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Assessing actin's growth rate

In 1981, Pollard and Mooseker used electron microscopy to measure the rate of actin polymerization.

At the beginning of the 1980s, researchers knew that actin filaments were present in all cell types and were important for force generation and cell movement. But relatively little was known about how actin monomers assembled into filaments. In fact, researchers didn't even possess information as fundamental as the rate constants of monomer association and dissociation. In 1981, however, Tom Pollard and Mark Mooseker successfully measured these rates using electron microscopy, a method not usually associated with a process as dynamic as actin polymerization (1).

Previous studies had used light-scattering spectroscopy to measure the elongation of actin filament populations in vitro. But in 1975, Tom Pollard and colleagues demonstrated that the two ends of actin filaments grew at different rates; the "barbed" end elongated rapidly while the "pointed" end grew slowly (2). "That meant it would be difficult to learn anything if you only had a cuvette full of actin filaments. You'd actually have to look at them directly to see what was going on at the two ends," explains Pollard, who now works at Yale University but was then affiliated with Johns Hopkins University School of Medicine.

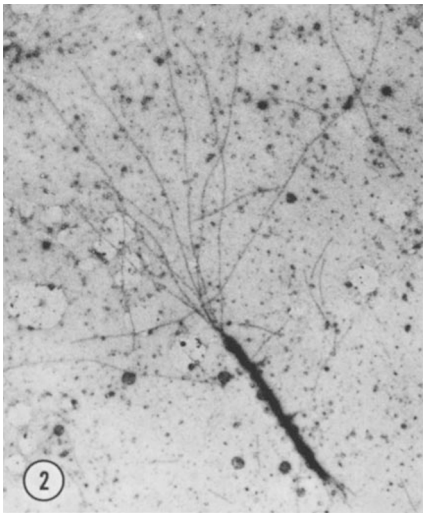
Lawrence Bergen and Gary Borisy had recently used electron microscopy to observe the growth of microtubules from isolated flagella axonemes (3). Pollard realized that he could use a similar approach to follow actin polymerization at both the barbed and pointed ends of actin bundles that Mark Mooseker, a former postdoc in Pollard's lab who was running his own group at Yale, was able to purify from intestinal brush borders. Pollard and Mooseker incubated these bundles with actin monomers and then used electron microscopy to measure the growth of filaments from both ends over time. "It worked pretty well, and, by looking at the rate of elongation as a function of actin concentration, we could calculate the kinetic rate constants at both ends," Pollard says. The numbers showed that actin monomers associated and dissociated at the barbed end much more quickly than they did at the pointed end.

As Pollard and Mooseker noted in their paper, however, one drawback to the electron microscopy method was that it was "tedious." Perhaps that is one reason why other researchers continued to measure actin elongation using bulk spectroscopy methods that produced rate constants that could only be explained by complicated actin polymerization reaction schemes. Pollard was skeptical of these schemes, however, so in 1986 he implemented several technical improvements—including the use of actin bundles isolated from the acrosomes of horseshoe crab sperm—to repeat his measurements with greater precision than before (4). "I looked over a much wider range of actin concentrations, compared different conditions, and contrasted the polymerization of ATP–actin and ADP–actin," Pollard says.

> "These rate constants have been the foundation for every subsequent actin polymerization paper."

Despite the technical improvements, measuring the growth of actin filaments by electron microscopy was still a laborious process. "It was hard to find the time to do this during the week, and I couldn't get anyone else to do it," Pollard recalls. "So I'd come into the lab every Saturday, turn on the Metropolitan Opera broadcast on the radio, and measure actin filaments all afternoon. After a while, I had these beautiful graphs. It was very satisfying!"

The new data refined the rate constants calculated five years previously and, by providing separate measurements for the polymerization of ATP–actin and ADP–actin at both the barbed and pointed ends, supported a simpler model of actin assembly in which the elongation rate is mainly determined by the nucleotide-binding state of actin monomers. "These rate constants have been the foundation for every subsequent actin polymerization paper," Pollard says.



An electron micrograph shows long filaments grown at the barbed end of an actin bundle, in contrast to the shorter filaments assembled at the pointed end.

The numbers obtained by electron microscopy also showed that, at steady state, there can be a net addition of actin subunits at the barbed end of filaments accompanied by a net loss of subunits from the pointed end, producing a treadmilling, or flux, of subunits from one end of the filament to the other. Pollard has since explained the molecular basis for this phenomenon (5), but he cautions that flux is probably too slow to be physiologically significant, a detail often overlooked by many researchers in the field.

In 2001, Pollard and colleagues were finally able to measure actin polymerization in real time using light microscopy (6), and they confirmed the rate constants obtained nearly 20 years before. "The '81 and '86 papers show that it was actually possible to do sophisticated experiments with primitive technology," Pollard reflects. "Back then, we didn't have so many resources, but we found a way to make these fundamental measurements."

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2. Woodrum, D.T., et al. 1975. *J. Cell Biol.* 67:231–237.
3. Bergen, L.G., and G.G. Borisy. 1980. *J. Cell Biol.* 84:141–150.
4. Pollard, T.D. 1986. *J. Cell Biol.* 103:2747–2754.
5. Fujiwara, I., et al. 2007. *Proc. Natl. Acad. Sci. USA.* 104:8827–8832.
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