CELL BIOLOGY

Catching up to the speed of signaling

A simple, continuous quench-flow system allows quantitative analysis of very early cellular signaling events.

It is perhaps not uncommon for scientists studying molecular aspects of cell function to wish that they could shrink themselves down, a fly on the membrane so to speak, and simply watch their favorite process as it unfolds. Although physical shrinkage of this sort is thankfully not part of the protocol, a new approach by researchers at the University of South Denmark now makes it possible to monitor very early signaling events, occurring within seconds of cell exposure to an external stimulus. "In the case of large-scale proteomics studies in particular," says Blagoy Blagoev, coauthor on the paper reporting this new approach, "it has been difficult to look very rapidly at what is going on because sample preparation takes such a long time. So we tried to find an alternative."

The researchers used a setup consisting of three peristaltic pumps, which deliver cells, a stimulus or a quenching reagent, to examine phosphorylation events downstream of epidermal growth factor (EGF) receptor signaling in HeLa cells. The stimulus and the cells are pumped into a common chamber, and then out of this chamber to meet the quenching reagent, 70% ethanol at –20 °C. The duration of stimulation is thus controlled by the flow rate and the length of tubing between the mixing chamber and the point of delivery of quencher. In this study, the scientists quenched cellular activity as rapidly as one second after EGF addition, but the device is theoretically capable of measurements within a few hundred milliseconds.

Cells that had been stimulated for different lengths of time could then be collected and analyzed. Using stable isotope labeling and a mass spectrometric method that they had previously developed, the researchers measured the relative abundance of phosphorylated peptides derived from the EGF receptor itself and from Shc and PLCγ-1, which are downstream components of the pathway. They were able to detect tyrosine autophosphorylation on the EGF receptor after less than five seconds of stimulus. Moreover, the phosphorylation of Shc followed immediately after, suggesting that Shc must be in very close proximity to the receptor. "So we can make predictions based on the timing of the events that we measure," says Blagoev. "And we could use this approach for other events that are known to be very fast, like G protein–coupled receptor signaling. We hope that some enigmas can be resolved."

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RESEARCH PAPERS

Dengjel, J. *et al.* Quantitative proteomic assessment of very early cellular signaling events. *Nat. Biotechnol.* **25**, 566–568 (2007).