

## Focal adhesions come unstuck

When cells let go of a surface, they don't let go of their integrins.

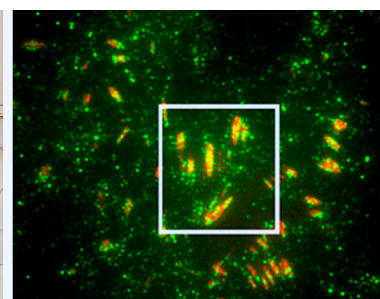
**C**rawling cells pick up after themselves before moving on. Ezratty et al. (1) show that cells use the endocytic protein clathrin to reabsorb the integrin receptors that attach them to surfaces, instead of simply leaving the molecules behind.

Cells on the go gain traction by forming focal adhesions (FAs), temporary attachments to the surface. But if they can't break these connections, cells get stuck. Scientists know much more about the assembly of FAs than about their disassembly. Microtubules appear to spur FA breakdown, possibly by shipping in an unidentified "relaxing factor" (2). Also unclear is what happens to the integrins that bunch up at FAs and fasten to the extracellular matrix. Cells might just crawl away from their integrins (3), leaving them behind like the pitons mountaineers leave in a rock face. But the leading hypothesis holds that cells absorb integrins through endocytosis, possibly allowing them to be reused later. Teasing out the molecular mechanism of FA disassembly has proven difficult because cells simultaneously create and break the connections. Four years ago, the researchers devised a way to sort out the two processes (4). They collapsed the cell's microtubules with nocodazole, triggering a flurry of FA formation. Removing the drug prompts the adhesions to break down as the tubules reassemble.

Using this same approach, Ezratty et al. tested whether clathrin—a protein that encapsulates vesicles and promotes endocytosis—takes part in FA breakdown. Not only did clathrin amass at FAs, two of the adaptor molecules that attach clathrin to its targets, Dab2 and ARH, also accumulated there. In cells lacking clathrin, FA disassembly decreased by up to 80%. The process also faltered if the adaptor proteins were absent. And cells missing clathrin or the adaptors stalled; their front ends advanced while



Ellen Ezratty (left), Gregg Gundersen, and colleagues tracked what happens to the integrin receptors of a crawling cell. They found that the endocytic protein clathrin (green) homes in on focal adhesions (red) and helps absorb the integrins.



### FOCAL POINT

their tails remained attached to the surface, stretching the cells out.

The researchers observed that integrins abandoned the cell surface upon FA disassembly. Surface levels of the  $\alpha 5$  integrin subunit plunged 50% after nocodazole removal, for instance. These absconding integrins showed up in vesicles carrying the small GTPases Rab5 and Rab11, evidence that the receptors had been absorbed through the endocytic pathway. And the cell surface levels of integrins that

were not part of cell attachments didn't change, showing that endocytosis was specific to FAs.

Clathrin's role as a shepherd for integrins became clear when the team observed the lower surfaces of crawling fibroblasts with total internal reflection fluorescence microscopy. They saw clathrin sidling up to integrins in FAs and the

two types of molecules departing the FAs together. Cells might use the reabsorbed integrins to build new FAs, although researchers haven't yet demonstrated this recycling.

"The work begins to get at the question of how focal adhesions are disassembled, something we know little about," says senior author Gregg Gundersen.

He says that clathrin could be the relaxing factor that microtubules deliver to FAs. Previous studies have reported that clathrin scoots along the trackways. But, he says, it's hard to imagine how clathrin could extract integrins, which associate with other proteins in FAs. So the relaxing factor might be a different molecule that loosens the packed proteins so that clathrin can gain access to them.

Other molecular details remain to be worked out. "Whether all focal adhesion integrin disassembles by this mechanism or through other pathways is another question," says Gundersen. The protease calpain is just one of the other molecules known to help break down FAs under certain conditions. How many adaptor proteins are involved is also unclear. A recent study suggested that the adaptor AP-2 helped spur integrin endocytosis in fibrosarcoma cells (5). The findings might have a practical payoff, providing drug designers with a new target, since blocking clathrin or other proteins that take apart FAs might immobilize cancer cells.

1. Ezratty, E.J., et al. 2009. *J. Cell Biol.* doi:10.1083/jcb.200904054.
2. Kaverina, I., et al. 1999. *J. Cell Biol.* 146:1033–1044.
3. Palecek, S.P., et al. 1996. *J. Cell Sci.* 109:941–952.
4. Ezratty, E.J., et al. 2005. *Nat. Cell Biol.* 7:581–590.
5. Chao, W.T., and J. Kunz. 2009. *FEBS Lett.* 583:1337–1343.

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