WEB WATCH

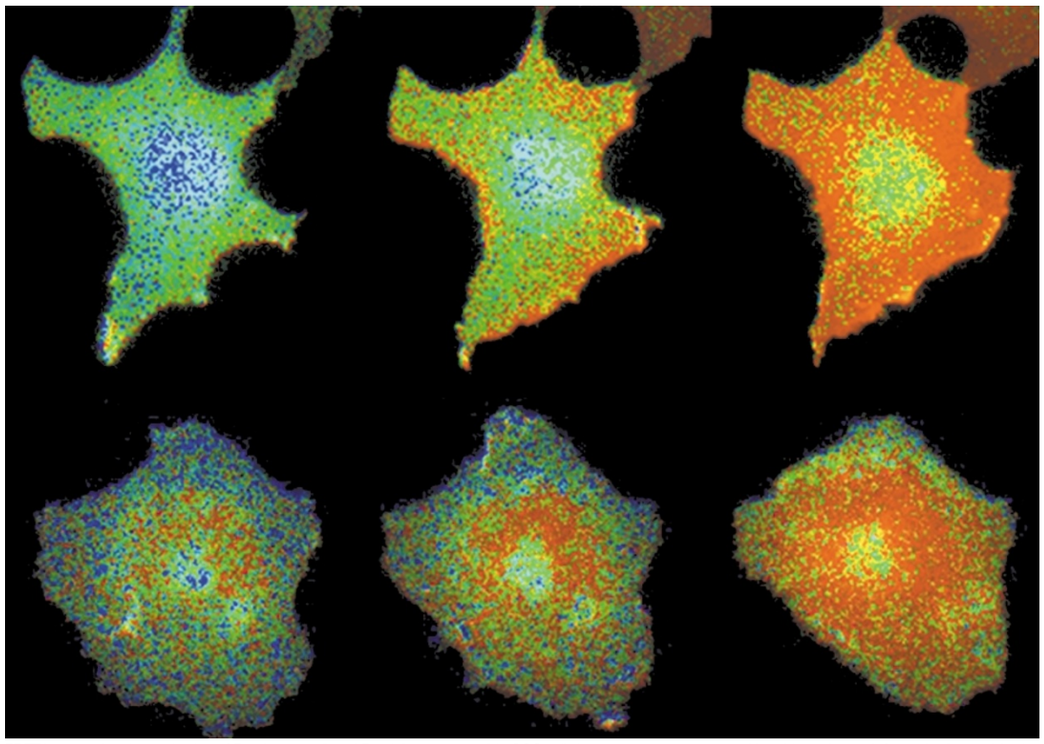
Shopping for antibodies

If you're looking for an antibody, look no further. The Antibody Resource Page promises to be "your complete guide to antibody research and suppliers", and it does exactly what it says. If you take longevity as an indication of success, this site must be doing something right, as it was created over five years ago and is still thriving. Monthly updates ensure that all links actually lead somewhere, and that new antibody resources don't go unnoticed for long.

The structure of the site is simple but effective: the homepage takes you to different listings, which, in turn, lead to external sources of information. You'll find links to: free online services that allow searching for antibodies across many companies; companies that sell antibodies; suppliers for custom antibodies; or other useful resources, such as hybridoma cell-culture databases or modelling software.

Don't waste time with the picture gallery, the Yahoo/Google searches for 'antibody news', or the links to journals and books. Instead, go directly to the educational resources page, which links to many useful (and a few not-so-useful) sites where you'll find information on how antibodies work, antibody structures and even animations of immune processes. So even if you're only window shopping, you should still be able to have a good time in this antibody shopping mall.

Raluca Gagescu



FRET images showing activation of Ras (top panel) and Rap1 (bottom panel) in COS cells in response to treatment with epidermal growth factor for 0, 5 or 30 min (from left to right). A red hue is associated with a high emission ratio and the intensity of this hue correlates with source image brightness. Image reproduced with permission from *Nature* © (2001) Macmillan Magazines Ltd.

CELL SIGNALLING

Right here, right now!

There's a time and place for most things — and intracellular reactions are no exception. Reporting in *Nature*, Matsuda and colleagues have shown that Ras and Rap1 are activated in a spatio-temporal manner in cells using fluorescent resonance energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP) techniques.

The key to their findings was the design of a protein comprising H-Ras, the Ras-binding domain of Raf (Raf RBD), and yellow- and cyan-emitting mutants of the green fluorescent protein, YFP and CFP, respectively. Intramolecular interactions between active, GTP-bound Ras and the Raf RBD bring YFP and CFP into close proximity, thereby increasing FRET. A probe for Rap1 was also made by substituting Rap1 for Ras. The proteins were designated Raichu-Ras and Raichu-Rap1 (Raichu standing for Ras and interacting protein chimeric unit). Co-expressing Raichu-Ras or Raichu-Rap1 with specific guanine-nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) effectively showed the increase and decrease in FRET, as did activating and inhibiting mutants of Ras and Rap1 substituted in the Raichu proteins.

Next the authors measured the activation of Ras and Rap1 in response to epidermal growth factor (EGF) in live COS cells. Using dual-emission microscopy to measure the emission intensities from CFP and YFP, FRET images were generated. EGF-induced activation of Ras occurred at the peripheral plasma membrane, and was lower at the sites of cell–cell contact — probably due to increased local Ras GAP activity. By contrast, EGF led to Rap1 activation at the internal perinuclear region. As a Raichu-Rap1 mutant lacking a farnesyl moiety was not activated by EGF, and pretreating the Raichu-Rap1-expressing cells with an inhibitor of clathrin-mediated endocytosis prevented Rap1's activation, it seems that Rap1 is activated at intracellular membrane compartments by internalization of the EGF receptor.

In PC12 cells, prolonged activation of Ras by nerve growth factor (NGF) stimulates neurite outgrowth. Early activation of Raichu-Ras occurred in the cell body, but at later time points active Ras persisted only in the extending neurites. This is consistent with the proposal that survival responses are mediated by activation of the NGF receptor, TrkA, at the cell surface, whereas differentiation occurs in response to TrkA in endosomes. Again, by contrast, active Rap1 was seen only in the intracellular region of these cells.

Is the sustained activation of Ras and the low activity of Rap1 in neurites a cause of retaining active Ras or inactive Rap1 here, or is it due to the activities of these two G proteins being determined locally? As the fluorescence intensity of PC12 neurites expressing YFP-Ras or YFP-Rap1 recovered quickly after photobleaching (FRAP technology), this suggests the latter.

The use of this technique to pinpoint active Ras and Rap1 proteins in specific regions of the cell at distinct times clearly also indicates that a large proportion of these proteins is inactive, emphasizing the importance of being in the right place at the right time.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Mochizuki, N. *et al.* Spatio-temporal images of growth-factor-induced activation of Ras and Rap1. *Nature* **411**, 1065–1068 (2001)