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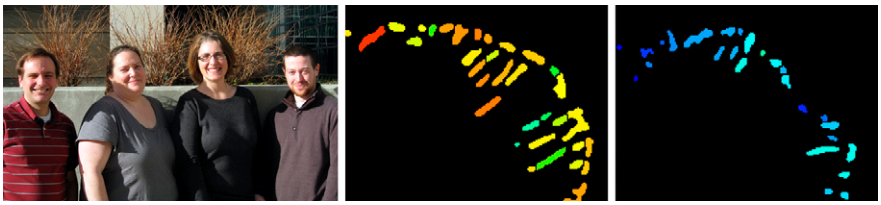
Stress fibers guide focal adhesions to maturity

Study suggests that actin bundles serve as templates for adhesion growth.

Focal Point



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(Left to right) Jonathan Stricker, Yvonne Beckham, Margaret Gardel, and Patrick Oakes reveal that focal adhesion–associated stress fibers aren't required to transmit force from the actin cytoskeleton to the extracellular matrix but they are essential for the growth and maturation of adhesions into stable cell–matrix attachments. The authors suggest that the stress fibers form a structural template that helps recruit focal adhesion components. Heat maps show that autophosphorylated focal adhesion kinase is more enriched at focal adhesions in a control cell (center) than in a cell lacking radial stress fibers (right).

Integrin-based focal adhesions initially form at the leading edge of a migrating cell and then mature into larger structures that stably attach and transmit force to the extracellular matrix (ECM). Oakes et al. describe how this maturation process is guided by the actin stress fibers that assemble at nascent adhesions (1).

Maturing focal adhesions grow in size and change their protein composition, and, in fibroblasts, they eventually develop into specialized fibrillar adhesions that can remodel the ECM (2). Maturation depends on the motor protein myosin II, which puts the adhesions under tension and promotes the assembly of adhesion-associated actin bundles called radial stress fibers. "It's been presumed that maturation is entirely tension dependent and that the stress fibers are important for transmitting the myosin-generated forces to the adhesions," explains Margaret Gardel, from the University of Chicago. But myosin generates tension and radial stress fibers simultaneously, making it difficult to dissect the roles of these two processes in adhesion maturation. "We wanted to investigate whether the radial stress fibers that form at focal adhesions are important for force transmission and the maturation process," Gardel recalls.

Gardel and colleagues, led by Patrick Oakes and Yvonne Beckham, blocked the formation of adhesion-associated stress fibers by inhibiting either the actin-nucleating formin Dia1 (3) or the filament-bundling protein α-actinin (4). Cells treated with inhibitors or shRNAs targeting these proteins failed to assemble radial stress fibers, despite having normal levels of active myosin II. Other actin structures in the cell were unaffected.

Cells lacking radial stress fibers formed small focal adhesions, which, says Gardel, "were still under a lot of tension, so the stress fibers aren't that important for force transmission." The adhesions' small size, however, suggested that, in the absence of radial stress fibers, tension isn't sufficient to drive adhesion maturation. Indeed, key components of mature focal adhesions weren't recruited when stress fiber formation was inhibited, and fibroblasts lacking Dia1 or α-actinin failed to assemble fibrillar adhesions capable of remodeling the ECM.

"So the recruitment of proteins [to form mature adhesions] is strongly correlated to the assembly of actin bundles at the focal adhesion plaque," Gardel says. Adhesions fail to mature in the absence of radial stress fibers, even though myosin II continues to exert significant amounts of force on nascent cell–matrix attachments.

> "Radial stress fibers... serve as structural templates to recruit other focal adhesion proteins."

Oakes et al. then examined the effect of myosin II–dependent tension on focal adhesion maturation. The researchers limited myosin II activity by treating cells with increasing concentrations of a Rho kinase inhibitor. Though some myosin II activity is required to form mature adhesions, motor function and intracellular tension could be reduced by as much as 80% without inhibiting adhesion growth and maturation. "So you only need a really minimal threshold of tension," Gardel says.

Gardel and colleagues think that this minimal level of myosin II activity is required to drive a "retrograde flow" of actin filaments away from the cell's leading edge. These filaments are captured at nascent cell adhesions and converted by Dia1 and α-actinin into dense actin bundles. "We think that these radial stress fibers then serve as structural templates to recruit other focal adhesion proteins," Gardel explains. "You need a sufficient amount of tension to form the template, but maturation isn't a tension-dependent process above that threshold."

Focal adhesions can be regulated by tension, however. Cells form smaller adhesions on soft matrices than they do on more rigid substrates, suggesting that ECM stiffness can regulate the assembly of radial stress fibers. "That's something we're investigating now," Gardel says. "How is stress fiber assembly regulated at focal adhesions, and why does that depend on ECM cues?"

1. Oakes, P.W., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201107042.
2. Gardel, M.L., et al. 2010. *Annu. Rev. Cell Dev. Biol.* 26:315–333.
3. Hotulainen, P., and P. Lappalainen. 2006. *J. Cell Biol.* 173:383–394.
4. Choi, C.K., et al. 2008. *Nat. Cell Biol.* 10:1039–1050.