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Not all vinculins are created equal

Cells modify force-transmitting protein for use in adherens junctions.

Focal Point

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Ashley Angell (back row, second from right), Kris DeMali (back row, right), Jennifer Bays (front row, center), Xiao Peng (front row, right), and colleagues (not pictured) discovered how cells distinguish vinculin molecules that help form the adherens junctions between cells. The team found that cells phosphorylate these vinculins at tyrosine 822. Vinculin molecules sporting this modification (red) congregate with E-cadherin (top right, green) at cell–cell junctions but don't associate with talin (bottom right, green), a marker of cell–extracellular matrix adhesions.

The actin-binding protein vinculin leads a double life in a cell, bolstering contacts with other cells and strengthening adhesions to its substrate. Bays et al. now explain what differentiates the protein for its distinct roles, revealing that cells phosphorylate vinculin molecules in cell–cell junctions (1).

When cells are stretched, squeezed, or bent, they don't just sit there passively. They stiffen in response to force, thus maintaining their shape. The signaling cascade that incites this reaction involves Rho GTPases, such as RhoA, which stimulate myosin II and actin filaments to produce internal forces that counteract the external ones (2). To detect outside forces, cells rely on adhesion receptors such as integrins and cadherins (3). Cadherins anchor the connections between cells, whereas integrins attach cells to the surrounding extracellular matrix (ECM). Cell–cell junctions share many proteins with cell–ECM adhesions, including vinculin, which relays external forces to the cytoskeleton and helps the cell retain its shape (4). But these two types of junctions have different functions and operate in different situations, raising the question of how similar molecular lineups elicit distinct behaviors. One answer, Bays et al. determined, is that cells customize vinculin for intercellular adhesions.

The observation that pointed the researchers toward this conclusion came when they were measuring the levels of vinculin phosphorylated at tyrosine 822. The team noticed that, in confluent cultures that are so crowded that the cells touch, the levels of this variant were four times higher than in less dense cultures. That difference suggested that phosphorylated vinculin has a role in cell–cell adhesions.

To confirm that possibility, the researchers forced the cells to undergo an epithelial-to-mesenchymal transition, a transformation that breaks intercellular connections. After the cells disengaged from each other, the levels of vinculin carrying a phosphate at tyrosine 822 plunged. Bays et al. saw a similar result when they disrupted cell–cell adhesions by lowering the level of calcium in the culture media. The researchers also created a vinculin mutant that can't be phosphorylated at position 822. Cells carrying this variant didn't form tight connections with other cells.

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To uncover the function of phosphorylated vinculin, the team gauged how external forces affected the protein's phosphorylation. Bays et al. used magnetic beads covered with antibodies to tug on cells' integrins or cadherins. Yanking on E-cadherin molecules, but not integrins, increased vinculin phosphorylation. The researchers then measured whether the modified vinculin enabled cells to stiffen in response to external force. Control cells firmed up when the researchers tugged on E-cadherin, but cells carrying the phosphorylation-resistant form of vinculin remained soft.

Preventing vinculin phosphorylation didn't appear to hamper integrins, the team showed. Cells carrying the nonphosphorylatable version attached tightly to surfaces, and they stiffened in response to tension on their integrins.

The researchers expected that the Src tyrosine kinase, which flips on when cadherins interlock, would phosphorylate vinculin. But they found that the Abelson (Abl) kinase does the job instead. The enzyme activates when cadherins respond to force, the team showed, and blocking it breaks down adherens junctions.

Cells stockpile vinculin in the cytoplasm and parcel it out to cell–cell junctions and cell–ECM contacts. "It's never been elucidated how vinculin functions at one adhesion complex versus the other," says senior author Kris DeMali. "Our data reveal a mechanism." The findings also explain some of the side effects of the anticancer drug Gleevec. Gleevec inhibits Abl, so it makes sense that patients receiving the drug sometimes suffer symptoms, such as edema, that indicate faulty connections between cells. An important question to answer now, DeMali says, is what signals spur cells to phosphorylate vinculin and tailor it for cell–cell adhesions.

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2. Goldyn, A.M., et al. 2009. *J. Cell Sci.* 122:3644–3651.
3. Chen, C.S., et al. 2004. *Annu. Rev. Biomed. Eng.* 6:275–302.
4. Grashoff, C., et al. 2010. *Nature*. 466:263–266.