Dynamic Microtubule Simulation, Seams and All

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The concept of dynamic instability of microtubules, introduced by Mitchison and Kirschner in 1984 (1) has profoundly influenced the direction of microtubule research in the past few years. With the recognition that microtubules exist in a mixed population of two radically different states of stability, the importance of observing the behavior of individual microtubules rather than a population average has become apparent. New techniques of optical microscopy have clearly demonstrated the occurrence of dynamic instability of microtubules in cells.

Microtubules exist in two states, one state exhibiting rapid growth, and the other rapid disassembly, both states coexisting under identical conditions of subunit availability. At arbitrary times, rapidly growing microtubules undergo a transition from growing to shrinking, known in the trade as *catastrophe*; the transition from shrinking to growing being is known as *rescue*. The exact mechanisms for rescue and catastrophe remain unsettled, but are generally believed to involve the incorporation into the microtubule lattice of tubulin molecules containing bound GTP and the hydrolysis of that GTP.

Key to understanding the mechanism is the timing of the GTP hydrolysis, which occurs after rather than synchronously with incorporation of an additional tubulin subunit in the lattice; thus the growing microtubule is "capped" by a layer of tubulin subunits containing bound GTP, while the shrinking microtubule terminates with tubulin containing the hydrolysis product, GDP. Mechanistic models may be classified as "large cap," for which the hydrolysis proceeds at a rate independent of tubulin subunit incorporation; when free tubulin becomes depleted, the rate of incorporation slows, hydrolysis may catch up and the cap disappears, giving rise to catastrophe. "Small cap" models suggest that hydrolysis is coupled to tubulin incorporation, but occurs on the previous terminal tubulin subunit when the next subunit is added, so that the newly added tubulin retains its GTP unhydrolyzed. The cap is then a single layer of tubulin subunits containing bound GTP at the growing end of the microtubule lattice. Catastrophe occurs infrequently when the terminal tubulin GTP dissociates from the microtubule before a new subunit can be added. The "lateral cap" hypothesis previously proposed by Bayley et al. (2) is a model of this type.

The experimental tools for investigating the size of the GTP cap have proven to be rather blunt. The molarity of microtubule ends is so low that direct measurement of GTP incorporation is lost against the background. Recent measurements correlating GTP hydrolysis rates with microtubule assembly generally support a small cap (3), but time resolution is too poor to make a definitive judgment. Finally, the real-time observation of individual microtubules by Walker et al. (4) also suggests a small cap but, limited by optical resolution, can't discriminate single layers of tubulin subunits at the lattice end.

Computer simulation of the microtubule assembly process provides a way around the limitations of bench experiments, and a well constructed computer model of the system can be a valid experimental tool in its own right. Previous simulations by Bailey et al. (2) were based on a simplified helical lattice model, with only longitudinal and a single omnibus lateral interaction between subunits. Microtubules readily form different lattices, some having a "seam" in which the lateral interactions between adjacent protofilaments are out of register with the rest of the lattice, a consequence of the quasiequivalence of the α- and β-tubulin subunits. In the paper that follows, Martin, Schilstra, and Bailey have constructed a more rigorous lattice model, one which successfully deals with microtubule lattice variations, seams and all.

The credibility of a computer simulation depends on minimum reliance on a priori assumption. Martin et al. have developed rate constants for the individual processes starting from intersubunit bond energies, along the lines of Erickson for the actin filament (5). For the microtubule to exist, these intersubunit bonds must also exist, and although the assignment of numeric values is arbitrary, in the absence of hard experimental values, it is the only way to proceed, and the case for the values assigned is well argued. Besides, as Erickson has commented, one or two orders of magnitude variation may not make much difference in the performance of the simulation.

What is impressive is the ability of the simulation to recreate many experimentally observed aspects of microtubule behavior, using only the preassigned values for bond energies. Many inappropriate models can simulate a predetermined behavior if parameters are fine-tuned until forced into compliance. The validity of a simulation can be judged on the basis of its ability to predict results (even if the "predictions" are actually known beforehand) if the model is set up, allowed to run, and results consistent with experiment simply fall out of the simulation. The ability of the new model to reproduce experimental catastrophe and rescue frequencies and growth rate dependence on concentration (a surprisingly complex function) gives strong support to a lateral cap hypothesis.

All this is not to say that the lateral cap model is 100% correct or proved by a computer simulation. Like kinetic experiments, simulations can sometimes disprove wrong ideas but merely support a correct hypothesis. What is particularly important about the Martin paper is the way it lays out a rigorous method for simulating the growth of the microtubule lattice. If others have different ideas about the nature of the GTP cap and its mechanism for catastrophe or rescue, these mechanistic details can be linked to a lattice model along the lines set down by Martin et al. It will be

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interesting to see how other mechanisms fare by comparison.

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