EXTRACELLULAR MATRIX

Being flexible

Many tissues require high elasticity for their function. To form elastic fibres, tropoelastin monomers are assembled and crosslinked into an insoluble extracellular matrix, which is organized into functional fibres. But the molecular mechanisms that control the supramolecular organization of elastic fibres have so far been a mystery. Two papers published in *Nature* now show that the integrin ligand fibulin-5 is essential for this process.

The two groups independently created *fibulin-5* knock-out mouse strains and found that the mice, although viable, had defects related to tissue elasticity, which worsened with age. Skin, lungs and large arteries — tissues that are normally highly elastic — were most affected. The mice had loose skin, severe emphysema (that resulted from expanded lungs with dilated alveoli), and a tortuous and elongated aorta. Microscopic inspection of the affected tissues indicated that, although elastic fibres were present, they were short and disorganized.

These defects pointed to the involvement of fibulin-5 in the organization of elastic fibres. Indeed, Olson and colleagues found that fibulin-5 interacts with elastin in a calcium-dependent manner in a solid-phase binding assay, and Chien and colleagues showed that it is recruited to elastic fibres produced by mouse-skin fibroblasts grown in culture.

Chien and colleagues also analysed the interaction between fibulin-5 and integrins, and found that fibulin-5 can promote cell attachment in cells that express αvb3, αvb5 or α9β1.

Both groups conclude that fibulin-5 probably links elastic fibres to the cell surface and thereby somehow organizes elastin polymers into functional elastic fibres. An added bonus of these studies is that the mutant mice might be a good model for studying ageing, as they have some of its more striking features — sagging skin, breathing difficulties and high-pulse pressure.

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References and links

ORIGINAL RESEARCH PAPERS Yanagisawa, H. *et al.* Fibulin-5 is an elastin-binding protein essential for elastic fibre development *in vivo*. *Nature* **415**, 168–171 (2002) | Nakamura, T. *et al*. Fibulin-5/DANCE is essential for elastogenesis *in vivo*. *Nature* **415**, 171–175 (2002)

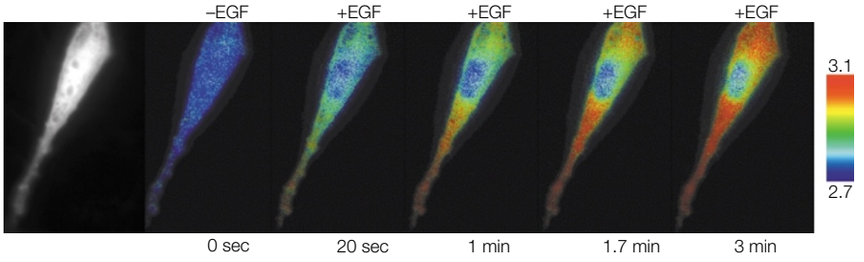
TECHNIQUE

Light up your kinase

Protein phosphorylation is tightly regulated in space and time but it has so far been almost impossible to study this aspect of cell signalling. Roger Tsien and colleagues have now developed a new class of genetically encoded fluorescent reporters that might allow us to follow the activation of virtually any kinase in living cells.

The new probes contain a substrate domain specific for a given protein kinase and a phosphorylation recognition domain that binds the phosphorylated substrate domain. This construct is sandwiched between two green fluorescent protein (GFP) variants that are compatible for FRET (fluorescence resonance energy transfer), for example cyan fluorescent protein and yellow fluorescent protein. If the substrate domain is phosphorylated on activation of the kinase, it undergoes an intramolecular interaction with the phosphorylation recognition domain, which induces a conformational change that alters the distance and/or the relative orientation between the two fluorescent proteins and hence the efficiency of FRET. In principle, the FRET change is reversible if a phosphatase dephosphorylates the substrate.

The authors used various chimaeras built according to this blueprint to follow the activity of four well known kinases: the serine/threonine kinase PKA (protein kinase A) and the tyrosine kinases Src, Abl and EGFR (epidermal growth factor receptor). They transfected cells with the constructs and then measured FRET before and after stimulation of kinase activity.



Adding growth factors to cells to stimulate individual tyrosine kinases caused, within minutes, a readily measurable 25–35% change in FRET. In the case of Src and EGFR, the signal started at the plasma membrane and then spread towards the cell centre. Abl, on the other hand was most active in membrane ruffles, consistent with its known function in cell migration.

Activation of PKA by forskolin, also caused a rapid 25–50% change in FRET, and, in this case, the kinase was active throughout the cytoplasm. However, when the reporter was relocalized to the nucleus by addition of a nuclear-localization signal, the FRET change was much delayed, whereas tethering the reporter to PKA accelerated the response. This shows how important substrate localization is for susceptibility to PKA *in vivo*.

The new probes show a lot of promise, and optimized versions — in particular with respect to specificity — will certainly become invaluable tools for studying targeting and compartmentation of signalling in living cells.

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References and links

ORIGINAL RESEARCH PAPERS Zhang, J. *et al.* Genetically encoded reporters of protein kinase A activity reveal impact of substrate tethering. *Proc. Natl Acad. Sci. USA* **98**, 14997–15002 (2001) | Ting, A. Y. *et al.* Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells. *Proc. Natl Acad. Sci. USA* **98**, 15003–15008 (2001)

WEB SITE

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