TECHNIQUE

DNA hairpins track traction

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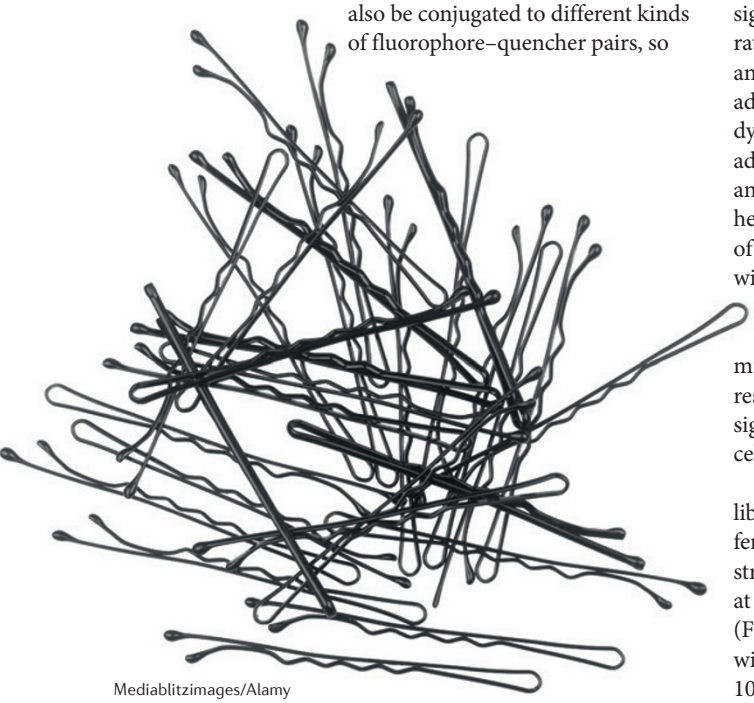
Mechanical forces such as cell traction control cell growth, differentiation, motility and embryonic development. The techniques available for measuring traction forces are restricted in spatial and/or temporal resolution, force sensitivity and tunability. To address these limitations, two research groups, reporting in *Nature Methods* and in *Nature Communications*, took advantage of the physical properties of DNA hairpins — they unfold in response to precise amounts of force — to develop optical, DNA-based tension probes that can be designed easily to report forces of varying intensities, with high spatiotemporal resolution and high signal-to-noise ratio.

DNA hairpins can be tuned to respond to different amounts of force by varying their sequence, length and secondary structure. They can also be conjugated to different kinds of fluorophore–quencher pairs, so that the fluorophores are quenched when the hairpins are folded, but fluoresce in reaction to forces that can open the hairpins. One terminus of the hairpins can additionally be conjugated to different adhesive peptides or proteins. Both research groups conjugated it to Arg–Gly–Asp-based peptides, which bind integrins (transmembrane receptors that relay mechanical signals from the extracellular matrix (ECM) to the cytoskeleton). The second terminus of the hairpin can be chemically modified to bind to various cell culture substrates. When cells are attached to a substrate through these tension probes, the emitted fluorescence enables cell traction forces to be measured accurately.



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By applying various hairpin sensors to mouse fibroblasts, Blakely *et al.* detected fluorescent signals with high signal-to-noise ratio that mirrored the sizes, shapes and distribution of focal (cell–ECM) adhesions. The imaged fluorescence dynamics resembled those of focal adhesion assembly and disassembly, and the authors found considerable heterogeneity in the distribution of traction forces between and within focal adhesions. Importantly, suppressing or enhancing cell contractility resulted in the dimming or brightening of fluorescence, respectively, suggesting that these signals indeed reflect changes in cellular traction forces.

Zhang *et al.* synthesized a small library of tension probes with different GC contents and secondary structures, to determine the force at which 50% of hairpins unfold ($F_{1/2}$). They found that for probes with GC contents of 22% and 100%, the experimental $F_{1/2}$ was 4.7 ± 1.7 piconewtons (pN) and 13.1 ± 2.4 pN, respectively. They then plated breast cancer cells onto a 22% GC probe surface and acquired time-lapse videos as cells initiated spreading and adhesion. Within minutes the tension signals localized to cell edges and increased in intensity, suggesting that cell adhesion forces rapidly reach and exceed 4.7pN. Furthermore, and similarly to the findings by Blakely *et al.*, these signals were highly dynamic and heterogeneous. Using rat fibroblasts expressing GFP-β3-integrin, Zhang *et al.* also showed that tension signals colocalize with GFP, indicating that hairpin unfolding is indeed mediated through integrin receptors. Interestingly, a subset of focal adhesions near the cell edge preferentially triggered the 100% GC probes over the 22% GC probes, suggesting that some integrins might prefer to bind more rigid ligands.

DNA hairpin-based tension probes are easily designable sensors of cellular forces that offer high resolution and the ability to couple variable ligands and substrates, including three-dimensional matrices that better mimic the ECM. As such, they should prove valuable in elucidating the contribution of mechanical forces to cell behaviour and function.

Eytan Zlotorynski

ORIGINAL RESEARCH PAPERS

Blakely, B. L. *et al.* A DNA-based molecular probe for optically reporting cellular traction forces. *Nature Methods* http://dx.doi.org/10.1038/nmeth.3145 (2014) | Zhang Y. *et al.* DNA-based digital tension probes reveal integrin forces during early cell adhesion. *Nature Communications* http://dx.doi.org/10.1038/ncomms6167 (2014)

FURTHER READING

Iskratsch, T., Wolfenson, H. and Sheetz, M.P. Appreciating force and shape — the rise of mechanotransduction in cell biology. *Nature Rev. Mol. Cell Biol.* http://dx.doi.org/10.1038/nrm3899 (2014)