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Arp2/3-deficient cells share their problems

Loss of the actin-nucleating complex activates a stress response that nonautonomously inhibits chemotaxis.

The Arp2/3 actin-nucleating complex assembles branched actin networks to drive membrane protrusion at the leading edge of migrating cells. Accordingly, cells lacking Arp2/3 move at slower speeds, but whether the complex is required to guide cells toward a chemoattractant is unclear. Wu et al. reveal that the loss of Arp2/3 inhibits chemotaxis cell nonautonomously by up-regulating the secretion of factors that perturb EGF signaling (1).

In 2012, James Bear, Congying Wu, and colleagues at the University of North Carolina–Chapel Hill generated fibroblasts lacking two key subunits of the Arp2/3 complex (2). Surprisingly, the cells were still able to navigate toward a source of the soluble chemoattractant PDGF. "The cells were slow, but they could sense and respond to this cue," Bear says.

Wu et al. subsequently found that Arp2/3-deficient fibroblasts also responded to gradients of EGF. Around the same time, however, a second group of researchers reported that Arp2/3-deficient fibroblasts were unable to migrate toward a source of EGF (3). "[Suraneni et al.] got the exact opposite result," says Bear. "We were totally confused by this."

A possible explanation arose when Wu et al. examined the expression profile of their Arp2/3 knockdown cells (1). "A whole host of chemokines, cytokines, and matrix metalloproteases were up-regulated [in the absence of Arp2/3]," Bear explains. "We thought this might explain the difference between our chemotaxis results and those from [Suraneni et al.]." Wu et al. performed their chemotaxis experiments using microfluidic chambers in which the cell supernatant was constantly replaced by fresh media. Suraneni et al., on the other hand, used sealed chambers in which the media remained unchanged throughout the experiment. Bear and colleagues wondered whether proteins secreted by Arp2/3-deficient fibroblasts would inhibit chemotaxis in sealed chambers but be washed away under continuous flow conditions, allowing chemotaxis to proceed as normal.

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To test this idea, Wu et al. funneled conditioned media, rather than fresh media, through their continuous flow chambers. Media collected from control cells had no effect on the movement of Arp2/3-deficient fibroblasts toward EGF, but media collected from Arp2/3-deficient cells disrupted the chemotaxis of both Arp2/3-deficient and control cells, apparently by hyperactivating the EGF signaling pathway so that the cells became insensitive to EGF gradients. "When you manipulate the cytoskeleton, you don't really think about nonautonomous effects," says Bear. "But our results show that when you inhibit the Arp2/3 pathway you affect not just those cells but the cells around them, too."

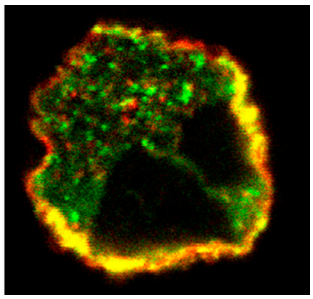
But how and why do Arp2/3-deficient cells up-regulate the secretion of chemokines and cytokines? The transcription factor NF-κB induces a similar array of inflammatory proteins in senescent cells, and Wu et al. found that NF-κB was activated in fibroblasts lacking Arp2/3. Inhibiting NF-κB blocked the up-regulation of inflammatory factors in these cells. The researchers also identified two proteins—the MAP kinase MEKK3 and the actin-binding scaffold protein CCM2—that activated NF-κB in the absence of Arp2/3.

MEKK3 and CCM2 have previously been implicated in the osmotic stress response (4). This pathway was hyperactivated in Arp2/3-deficient cells, suggesting that the Arp2/3 complex helps cells adapt to osmotic stress. "Osmotic stress responses depend on the actin cortex," says Bear. "So we tested the role of Arp2/3 at the cell cortex."

Arp2/3 was recruited to the cell cortex under hyperosmotic conditions, and the amount of cortical actin was reduced in cells lacking Arp2/3. Accordingly, Arp2/3-deficient cells were unable to adjust their volume and were more likely to die when placed in hyperosmotic media. Treating the cells with hypoosmotic media, on the other hand, reduced the activity of the osmotic stress pathway and NF-κB.

"So we think that, in response to hyperosmotic shock, Arp2/3 builds a robust actin cortex that will alter the signaling and mechanical properties of the cell," Bear explains. "Without Arp2/3, the stress response runs amok. It opens up a new way of thinking about Arp2/3's role in cell physiology."

Focal Point



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(Left to right) Sreeja Asokan, James Bear, Liz Haynes, Congying Wu, and colleagues (not pictured) resolve conflicting data about whether or not the actin-nucleating Arp2/3 complex is required for chemotaxis. Loss of Arp2/3 induces the secretion of numerous inflammatory proteins that perturb EGF signaling, thereby inhibiting chemotaxis cell nonautonomously. The researchers reveal that Arp2/3 (green) promotes assembly of the actin cortex (red), which helps cells adapt to osmotic stress. In the absence of Arp2/3, the osmotic stress response runs amok, activating the transcription factor NF-κB to induce expression of chemokines and cytokines.

1. Wu, C. et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201306032.
2. Wu, C., et al. 2012. *Cell*. 148:973–987.
3. Suraneni, P., et al. 2012. *J. Cell Biol.* 197:239–251.
4. Uhlik, M.T., et al. 2003. *Nat. Cell Biol.* 5:1104–1110.