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MECHANOTRANSDUCTION

Switch and stretch

> How mechanical force results in a chemical response…

How mechanical force results in a chemical response is poorly understood. Two papers in *Science* provide new insights into the mechanisms that underlie the activation of the adhesion receptor α5β1 integrin (α5β1) by mechanical force and the interaction of the force-bearing cytoskeleton protein talin with its binding partner, vinculin.

Friedland *et al.* analysed the mechanoresponsive properties of α5β1 bound to its ligand fibronectin in the presence and absence of mechanical tension. The ability or inability of specific crosslinkers to crosslink α5β1 to fibronectin provides a chemical basis for distinguishing between two types of bonds, referred to as the relaxed and tensioned states. For fibroblast-like cells that are plated on stiff (plastic or glass) fibronectin-coated surfaces, both the proportion of α5β1 that can be crosslinked and the force that is required to detach the cell are proportional to the number of adhesive α5β1–fibronectin bonds. But when myosin II function is inhibited in applying tension to the cell through actin filaments, the bond is relaxed and can no longer be crosslinked. The application of tension with a spinning disc device returns it to the tensioned form.

How does α5β1–fibronectin respond to tension? Mutations in the RGD domain of fibronectin prevent any binding to α5β1, whereas mutations in the synergy site reduce binding by 90%. Because the effect of the synergy site is only seen under tension, this suggests that the relaxed form engages the RGD domain, whereas the tensioned form engages both the RGD and synergy sites — resulting in a stronger bond.

The ability of cells to sense the stiffness of a substrate is a key feature of mechanosensing. But how do cells distinguish between soft and stiff substrates? The authors show that the proportion of α5β1 bound to fibronectin is not affected by substrate stiffness, but that the proportion of α5β1 in the tensioned state is directly dependent on substrate stiffness. In addition, signalling from α5β1, as measured by phosphorylation of focal adhesion kinase on residue Y397, correlates with the tensioned form. This implies that it is the conversion of α5β1 from the relaxed to the tensioned state, rather than the binding of α5β1 to the ligand, that generates the downstream signals. These findings show that the α5β1 adhesion receptor is not only the 'anchor' in a mechanosignalling system, but it also has mechanosensing and mechanoresponsive properties.

At a molecular level, del Rio *et al.* show that stretching of cytoskeletal molecules can expose binding sites for adhesion complex proteins. The interaction of the cytoskeletal protein talin, which links membrane integrins to the cytoskeleton through its actin-binding site, recruits additional vinculins upon stretch. The talin rod lies between the integrin- and actin-binding sites and contains multiple α-helical bundles with many hidden vinculin-binding sites.

The authors used magnetic tweezers to mechanically stretch single talin rod molecules, and determined the number of fluorescently labelled vinculin molecules that are bound to the talin rod by counting single-molecule photobleaching events. In the absence of force, the authors observed either no events or just one, which increased to three in the presence of force — that is, upon exposure of up to two additional vinculin-binding sites.

Atomic force microscope measurements of talin stretching showed that it would rapidly stretch with physiological forces. Thus, force-dependent talin stretching and unfolding exposes cryptic vinculin-binding sites, leading to downstream intracellular responses. Since many cytoskeletal proteins contain multiple domains of α-helical bundles, this is potentially a general mechanism of force transduction into biochemical signals.

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STOCKBYTE

ORIGINAL RESEARCH PAPERS del Rio, A. *et al.* Stretching single talin rod molecules activates vinculin binding. *Science* **323**, 638–641 (2009) | Friedland, J. C. *et al.* Mechanically activated integrin switch controls $\alpha_5\beta_1$ function. *Science* **323**, 642–644 (2009)

FURTHER READING Geiger, B. *et al.* Environmental sensing through focal adhesions. *Nature Rev. Mol. Cell Biol.* **10**, 21–33 (2009) | Vogel, V. & Sheetz, M. Local force and geometry sensing regulate cell functions. *Nature Rev. Mol. Cell Biol.* **7**, 265–275 (2006)