RESEARCH NEWS AND VIEWS

Chipping away at GPCR function

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Many cell signaling events depend on the complex and regulated interaction of proteins. A well-studied example of this is the association of the ubiquitous G protein–coupled receptors (GPCRs) with membrane-bound subunits of GTP-binding proteins (G proteins). Traditionally, the activity of GPCRs has been studied by measuring the binding of radiolabeled GTP to membranes, or by following downstream cell signaling events, such as changes in intracellular Ca^{2+} or cAMP, or changes in cellular transcription profiles. Until now, simple, direct ways of measuring the interaction of G proteins with GPCRs have been lacking. In this issue, Bieri and colleagues[1] describe the development of a novel assay of GPCR activity that employs the "chip" concept to immobilize receptors on a solid surface to enable easy detection of their activity.

Following activation by an external signal, the GPCR physically engages an inactive heterotrimeric G protein complex, composed of α, β, and γ subunits. It catalyzes the dissociation of GDP from the α subunit, which allows binding of GTP—which is at relatively high concentrations in the cell—and the rapid dissociation of the activated Gα subunit from the β and γ subunits. Both the GTP-bound α subunit and the βγ complex are then free to act as cell signaling effectors. It is this release of the G protein subunits that Bieri and coworkers have exploited to measure G protein signaling. The release of the G protein results in a reduction in mass of the immobilized receptor–G protein complex, which can be detected by surface plasmon resonance (SPR), as seen by a change in the reflection angle of light from the bottom of the gold film "chip"[2].

The key feature of this system is that the receptor–G protein complex is immobilized on the sensor chip in a functional form. To achieve this, Bieri et al. used a specific labeling procedure to biotinylate the carbohydrate located in the glycosylated extracellular N-terminal region of the rhodopsin receptor, a GPCR responsible for dim light detection in animals. To create an attachment site for the biotinylated receptor, they coated the sensor surface with a monolayer of ω-hydroxy-undecanethiol—a substrate to reduce non-specific attachment sites on the surface—and then printed a pattern of biotinyl-thiolipid on the chip using lithography. The result was an array of streptavidin attachment sites. Streptavidin was then added to the monolayer, creating a patterned surface to which the biotinylated rhodopsin, solubilized from bovine retinal membranes, was attached (see Figure).

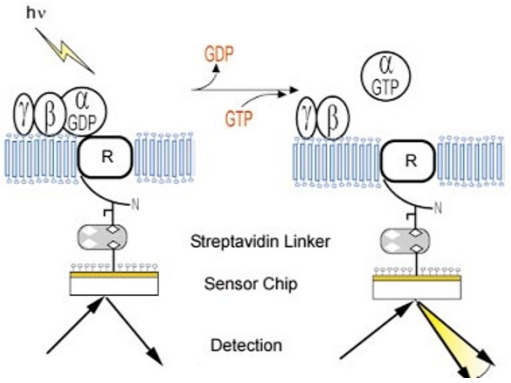

Figure 1. Scheme for direct detection of rhodopsin receptor activation (adapted from Bieri et al. this issue). A "self-assembled monolayer" of lipid and a printed pattern of biotin-thiol is created on the surface of a gold film. Purified biotinylated rhodopsin receptor (complexed with *cis*-retinal) is attached to the biotin via a streptavidin linker, and the G protein complex is added along with lipid micelles to complete the preparation (left panel). Upon activation of the GPCR with light, the G protein α subunit is activated and released, lowering the mass of the system. This is detected as a shift in the angle of a reflected light beam using surface plasmon resonance (right panel).

With its extracellular N terminus oriented toward the chip, the G protein binding domain of rhodopsin was oriented away from the sensor surface, facilitating the interaction with G protein. Rhodopsin interacts with a specific Gα subunit, transducin, which normally couples rhodopsin to activation of retinal cGMP phosphodiesterase. A substantial portion of the transducin is released from the membrane when rhodopsin is activated by light[3]. One of the unusual features of rhodopsin is that its ligand, *cis*-retinal, is associated with the receptor through a covalent Schiff's base linkage, and is activated by photons. Bieri et al. were thus able to activate the immobilized rhodopsin on the sensor chip with a flash of light, and monitor receptor activation through the release of transducin from the chip surface. The sensitivity of the system was calibrated by varying the intensity of the light flash, or the concentration of *cis*-retinal incubated with rhodopsin before the light flash.

One of the key advances in GPCR technology demonstrated here is the patterned immobilization of GPCRs on a chip surface and the direct detection of a functional response. Possible applications of this approach include screening for small molecules or peptides that can activate either known or orphan receptors. Antagonists would not be recognized, however, since the receptor must be active to cause G protein release. Extension of the promising immobilization strategy to chip-based screening will need to address several technical issues, however. For example, incorporation of the technology into a high-throughput platform will require the development of flowthrough methods of exposing the chip-attached receptor to multiple compounds, including soluble agonists. Perhaps even before addressing throughput, however, it remains to be determined whether the hormone/ligand binding site for a diverse range of GPCRs will remain functional and accessible to soluble ligand when the N terminus is coupled to a support by the method used here. Moreover, while transducin detaches from the cell membrane upon activation, allowing its desorption from the chip to be monitored by SPR, it is not clear that other Gα subunits do likewise. Therefore, the generality of the SPR approach will need to be proven. However, as Bieri et al. point out, other optical technologies, such as the displacement of fluorescent probes, may be suitable for detecting G protein activation, especially when there is a requirement for high sample throughput.

Regardless of the future technical applications and detection schemes for GPCRs, the implications of this study extend to other protein systems as well. The use of sensor chips and patterned arrays of proteins has some intriguing analogies with the development of DNA chips in terms of providing information. If proteins can be reliably "printed" onto solid surfaces while retaining the conformational features that impart function, various signaling systems could become amenable to chip-based assays.

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