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Stochastic Sensing of IP_3 Has Far-Reaching Consequences

Hagan Bayley's group at Texas A&M University has devised a stochastic sensing methodology for the quantitation of the second messenger inositol 1,4,5-trisphosphate. The unique sensing scheme is very selective and has the potential to measure cytosolic concentrations of IP_3.

Inositol 1,4,5-trisphosphate (IP_3) is a second messenger used in a variety of signal transduction events [1]. The binding of extracellular signals, such as hormones acting as agonists, can elicit a cascade of biochemical processes, leading to the production of *sn*-1,2-diacylglycerol (DAG), which remains associated with the inner leaflet of the plasma membrane, and IP_3, a diffusable cytosolic messenger capable of releasing intracellular stores of Ca(II). This release can have various effects on cellular metabolism, including the activation of protein kinase C (PKC), which has a heightened sensitivity to Ca(II) levels in the presence of DAG. PKC catalyzes the phosphorylation of various serine and threonine residues, leading to the modulation of activity for additional enzymes and proteins. Known phosphorylation targets for PKC include the insulin receptor, β-adrenergic receptor, cytochrome P-450, and tyrosine hydroxylase. Clearly, IP_3 is a crucial cellular player, serving as a key control point for a wide range of cellular processes.

The ability to quantitatively monitor the intracellular levels of various messengers would be useful for developing accurate models of diverse cell functions, development, growth, and responses to stimuli. Although there are now outstanding methods for following Ca(II) using fluorescent probes [2], progress in monitoring IP_3 has been much slower. In one interesting approach, Allbritton and coworkers at the University of California, Irvine, have demonstrated the capability to use cultured cells as detectors for IP_3 sampled from oocytes [3]. In Allbritton's approach, the effluent from a sampling/electrophoresis capillary is directed onto a partially permeabilized cell, allowing IP_3 to release Ca(II) from intracellular stores; the resultant spike in [Ca(II)] can then be measured using fluorescence imaging. A second existing strategy for sensing IP_3 uses a synthetic receptor that binds IP_3 strongly in water and methanol mixtures [4] and is currently being used to analyze IP_3 with capillary electrophoresis (E.V.A. and J.B.S., unpublished results).

In this issue of *Chemistry & Biology*, an article from Stephen Cheley and Li-Qun Gu in Hagan Bayley's group at Texas A&M University reports sensing of IP_3 [5]. In this case, the sensing approach utilized a transmembrane pore, αHL, that was engineered to have affinity and selectivity for IP_3. The pore allows ions to flow through the membrane, and a planar bilayer device measures a current modulation that is indicative of the activity of the pore. When the ion of interest binds the interior of the pore, the channel is effectively blocked, reducing the flow of current. The frequency with which the analyte binds to the pore is indicative of the concentration of that analyte, while the amplitude and duration of the current modulation steps reveals the identity of the analyte.

Cheley and coworkers engineered αHL to have affinity for phosphate and IP_3 by placing guanidinium groups into the lumen of the pore. This was inspired by biological phosphate receptors, which are known to often contain the amino acid arginine. A variety of amino acids in the lumen and on the mouth of the pore were modified in an incremental fashion as a means to change the behavior of this channel and tune it toward IP_3 binding. The final design consisted of 14 arginines near the *cis* end of the barrel-shaped pore. The refined pore was unaffected by simple anions, such as chloride and nitrate, and remarkably was also unresponsive to cAMP and only slightly affected by ADP and inositol-2-monophosphate. However, when the pore was presented with IP_3, a dramatic change in conductivity was detected. In an attempt to mimic intracellular conditions, salts, ATP, Mg(II), and buffer were used at cellular levels, while IP_3

levels were varied between 0 and 500 nM. Beautiful calibration curves show that IP_3 concentrations as low as 100 nM are easily detected.

The impact of the work from Cheley and colleagues could be far reaching. As with the Allbritton studies, one could imagine using this sensor at the outlet of an electrophoresis capillary to probe IP_3 sampled from a cellular environment. Alternatively, it may be feasible to insert a patch pipette into the cytosol to probe cytosolic concentrations of this compound. If so, this technique would be a welcome tool to researchers involved in the analysis of signal transduction pathways.

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