Technology watch

HUNTING PHOSPHOPROTEINS

Phosphorylation has a significant role in a wide range of cellular processes by regulating the activity of key signalling proteins. Although mass spectrometry (MS) is a useful technique for identifying protein phosphorylation sites, it is limited by the fact that non-phosphorylated proteins are vastly more abundant than phosphoproteins in most samples. Phosphoproteins thus need to be enriched or labelled for their identification by MS, which prohibits physiologically relevant screens. Old *et al.* now describe a strategy for quantitatively detecting phosphoproteins that does not depend on their enrichment or labelling. They applied a technique called –79 Da precursor ion scanning MS, which has previously only been used on single proteins or simple protein mixtures, to complex human cell lysates. A hybrid mass spectrometer in negative ionization mode detects phosphoproteins by the presence of a negatively charged phosphite ion (PO_3^-) that is released upon protein proteolysis. Following detection of this precursor ion, the instrument is switched to positive mode, which allows for the tandem MS sequencing of phosphopeptides. The intensity of the –79 Da signal generated by PO_3^- allows changes in protein phosphorylation state to be quantified between samples. Confirming the power of this technique, the authors discovered that as many as 90 phosphorylation events might be regulated by B-Raf — an oncogenic kinase that is constitutively active in melanomas and other cancers.

ORIGINAL RESEARCH PAPER Old, W. M. *et al.* Functional proteomics identifies targets of phosphorylation by B-Raf signaling in melanoma. *Mol. Cell* **34**, 115–131 (2009)

SEE THE FORCE

Atomic force microscopy (AFM) produces images of surfaces by using a physical probe, which scans the specimen and records information about the probe–surface interaction in response to mechanical force. AFM can be combined with brightfield and fluorescence microscopy, which allows the imaging of cell shape in parallel with the measurement of force. However, the information gained is limited by the fact that such microscopy provides a view along a plane that is parallel to the surface — even though the most significant changes in cell morphology in response to force occur in a plane perpendicular to the surface, along the direction of the applied force. Chaudhuri *et al.* now combine AFM with a side view fluorescent optical path and show its efficiency in studying cell shape in relation to mechanical force. The authors measured the contractile force during adherent cell contraction of a single cell while successfully imaging the reorganization of the cytoskeleton and the formation of stress fibres. The development of side view AFM will be useful for studying the role of mechanics in biological processes, such as cell contractility and cell–cell adhesion.

ORIGINAL RESEARCH PAPER Chaudhuri, O. *et al.* Combined atomic force microscopy and side-view optical imaging for mechanical studies of cells. *Nature Methods* **6**, 383–387 (2009)