TECHNIQUE

Cellular cartography

Cell imaging, through the use of a plethora of imaging techniques and an ever-improving and -expanding range of fluorescent-molecule technologies, has become an essential and fast-moving area of modern cell biology. In the *Journal of Cell Science*, Paul Wiseman, Claire Brown and colleagues describe their contribution to this exciting field through their development and extension of the technique of image correlation microscopy (ICM), which they used to analyse integrin dynamics and interactions during cell migration.

ICM is derived from fluorescence correlation microscopy (FCS), and is used to measure spatial fluctuations in fluorescence intensity from images that have been obtained by laser scanning confocal microscopy. Correlation analysis is then used to calculate the various properties and interactions of a fluorescently tagged molecule. In the past, ICM was used mainly on fixed cells, but this research team have improved the technique by using 'retrospective image correlation', in which a time series of images taken from living (and, in this case, migrating) cells is analysed retrospectively. This allows the production of spatial maps of the concentration, aggregation state, interactions and transport properties of fluorescently tagged molecules within a cell. By using ICM to calibrate fluorescence intensities, the authors were also able to estimate the molecular densities (which includes information on molecular clustering) of integrins in adhesions. And, directional correlation analyses were used to study the net direction and speed of non-random molecular movements.

In this study the authors used ICM to analyse the behaviour of α_5-integrin in migrating cells. They showed that integrins are present in sub-microscopic clusters of 3–4 molecules throughout the cell before they become discernably organized. In nascent focal adhesions, integrins become much more clustered and concentrated as well as less mobile, and, in fact, they seem to nucleate focal adhesions. Image cross-correlation microscopy (ICCM), in which the cross correlation between two images with different fluorescent signals is computed, was also used to show that α_5-integrin and α-actinin reside in a complex even when they are not visibly organized. Furthermore, ICM was able to reveal interesting differences in the transport properties of the focal-adhesion components integrin, α-actinin and paxillin during focal-adhesion disassembly.

ICM and ICCM are extremely powerful techniques that can be used to provide spatial and temporal maps of several physical properties of, and interactions between, cellular components. And, as the images can be analysed both retrospectively and quantitatively, it has important implications for techniques such as high-throughput cellular screens and cellular modelling. This new cellular-imaging technology therefore promises huge potential for the analysis of complex biological phenomena.

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References and links

ORIGINAL RESEARCH PAPER Wiseman, P. W. *et al*. Spatial mapping of integrin interactions and dynamics during cell migration by image correlation microscopy. *J. Cell Sci.* **117**, 5521–5534 (2004)

WEB SITE

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IN BRIEF

AUTOPHAGY

The role of autophagy during the early neonatal starvation period.

Kuma, A. *et al. Nature* 3 Nov 2004 (doi:10.1038/nature03029)

After birth, neonates face starvation until they can obtain nutrients from milk, so how do they survive this period? This paper shows that the level of autophagy in mice is upregulated in various tissues straight after birth. Mice deficient in a gene that is essential for autophagosome formation die within a day of birth and show reduced amino-acid concentrations in plasma and tissues. So, neonates seem to survive by producing amino acids through the autophagic degradation of 'self' proteins.

MICRORNA

Processing of primary microRNAs by the Microprocessor complex.

Denli, A. M. *et al*. *Nature* 7 Nov 2004 (doi:10.1038/nature03049)

The Microprocessor complex mediates the genesis of microRNAs.

Gregory, R. I. *et al*. *Nature* 7 Nov 2004 (doi:10.1038/nature03120)

Micro (mi)RNA processing involves two steps: primary miRNA transcripts (pri-miRNAs) are first cleaved into shorter, precursor miRNAs (pre-miRNAs) by an enzyme known as Drosha; and they are subsequently processed by another enzyme, Dicer, into ~22-nt miRNAs. Two papers now report the existence of a Drosha-containing complex in humans and *Drosophila melanogaster*, which was named Microprocessor. Denli *et al*. identified a double-stranded RNA-binding protein — Pasha — in the *D. melanogaster* Microprocessor complex, which is required for pri-miRNA processing and for repression of miRNA-mediated genes. Gregory *et al*. identified two Drosha-containing complexes in human cells, of which the smaller one — Microprocessor — contained the double-stranded RNA-binding protein DGCR8, which is deleted in DiGeorge syndrome. Of the two complexes, only the Microprocessor complex seems to be required for miRNA processing.

CYTOSKELETON

Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis.

Romero, S. *et al. Cell* **119**, 419–429 (2004)

The authors showed that the rapid, processive growth of actin filaments that is mediated by formins requires the nucleation enhancer profilin to be bound to actin–ATP, and for this profilin–actin complex to associate with the formin homology (FH) domains 1 and 2 of formin. FH1–FH2 accelerates the hydrolysis of ATP that is coupled to profilin–actin polymerization, and the free energy that is derived from this increases the rate constant for the binding of profilin–actin to barbed filament ends.