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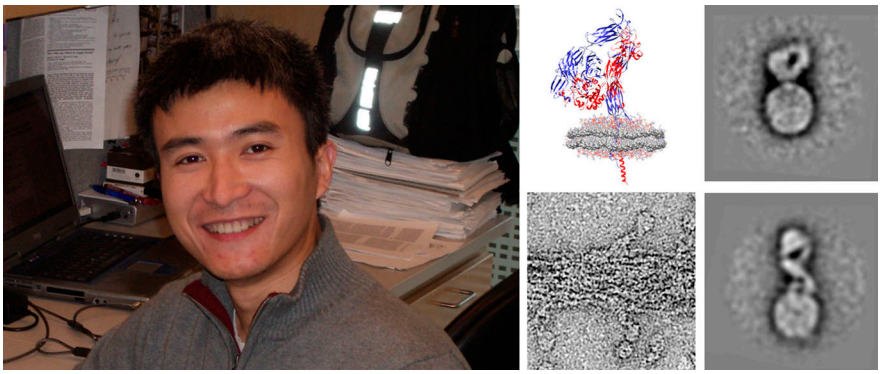
The integrin reconstruction act

Recreating integrin activation in vitro resolves several long-running controversies.

Focal Point



Feng Ye (above) and colleagues from Florida State University, the University of Illinois, The Burnham Institute, and UC San Diego resolve several long-standing controversies over the mechanism by which integrin adhesion receptors are activated to bind their extracellular matrix ligands with higher affinity. The researchers recreated the process in vitro by inserting single integrin molecules into phospholipid nanodiscs (top row). The cytoplasmic protein talin was sufficient to activate these integrins, causing them to adopt an extended conformation (bottom right) capable of binding extracellular matrix (bottom left).

"In biology, you really know what you're talking about when you can reconstruct a system synthetically," says Mark Ginsberg from UC San Diego. That hasn't been possible in the case of integrin activation—the process by which integrin adhesion receptors gain an increased affinity for their extracellular matrix ligands. The activation mechanism has been highly controversial for more than a decade, partly because the phenomenon hasn't been accurately recapitulated with purified proteins in vitro. But a team of scientists has now successfully reconstructed the events leading to integrin activation, which may help settle some of the field's major disputes (1).

Integrin activation occurs from the inside out: events in the cytoplasm change the behavior of the integrins' extracellular domains (2). A key event is the binding of a protein called talin to the integrin-β subunit's cytoplasmic tail. According to different structural, genetic, and cell biological studies, talin binding may or may not be sufficient to activate the adhesion receptors—recent reports suggest that a family of proteins called kindlins may be required, too (3). It's also unclear whether activation involves a change in integrin conformation, or whether receptor clustering increases affinity for the extracellular matrix. On top of that, the role of force in integrin activation has remained elusive, with some suggestions that tension may be required to pull integrins into an active conformation (4).

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Ye et al. directly tested the contribution of talin to integrin activation by reconstituting the process in vitro with purified integrins inserted into phospholipid liposomes. Addition of the head domain of talin—the key component for integrin activation—was sufficient to induce the integrins to bind their ligand in greater amounts. Whereas kindlins may be involved in integrin activation in cells, it seems talin doesn't need their help in vitro. However, the integrins must be membrane embedded, and mutating a phospholipid-binding site in talin reduced its ability to activate integrins in vitro. The group proposes that the membrane helps orient the talin and its bound integrin-β subunit to effect activation (5).

But what happens to integrins when they're activated by talin in vitro? Do they undergo a conformational change or do they cluster together? They didn't seem to be clustering on the liposome surface, but to answer the question definitively, Ye and colleagues immobilized single integrins in lipid nanodiscs—10–13-nm phospholipid bilayers stabilized by a membrane scaffold protein. Again, the talin head was sufficient to increase ligand binding, even though electron microscopy (EM) revealed that individual integrins remained isolated in separate nanodiscs.

Thus, talin increases the affinity of single, unclustered integrins. It seems to do this by inducing a conformational change in integrin, so that its extracellular domain extends away from the membrane surface instead of forming a compact, bent shape. The team's EM analysis of nanodisc-embedded integrins shows that talin shifts the adhesion receptor to a more extended conformation even in the absence of ligand or pulling forces. "This is the first direct proof that talin binding alone activates a single integrin and shifts its equilibrium toward the extended form," says Ginsberg.

The establishment of an in vitro system that recreates integrin activation with purified proteins should help solve many more questions about the process. How is talin regulated? Do changes in membrane lipid composition affect activation? And what exactly do kindlins do? "Genetics tells us that they're doing something," explains Ginsberg. "Are they working directly to activate integrins, or do they have a more indirect effect? With this system, we can add back proteins and figure out who is doing what to whom."

The ultimate goal is larger still. "I think this is the first step toward the synthetic recreation of integrin-based focal adhesions," Ginsberg says. "That's something that a lot of people will be trying to do in the next five years."

1. Ye, F., et al. 2010. *J. Cell Biol.* doi:10.1083/jcb.200908045.
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3. Moser, M., et al. 2009. *Science.* 324:895–899.
4. Zhu, J., et al. 2008. *Mol. Cell.* 32:849–861.
5. Wegener, K.L., et al. 2007. *Cell.* 128:171–182.