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The instability of stabilization

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Actin filaments and microtubules control the shape of cells and organize their contents. The proteins that form these cytoskeletal polymers were identified and isolated decades ago—actin in 1942 (1) and tubulin in 1968 (2)—but we still argue about some of their basic properties. New work from Niedermayer et al. (3), however, puts us on the right track, or more properly back on the right track, to understanding how actin filaments fall apart. After years of study, how is it still possible for otherwise reasonable people to disagree about how actin filaments and microtubules assemble and disassemble? The shortest answer I can think of has two parts: (*i*) the details matter, and (*ii*) the experiments are hard. The details matter because small differences in the rate, mechanism, or geometry of polymer assembly and disassembly produce cytoskeletal networks with different architectures. Ultimately, we want to understand how cells manipulate such basic biochemical properties to produce different cytoskeletal structures with different biological functions. How, for example, does one cell construct a 3D pseudopod that pushes forward the plasma membrane; a linear stress fiber that pulls on cell adhesions; and a contractile ring that splits the cell in two—all from actin filaments? The experiments are hard, in part, because cytoskeletal polymers are constructed from large numbers of weak, noncovalent interactions, and they are dynamic, sometimes even ephemeral structures. To study assembly and disassembly of cytoskeletal polymers, we require techniques that maintain their native structure: techniques that often push technical boundaries in both biochemistry and physics. It is not surprising then, that the work of Niedermayer et al. represents the joint effort of two groups, one led by biochemist Marie France Carlier and the other by physicist Rheinhard Lipowsky.

The first single-polymer studies of cytoskeletal systems relied on static samples, often imaged by electron microscopy. Investigators used snapshots of polymer length distributions at different times to deduce rates of polymer growth and shortening and to demonstrate that both actin filaments and microtubules are kinetically polarized, with one end growing faster than the other. In the middle of the 1980s, static snapshots also revealed dramatic differences in the assembly dynamics of actin filaments and microtubules. Mitchison and Kirschner (4) uncovered a kinetic property they called "dynamic instability," whereby microtubules switch abruptly between stable growth and rapid, catastrophic disassembly. This switching is related to hydrolysis of GTP near the end of the microtubule. In contrast, all of the available data argued that actin assembly is a simpler process, in which rates of growth and shortening depend only on the filament polarity and the bound nucleotide. In this view, actin filament dynamics can be completely described by eight rate constants (5), governing growth and shortening of ATP- and ADP-actin filaments at fast- and slow-growing ends (Fig. 1).

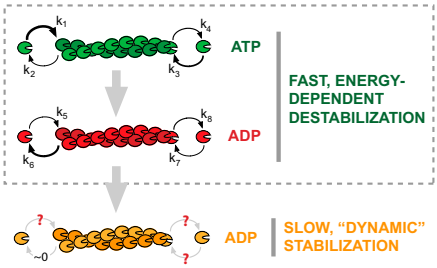

Fig. 1. Actin filament dynamics. (*Upper*) Chemical kinetic model for actin assembly, in which rates of assembly and disassembly depend only on filament polarity and bound nucleotide (ATP or ADP-Pi vs. ADP). Fast-growing, barbed ends are on the left and slow-growing, pointed ends on the right. (*Lower*) Slow conformational rearrangement (10) proposed to explain pauses observed during filament disassembly (9, 11).

Because microtubules are easier to detect than actin filaments, they were first to be filmed in the act of assembling and disassembling (6). Time-lapse movies of microtubules, imaged by dark-field and differential interference contrast microscopy, revealed structures with a complex personality, polymers that do indeed switch abruptly between stability and catastrophe. Later, time-lapse fluorescence microscopy of dye-labeled actin filaments only served to reinforce their reputation as stable structures: staid, generally predictable, and lacking the dynamic flair of microtubules.

Nowadays, state-of-the-art studies of actin and microtubule dynamics rely on holding fluorescent molecules near the surface of a glass coverslip and imaging them by total internal reflection fluorescence (TIRF) microscopy. Coupled with modern, electron-multiplying CCD cameras and image analysis algorithms, this approach can determine lengths of single polymers with nanometer accuracy and millisecond time resolution. One would imagine that actin filaments and microtubules, tackled with tools of this sophistication, could keep no more secrets, and indeed, long-standing mysteries are now being solved. For example, recent studies of microtubule assembly shed new light on the molecular mechanism of dynamic instability (7) and might, for the first time, lead to a satisfying mathematical description of the process. A mathematical model that describes the phenomenology of microtubule dynamics in terms of the basic biochemical properties of tubulin would be a powerful tool. It could be used to study the effects of microtubule binding proteins and to understand how microtubule-based structures assemble themselves in vivo. In a similar vein, elegant studies of actin filaments falling apart after dilution recently demonstrated that, after they hydrolyze ATP, subunits of an actin filament release the resulting phosphate randomly, with no regard for whether their neighbors have already given up their own phosphates (8). This may sound like a picayune detail but, as noted earlier, to understand how polymer dynamics controls cell architecture, we need to get the details right. In this case, actin filaments are taken apart by accessory factors, such as cofilin, which bind and disassemble filaments only after ATP hydrolysis and phosphate release. To understand patterns of filament disassembly we must, therefore, understand patterns of ATP hydrolysis and phosphate release.

Technical advances often solve old mysteries, but sometimes they create new ones. Watching fluorescent actin filaments depolymerize by TIRF, Mitchison and coworkers (9) saw something strange. Initially the actin filaments shortened as predicted by the rate constants measured 20 years earlier. As time went on, however, one by one, the shortening filaments abruptly slowed or paused in their disassembly. The authors argued that these age-dependent pauses are related to age-dependent rearrangements of actin filament architecture observed by electron microscopy (10) and that pausing was overlooked in earlier studies of actin assembly. This result, if true, would initiate

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a sort of catastrophic deconstruction of the standard model of actin dynamics (11). Rather than becoming less stable as subunits hydrolyze bound ATP, actin filaments would become more stable with time. Rather than obeying a single set of rules for growing and shortening, actin filament would exhibit dynamic switching. Because the proposed switching behavior was almost the mirror image of microtubule dynamic instability, the authors called it "dynamic stabilization."

Extraordinary claims call for extraordinary proofs, or at the very least exceptionally solid controls. Mitchison and colleagues, therefore, worked hard to demonstrate that dynamic stabilization was not an artifact of their experimental system. One source of stabilization they considered was electrostatic attachment of filaments to the nearby coverslip. Incomplete passivation of the glass surface might leave patches of bare glass to which filaments could adhere, and this might halt depolymerization. Mitchison and colleagues argued convincingly against this possibility by noting that the ends of paused filament often moved freely and did not appear tacked to the coverslip. They also performed depolymerization experiments in bulk solution using pyrene-labeled actin and observed a time-dependent slowing of the overall depolymerization rate, consistent with dynamic stabilization. In these bulk solution experiments the majority of actin filaments are far from a glass surface.

Dynamic stabilization was an obviously provocative and potentially important idea, so it is no surprise that other groups began to investigate it. Niedermayer et al. (3) grapple with the underlying mechanism using the most powerful and sophisticated experimental system yet developed for studying actin dynamics (12). This system enables the authors to not only record the dynamics of single actin filaments but also to rapidly switch buffer conditions and soluble protein concentrations. Although they image actin filaments close to a glass coverslip using TIRF microscopy, Niedermayer et al. minimize interaction between proteins and glass surfaces by attaching filaments to the coverslip only at one end, using spectrin–actin complexes. The filaments are housed inside a microfluidic device that flows fresh solution over the coverslip, at a constant rate. Fluid flow aligns the filaments and stretches them out so that their lengths can be measured, and microfluidics enable Niedermayer et al. to rapidly switch solutions perfusing the chamber. The authors first grow filaments by perfusing attached seeds with monomeric actin and then initiate filament disassembly by switching to a solution that contains no monomeric actin. Initially, they reproduce the results of the Mitchison group, observing long pauses in the process of disassembly. When they analyze the statistics of these pauses, however, Niedermayer et al. notice that they are not consistent with a global change in filament architecture but rather with a single, stabilizing event, occurring at a fixed position in the filament. They also note that the rate of pausing was not strictly related to the age of the filaments but to the length of time they were irradiated with intense excitation light. Pausing also increased with increasing amounts of fluorescently labeled protein, leading to the conclusion that "dynamic stabilization" is caused by photon-dependent chemical changes in the fluorescent probes attached to actin. These photo-chemical reactions can covalently cross-link adjacent subunits, creating stable dimers that can "cap" the end of a depolymerizing filament. In support of this idea, the authors find that irradiating dye-labeled actin generates cross-linked dimers. They also show that the rate at which paused filaments begin to shorten again fits kinetics expected for dissociation of a stable actin dimer.

The experimental system used by Niedermayer et al. sets a new gold standard for the study of actin filament dynamics.

The lesson of "dynamic stabilization" is that new and complex experimental systems may have great power, but they are also susceptible to new and wonderfully seductive artifacts. Some of these artifacts can be predicted, whereas others must be rooted out by statistical analysis. The experimental system used by Niedermayer et al. (3) sets a new gold standard for the study of actin filament dynamics (12). It is their careful data analysis, however, that ultimately uncovers the true nature of actin filament stabilization. In the end, those of us studying the cytoskeleton can rest easy, knowing that actin filaments are no closer to being microtubules and that they remain as stodgy and dependable as ever.

1. Straub FB (1942) Actin. *Stud Inst Med Chem Univ Szeged* II:3–15.
2. Weisenberg RC, Borisy GG, Taylor EW (1968) The colchicine-binding protein of mammalian brain and its relation to microtubules. *Biochemistry* 7: 4466–4479.
3. Niedermayer T, et al. (2012) Intermittent depolymerization of actin filaments is caused by photo-induced dimerization of actin protomers. *Proc Natl Acad Sci USA* 109:10769–10774.
4. Mitchison T, Kirschner M (1984) Dynamic instability of microtubule growth. *Nature* 312:237–242.
5. Pollard TD (1986) Rate constants for the reactions of ATP- and ADP-actin with the ends of actin filaments. *J Cell Biol* 103:2747–2754.
6. Horio T, Hotani H (1986) Visualization of the dynamic instability of individual microtubules by dark-field microscopy. *Nature* 321:605–607.
7. Gardner MK, et al. (2011) Rapid microtubule self-assembly kinetics. *Cell* 146:582–592.
8. Jégou A, et al. (2011) Individual actin filaments in a microfluidic flow reveal the mechanism of ATP hydrolysis and give insight into the properties of profilin. *PLoS Biol* 9:e1001161.
9. Kueh HY, Brieher WM, Mitchison TJ (2008) Dynamic stabilization of actin filaments. *Proc Natl Acad Sci USA* 105:16531–16536.
10. Orlova A, et al. (2004) Actin-destabilizing factors disrupt filaments by means of a time reversal of polymerization. *Proc Natl Acad Sci USA* 101:17664–17668.
11. Kueh HY, Mitchison TJ (2009) Structural plasticity in actin and tubulin polymer dynamics. *Science* 325: 960–963.
12. Jégou A, Carlier MF, Romet-Lemonne G (2011) Microfluidics pushes forward microscopy analysis of actin dynamics. *BioArchitecture* 1:271–276.