

Lamellipodial actin branches out

In the late 1990s, Tatyana Svitkina and colleagues described how actin is organized at the leading edge of migrating cells.

Many cell types, including fibroblasts and epithelial cells, move forward by forming an actin-rich membrane protrusion, called a lamellipodium, at the front of the cell. In the early 1990s, researchers thought that lamellipodial protrusion was driven by the polymerization of linear actin filaments. In two *JCB* papers published in 1997 and 1999, however, Tatyana Svitkina and colleagues revealed that the actin-nucleating Arp2/3 complex and the depolymerizing factor cofilin help organize lamellipodial actin into a dynamic network of branched actin filaments (1, 2).

Svitkina visualized lamellipodial actin using a platinum-replica electron microscopy technique she had developed as a staff scientist at Moscow State University in Russia. In the mid-1990s, she was invited to help a former colleague, Alex Verkhovskiy, establish the technique in Gary Borisy's laboratory at the University of Wisconsin–Madison. The initial aim was to understand the organization and function of myosin II in migrating fish keratocytes (1). “But the lamellipodia of these cells were so beautiful that I couldn't resist looking at them,” Svitkina, now at the University of Pennsylvania, recalls. “The actin filaments looked branched and that was kind of heretical at the time. I couldn't believe it.”

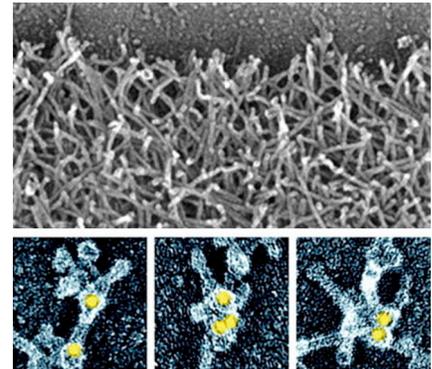
After convincing herself that this dendritic network was real, however, Svitkina realized that it would actually be much better at driving membrane protrusion than the linear actin filaments proposed to form in lamellipodia. Shorter filaments often branched off from longer ones, suggesting that new filaments might be nucleated from the sides of preexisting ones. This would allow cells to rapidly increase the number of filaments at the leading edge, and, because they were mechanically integrated into the entire actin network, even short filaments would be capable of pushing the plasma membrane outwards. “From a mechanical point of view, it looked much more productive,” Svitkina says.

One candidate for forming this dendritic network was the actin-nucleating Arp2/3

complex, which localized to lamellipodia (3), and could bind to the sides of actin filaments and assemble branched networks in vitro (4, 5). In 1998, Mullins et al. proposed a “dendritic nucleation” model, in which the Arp2/3 complex would nucleate new actin filaments and crosslink their pointed ends to the sides of existing ones (5). In 1999, therefore, Svitkina and Borisy took a closer look at lamellipodial actin and its relationship to the Arp2/3 complex (2).

The researchers found that lamellipodial actin filaments branched off from each other at an angle of 67°, similar to the actin branches formed by Arp2/3 in vitro. Because there were no antibodies to fish Arp2/3 subunits, Svitkina switched to *Xenopus* cells to examine by immunoelectron microscopy whether the complex localized to actin branch points. “But the lamellipodial actin network is so dense that it was hard to tell exactly where the Arp2/3 complex localized,” Svitkina recalls. However, by using low concentrations of actin-depolymerizing drugs to thin out the lamellipodial network, Svitkina saw that the Arp2/3 complex did indeed localize to filament branch points, strongly suggesting that the complex helped to form and maintain them. “I was very happy that I could prove it was there,” Svitkina says.

Svitkina and Borisy also suggested how the dendritic network might be disassembled at the rear of the lamellipodium. In the then prevalent model of lamellipodial protrusion, individual filaments “treadmilled” by simultaneously growing at their barbed ends near the leading edge and depolymerizing at their pointed ends further back in the cell (6). In the dendritic model, however, the filaments' pointed ends were localized throughout the lamellipodium, but were stabilized by their association with the Arp2/3 complex and the sides of other filaments. Svitkina and Borisy proposed that the network as a whole could treadmill if the Arp2/3 complex was displaced at the rear of the lamellipodium, allowing the actin depolymerizing factor cofilin to promote filament disassembly. Accordingly, cofilin was absent from the leading edge of keratocyte lamellipodia.



(Top) Electron microscopy shows the dendritic actin network at the leading edge of a keratocyte lamellipodium. (Bottom) Immuno-EM shows that the Arp2/3 complex (yellow dots) localizes to the network's branch points.

Dynamic, Arp2/3-dependent branched actin networks have now been shown to promote a variety of cellular processes, including endocytosis and cell–cell junction formation. “Wherever a cell needs to push hard, it engages this dendritic nucleation machinery,” Svitkina says. Nowadays, she adds, researchers are focused on how this machinery is adapted to different tasks, and how it is regulated by various accessory proteins. It also remains unclear precisely how the barbed ends of actin filaments manage to incorporate new subunits while they are pushed up against the leading edge plasma membrane.

“The discovery of branched actin networks within lamellipodia was met with much enthusiasm and some skepticism,” says Matt Welch, a *JCB* academic editor and expert in actin dynamics. “But in the end even skeptics were won over when these networks were observed by other electron microscopy methods. These landmark papers opened new avenues for modeling the biophysical mechanisms of force generation during lamellipodia protrusion, for revealing the molecular mechanisms that modulate actin dynamics, and for appreciating how microbial pathogens can hijack the cytoskeleton to cause disease.”

1. Svitkina, T.M., et al. 1997. *J. Cell Biol.* 139:397–415.
2. Svitkina, T.M., and G.G. Borisy. 1999. *J. Cell Biol.* 145:1009–1026.
3. Welch, M.D., et al. 1997. *J. Cell Biol.* 138:375–384.
4. Mullins, R.D., et al. 1997. *J. Cell Biol.* 136:331–343.
5. Mullins, R.D., et al. 1998. *Proc. Natl. Acad. Sci. USA.* 95:6181–6186.
6. Small, J.V. 1995. *Trends Cell Biol.* 5:52–55.