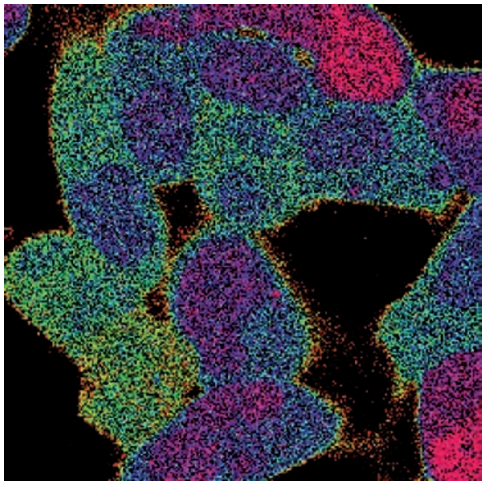
MILESTONE 12

Calcium, a messenger under the spotlight



Carbachol-evoked Ca^{2+} signals in human embryonic kidney cells recorded using the fluorescent indicator Fluo-4. Warmer colours denote a higher Ca^{2+} concentration. Image courtesy of Z. Ding and C. W. Taylor, University of Cambridge, UK.

"It is scarcely necessary any longer to stress the importance of intracellular free Ca^{2+} as a second messenger" reads the opening line of Roger Tsien's 1980 paper, reporting for the first time the synthesis of rationally designed fluorescent Ca^{2+} probes for intracellular use. Indeed, studies on calcium date back to the late nineteenth century, underscoring its significance. Reliable methods to measure intracellular Ca^{2+} concentrations first appeared in the 1960s, but it was not until the 1980s that the rapid expansion of new Ca^{2+}-measurement techniques opened the way to spatial and temporal analysis of the complex dynamics of Ca^{2+} concentrations in living cells.

In his original paper, Tsien introduced a new generation of fluorescent polycarboxylate dyes derived from the selective Ca^{2+} chelator ethyleneglycoltetraacetic acid (EGTA). Despite their groundbreaking potential, the prototype BAPTA and its derivative Quin2 had relatively limited application owing to restrictions imposed by their absorption spectrum, low selectivity and fluorescence intensity. However, all of the polycarboxylate dyes currently available are derived from this original design, the flexibility of which enabled the synthesis of many improved dyes within a few years of the original report.

In 1985, Tsien and his colleagues introduced six new indicators, among which Fura-2 and Indo-1 were the most successful. Compared with Quin2, the new dyes showed much stronger fluorescence, higher wavelength shift on Ca^{2+} binding, weaker affinity for Ca^{2+} and better selectivity against other ions. Soon after, high-resolution digital imaging of single smooth muscle cells labelled with Fura-2 allowed Tsien, Fredric Fay and their co-workers to perform unprecedented spatiotemporal measurements of Ca^{2+} gradients in subcellular compartments, revealing the differential regulation of Ca^{2+} concentrations in the cytoplasm, nucleus and sarcoplasmic reticulum of these contracting cells.

Critical to the widespread popularity of the polycarboxylate Ca^{2+} indicators was the synthesis of their acetoxymethyl esters, which are membrane permeable and allow non-disruptive loading and efficient trapping of the dyes in intact cells once the esterifying groups are hydrolysed (Tsien, 1981). One disadvantage of the Fura-2 family of dyes was that they required excitation at ultraviolet wavelengths, which limited their range of potential applications.

> "… Ca^{2+} measurement techniques opened the way to spatial and temporal analysis …"

In 1989, Tsien and colleagues further developed the visible-wavelength indicators Rhod-2, Fluo-2 and Fluo-3, which are among the most widely used non-ratiometric Ca^{2+} indicators so far, thanks in part to their general applicability to many cell types, including neurons.

Besides their crucial role in dogma-changing discoveries, synthetic Ca^{2+} indicators have driven technological advances such as fast cameras and automated filter wheels, and their uses have been further expanded by the concomitant development of imaging technologies such as fluorescence lifetime imaging and multiphoton microscopy. Today, fluorescent calcium dyes remain key to studies that combine imaging with electrophysiological analyses.

Silvia Grisendi, Associate Editor,
Nature Cell Biology

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