BIOPHYSICS

Soft cells

The bulk properties of a cell's cytoplasm have long been thought to resemble those of hydrogels, such as agar or children's party jelly. Now, using measurements on single cells, Ben Fabry and colleagues show that, in fact, they more closely resemble a soft glass.

Fabry and colleagues attached ferrimagnetic beads to the surface of single cells, held them in place with optical tweezers, and then rolled them back and forth in an oscillating electric field. By following the movement of these beads, a number of physical properties of the cytoplasm, such as the elastic modulus, could be estimated.

What emerged was a complex dependence on the speed of the oscillations — not what would be anticipated for a gel. Rather, the cells behaved like members of the 'soft glassy' class of materials, which also includes foams and slurries.

This could be because the cytoskeleton is never at equilibrium but is actually in constant flux. Microfilaments and microtubules assemble and disassemble, dissipating energy by ATP and GTP hydrolysis. Their crosslinks are also dynamic and many are formed by molecular motors — again powered by ATP.

If successful, this soft-glass model would replace the current view in which cell movements arise from phase transitions between stationary gels and fluid-like sols. However, it will take many more rheological studies, such as that of Fabry and colleagues, to fully test and define the bulk properties of single cells.

Christopher Surridge, Senior Editor, Nature

References and links

ORIGINAL RESEARCH PAPER Fabry, B. *et al.* Scaling the microrheology of living cells. *Phys. Rev. Lett.* **87**, 148102 (2001)

WEB SITE Ben Fabry's laboratory: http://www.hsph.harvard.edu/physiology/projects/bfabry/

DEVELOPMENT

Guidance suspects in the dock

The *Drosophila melanogaster* Dock protein — identified several years ago for its crucial function in photoreceptor axon guidance, does not act alone. Reporting in the *Journal of Biological Chemistry*, Dixon, Worby and colleagues have identified a *Drosophila* sorting protein, which, when tyrosine phosphorylated, can interact with this adaptor protein.

To unmask Dock's gang of accomplices, the authors stably expressed the src-homology-2 (SH2) domain of Dock as a tagged protein in *Drosophila* S2 cells and analysed proteins that complexed with it by affinity chromatography. Of the five protein bands that were visible, a protein of 63 kDa was found to contain an amino-terminal SH3 domain, an internal Phox homology (PX) domain, a proline-rich motif and a predicted coiled-coil domain. Based on its homology with human sorting nexin 9, also known as SH3PX1, the 63-kDa protein was renamed DSH3PX1. The interaction between Dock and DSH3PX1 — which the authors confirmed by co-immunoprecipitation — occurs mainly through the SH2 domain of Dock.

As other sorting nexins can associate with some cell-surface receptors, Dixon and colleagues then investigated whether DSH3PX1 could interact with another of the five proteins found in the Dock complex, called Dscam — the *Drosophila* orthologue of the human Down Syndrome cell-adhesion molecule (DSCAM). Not only did they find an interaction, but they also showed that Dock wasn't needed for the association by using RNA interference to eliminate expression of Dock. Further analysis showed that DSH3PX1 can also interact with AP-50, a member of the AP-2 complex that recognizes proteins that are destined to be endocytosed in clathrin-coated pits. So, DSH3PX1 might function to bridge Dscam to proteins in these pits.

Dscam is known to be required for embryonic axonal guidance, so Dixon *et al.* reasoned that by investigating the interactions of DSH3PX1, they might get an insight into the mechanisms by which Dscam mediates this effect. So, using full-length DSH3PX1, they carried out a two-hybrid screen. Among the interacting proteins was DSH3PX1 itself, indicating that this protein could dimerize. More significantly, however, Wasp — a regulator of the actin cytoskeleton, with a known function in endocytosis — was also identified as a positive binding partner. Wasp contains several protein–protein interaction domains (a Cdc42/Rac-binding (CRIB) domain, a proline-rich domain, two verprolin homology domains, a cofilin homology domain, a pleckstrin homology domain and an IQ domain), but the interaction with DSH3PX1 occurs through DSH3PX1's SH3 domain. This is consistent with previous observations that Wasp binds to several SH3-domain-containing proteins through its proline-rich sequence. Moreover, no competition was seen between the binding of Wasp and Dock to DSH3PX1, indicating that DSH3PX1 can interact with Wasp through its SH3 domain, whereas Dock uses an SH2-domain interaction.

So it is quite possible to imagine a scenario in which axonal-guidance cues induce tyrosine phosphorylation of DSH3PX1, which thereby enables it to associate with Dock. Dock, in turn, is bound to Dscam, so that, in conjunction with AP-50 and Wasp, DSH3PX1 might be involved in the cytoskeletal rearrangements that are required to endocytose Dscam. In this way, guidance receptors on the plasma membrane would undergo timely removal — a critical aspect of growth-cone remodelling.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Worby, C. A. *et al.* The sorting nexin, DSH3PX1, connects the axonal guidance receptor, Dscam, to the actin cytoskeleton. *J. Biol. Chem.* 2001 Sep 6 [epub ahead of print]

WEB SITE Jack Dixon's laboratory http://dixonlab.biochem.med.umich.edu/