IN BRIEF

TECHNIQUE

Lysozyme gets wired

Dynamic changes in protein conformation are commonly tracked through the imaging of a photon flux, for example by single-molecule fluorescence resonance energy transfer (smFRET). Here, Choi *et al.* describe a technique to image single-molecule dynamics through the detection of changes in an electron flux. A T4 lysozyme molecule was conjugated to a carbon nanotube, which amplifies electrical signals. As the lysozyme is charged, its conformational changes during the binding and processing of its substrate were sensed as electrostatic changes. This approach allowed high-sensitivity dynamic imaging of both the catalytic and non-productive lysozyme conformational motions. With the advantages of high time resolution, lack of signal bleaching and fluorophore independence, this method can be used as complementary to smFRET to monitor charged molecules.

ORIGINAL RESEARCH PAPER Choi, Y. *et al.* Single-molecule lysozyme dynamics monitored by an electronic circuit. *Science* **335**, 319–324 (2012)

CELL SIGNALLING

Tuning the Hedgehog pathway

Three recent studies identify mechanisms by which a Hedgehog (HH) gradient can finely tune downstream signalling. HH binding to its receptor leads to the phosphorylation of the transmembrane protein Smoothened (SMO). Li *et al.* observed that in *Drosophila melanogaster* the cell surface levels of SMO are downregulated through ubiquitin-mediated endocytosis and degradation. Its phosphorylation in the presence of HH prevents the ubiquitylation of SMO, leading to its stabilization on the plasma membrane. Interestingly, Ranieri *et al.* report that the extent of SMO activation by phosphorylation (which reflects the levels of extracellular HH) can influence the downstream transcriptional activity through differential phosphorylation of Costal 2 (COS2) by the kinase Fused; low-magnitude SMO activation results in COS2 phosphorylation at Ser572, whereas high-magnitude SMO activation induces COS2 phosphorylation at both Ser572 and Ser931. Futhermore, COS2 phosphorylated at Ser931 localizes to the plasma membrane, where it forms a stable complex with SMO and Fused, and promotes nuclear translocation and transcriptional activity of Cubitus Interruptus. Finally, with the use of reporter mice Balaskas *et al.* observed that the activity of Gli transcription factors (which are directly activated by HH signalling) in the embryonic neural tube decreases after embryonic day 8.5–9 despite the presence of a HH gradient. Thus, the dynamics of Gli activity may differ from the dynamics of the HH extracellular gradient. Moreover, *in silico* and *in vivo* studies revealed that the gene expression pattern of mouse neural progenitor cells is not determined by Gli activity but by a network of mutually regulated transcription factors, including NKX2.2, OLIG2 and PAX6. The authors suggest that this gene regulatory network ensures that the fate of neural progenitor cells is determined by both the level and the duration of HH signalling and Gli activity and, thus, cannot be affected by transient morphogen fluctuations.

ORIGINAL RESEARCH PAPERS Li, S. *et al.* Hedgehog-regulated ubiquitination controls Smoothened trafficking and cell surface expression in *Drosophila*. *PLoS Biol.* **10**, e1001239 (2012) | Ranieri, N. *et al.* Distinct phosphorylations on kinesin Costal-2 mediate differential Hedgehog signaling strength. *Dev. Cell* 2 Feb 2012 (doi:10.1016/j.devcel.2011.12.002) | Balaskas, N. *et al.* Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. *Cell* **148**, 273–284 (2012)