described as a linear sequence of states, this concept can apply just as well to a network or web of states, with the probability of being in a catastrophe-vulnerable state evolving slowly over time as the system reaches steady state.

For the cognoscenti, there are debates to be had regarding the shape of the potential wells governing tubulin-tubulin interactions, the relatively large tubulin stiffnesses used, the nature of the nucleotide-dependent conformational changes, and other details. More importantly, new structural data and the effects of different microtubule binding proteins can be investigated using this model and later permutations of it, and advances in supercomputer power should facilitate this ongoing modeling work. One of the pleasing aspects of the microtubule dynamics field is that there is an intertwined evolution of experiments and quantitative models, and as it should, the Zakharov model provides testable predictions for future experimental work. In the larger picture, this study demonstrates the intuitively pleasing idea that a system having many possible chemical or structural states can evolve slowly over time even if the specific transitions are quite rapid. Hence, just as the model bridges from the angstrom-level of tubulin binding potentials up to protofilament curls of

tens of nanometers, it demonstrates the general concept that kinetic transitions occurring over subsecond timescales can lead to evolution of behavior occurring over many minutes.

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