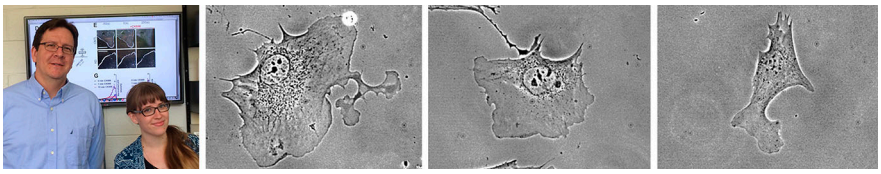
Text by Ben Short
bshort@rockefeller.edu

GMFβ prunes actin branches

Study reveals that turnover of lamellipodial actin networks helps fibroblasts follow adhesive guidance cues.

Focal Point



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James Bear (left), Elizabeth Haynes (right), and colleagues reveal that a protein called GMFβ helps disassemble the branched actin networks generated by the Arp2/3 complex at the leading edge of migrating fibroblasts, promoting lamellipodial retraction and the cells' ability to navigate towards adhesive directional cues. Compared with a control fibroblast (center), a cell lacking GMFβ (left) forms large lamellipodia that rarely retract. In contrast, a fibroblast overexpressing GMFβ (right) forms small, highly dynamic lamellipodia. In either case, the cells' ability to follow a gradient of the extracellular matrix protein fibronectin is perturbed.

Migrating fibroblasts assemble a dendritic network of branched actin filaments that drives the formation of a lamellipodial protrusion at their leading edge. Haynes et al. reveal that, like a topiarist guiding the growth of a tree, a protein called GMFβ "prunes" this actin network in order to guide the cell's migration towards certain directional cues (1).

Branched actin networks form when nucleation-promoting factors such as WASP and SCAR/WAVE activate the Arp2/3 complex, which nucleates the assembly of new actin filaments from the side of preexisting ones. "We know a lot about how you make actin branches," says James Bear, from the University of North Carolina at Chapel Hill, "but we don't know much about how this network gets turned over." Several proteins have been shown to disassemble Arp2/3-generated actin networks, including the actin-severing protein cofilin (2). A distant relative of cofilin, known as glia maturation factor (GMF), has also been implicated in the turnover of branched actin networks, even though this protein is unable to bind or sever actin filaments (3, 4). Instead, experiments in vitro and in budding yeast suggest that GMF can inhibit the Arp2/3 complex's activation by nucleation-promoting factors and destabilize the complex's interaction with actin filaments at network branch points (3–5). Bear and colleagues, led by graduate student Elizabeth Haynes, set out to investigate GMF's function in migrating fibroblasts (1).

> "The cells couldn't pick up the directional cue of the matrix."

Haynes et al. first determined that GMFβ—a ubiquitously expressed isoform of the protein and the only isoform expressed in fibroblasts—transiently localized to lamellipodia generated by the Arp2/3 complex. "Fibroblasts undergo cycles of protrusion and retraction," explains Bear. "GMFβ was recruited during the retraction phase when the branched actin network was being taken apart. When we knocked down GMFβ, the lamellipodia almost never retracted, and when they did, they retracted incredibly slowly." Lamellipodia were therefore larger in GMFβ-deficient fibroblasts, and Arp2/3-generated actin networks extended over a greater proportion of the cell perimeter. In contrast, fibroblasts overexpressing GMFβ formed smaller, more dynamic lamellipodia than control cells.

GMFβ therefore counteracts the assembly of branched actin networks by the Arp2/3 complex. To determine whether the protein does this by destabilizing the network branch points, or by preventing Arp2/3 activation, Haynes et al. treated fibroblasts with a small molecule inhibitor of the Arp2/3 complex called CK-666. "This stops actin branching and, in wild-type cells, the branched actin network disassembles fairly rapidly," Bear says. "But there was a distinct delay in network disassembly in GMFβ knockdown cells." Removing CK-666 induces a burst of actin branching and lamellipodial protrusion. These processes occurred at the same rate in wild-type and GMFβ-deficient cells, indicating that, at the leading edge of fibroblasts, GMFβ mainly debranches existing actin filaments rather than inhibiting the formation of new actin branches. Accordingly, a GMFβ mutant predicted to lack debranching activity was unable to rescue the lamellipodial dynamics of GMFβ knockdown cells.

The researchers then investigated whether GMFβ's ability to regulate lamellipodial dynamics affected fibroblast motility. Cells lacking GMFβ migrated slower than wild-type fibroblasts, but they were still able to navigate towards a source of the soluble chemoattractant PDGF. However, Haynes et al. then tested the cells' ability to follow a gradient of the adhesive extracellular matrix protein fibronectin, a process known as haptotaxis. "Knocking down or overexpressing GMFβ completely blocked haptotaxis," Bear says. "The cells couldn't pick up the directional cue of the matrix."

This is consistent with a previous study from Bear's lab demonstrating that Arp2/3-generated lamellipodia are required for haptotaxis, when cells need to actively seek out immobilized directional cues, but dispensable for chemotaxis, when cells can passively receive signals from soluble chemoattractants (6). "Our hypothesis is that the dynamic protrusion and retraction of lamellipodia is a sensing mechanism for the extracellular matrix," Bear explains. "Is there a mechanism to regulate GMFβ that helps cells tune when and where they make lamellipodia?"

1. Haynes, E.M., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb. 201501094
2. Chan, C., et al. 2009. *Curr. Biol.* 19:537–545.
3. Gandhi, M., et al. 2010. *Curr. Biol.* 20:861–867.
4. Ydenberg, C.A., et al. 2013. *Curr. Biol.* 23:1037–1045.
5. Luan, Q., and B.J. Nolen. 2013. *Nat. Struct. Mol. Biol.* 20:1062–1068.
6. Wu, C., et al. 2012. *Cell.* 148:973–987.