SENSORS AND PROBES

Dispatches from the interior

Fluorescent biosensors that catch their targets at just the right moment reveal an unusual cell signaling mechanism.

G protein–coupled receptors (GPCRs) initiate signal cascades that drive critical cellular processes throughout the body. After activation, GPCRs are internalized via endocytosis, which temporarily takes them out of circulation to prevent cells from 'overreacting'.

Some data suggest that GPCRs may continue signaling even after being internalized, but definitive proof has remained elusive. "Essentially all the data were indirect," says Mark von Zastrow, of the University of California at San Francisco. However, his group has now published a striking demonstration of endosomal signaling by the β_2-adrenoceptor (β_2-AR).

GPCR signaling behavior is challenging to study: activated receptors interact with nucleotide-free G proteins, which quickly bind a guanosine triphosphate payload and dissociate from the receptor. "You don't get a stable complex under physiological conditions," says von Zastrow. "It's this very fleeting intermediate."

von Zastrow built on work from Brian Kobilka at Stanford University. Kobilka's team has used nanobodies—tiny, single-chained antibodies derived from camelid species—to stabilize GPCR activation complexes bound to G proteins, thus enabling a landmark series of structural analyses of GPCR activation. von Zastrow's group has now transformed these reagents into powerful biosensors for GPCR activation.

Nanobodies are so small that they can bind targets within living cells, and the researchers used two nanobody-derived fluorescent biosensors to test their hypothesis *in vivo*. The first, Nb80-GFP, selectively recognizes ligand-bound, activated β_2-AR. This biosensor revealed two phases of activation: one upon initial ligand binding and a second phase after receptor internalization. The group verified these results with the second biosensor, Nb37-GFP, which exclusively binds nucleotide-free G protein—finally capturing this elusive intermediate. Blocking endocytosis effectively eliminated this second phase, confirming its endosomal origin.

von Zastrow's team hopes to use nanobody sensors to further characterize endosomal signaling from β_2-AR and other GPCRs. However, nanobody tools should prove broadly applicable for drug discovery, functional biology and other research areas. "Pick your receptor, pick your signaling pathway and—in principle, at least—you should be able to interrogate activation locations and timing using this approach," says von Zastrow.

Michael Eisenstein

RESEARCH PAPERS
Irannejad, R. *et al.* Conformational biosensors reveal GPCR signalling from endosomes. *Nature* **495**, 534–538 (2013).