BIOCHEMISTRY

Signaling Across the Cell Membrane

Structural and functional studies shed light on how G protein–coupled receptors sense external stimuli.

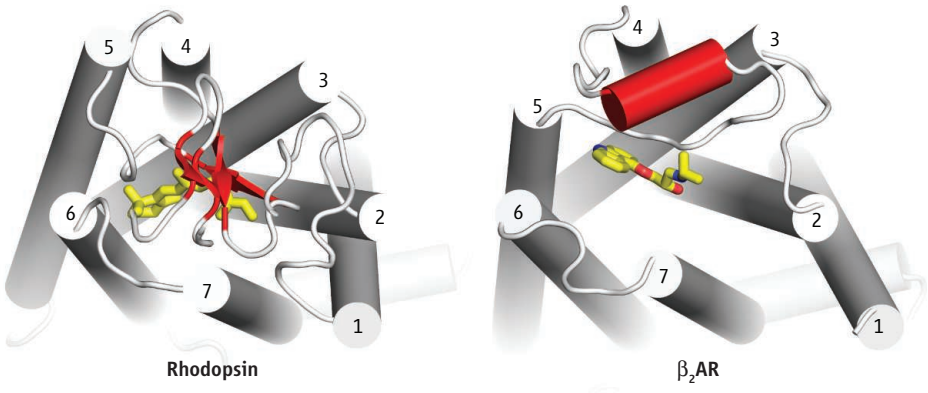
Rama Ranganathan

Cells contain a panoply of transmembrane receptor molecules that can recognize external signals and initiate intracellular signaling events. G protein–coupled receptors (GPCRs)—the largest and most diverse group of these receptors—occur in nearly every eukaryotic cell and can sense photons, cations, small molecules, peptides, and proteins (*1, 2*). Understanding how GPCRs operate has therefore been a major goal in signaling research for more than two decades (*3*). Two research articles in this issue (*4*, *5*) and a recent article in *Nature* (*6*) report important steps toward this goal.

How can GPCRs recognize such a diversity of extracellular stimuli? How is information about ligand binding (or light absorption) at a site facing the outside of the cell transferred to sites within the cell that mediate interaction with downstream signaling proteins? The structure of the inactive state of bovine rhodopsin (*7*)—a light-sensing GPCR—provided a critical initial step in addressing these questions. Together with functional studies, it led to basic models of GPCR action (*8*). However, it has been difficult to solve structures of more typical GPCRs that bind to diffusible ligands.

In addition to the usual problems encountered in producing membrane proteins suitable for crystallization, many GPCRs have an inherent conformational plasticity (*9*, *10*). Unlike rhodopsin, which is tightly locked into an inactive state by its covalently bound ligand, 11-*cis*-retinal, more typical GPCRs that are activated by diffusible molecules may assume an ensemble of different inactive and active states. This property could be physiologically important, accounting for the ability of different ligands to elicit a range of biological responses from the same GPCR by stabilizing different conformational states within these ensembles.

Given all this, the high-resolution structure determination of an engineered β_2-adrenergic receptor (β_2AR), a canonical GPCR family member activated by diffusible ligands, represents a spectacular advance (*4*–*6*). To minimize conformational heterogeneity and maximize crystal contacts, the authors made several modifications to the receptor that included cocrystallization with the ligand carazolol, removal of a flexible C-terminal tail, and either binding of a Fab fragment of a monoclonal antibody (*11*) or insertion of a small globular protein (T4 lysozyme, T4L) into the flexible third intracellular loop. Further key technical features included addition of cholesterol (known to stabilize β_2AR) as a crystallization additive and the use of the lipidic cubic phase to facilitate crystal growth (*12*). The structure determination itself was a tour de force of advanced techniques in crystal growth, screening, and diffraction. The current papers describe two structures of β_2AR bound to carazolol—a lower-resolution complex with Fab (*6*) and a high-resolution chimera with T4L reported by Cherezov *et al.* [p. 1258, (*4*)]—and a detailed functional characterization of the β_2AR-T4L protein by Rosenbaum *et al.* [p. 1266, (*5*)].



Similar yet different. Rhodopsin (**left**) and the β_2AR (**right**) share overall structural features and a binding pocket for their cognate ligands, 11-*cis*-retinal and carazolol, respectively (in yellow), at a site located deep within the transmembrane helices. However, the extracellular loops are distinctly structured, a result that may explain how diffusible ligands gain access to the binding pocket in the β_2AR.

Although the structures are similar in overall fold to rhodopsin—a roughly ellipsoid arrangement of seven membrane-spanning α-helical segments surrounding the ligand binding site (see the figure)—there are several new findings. With regard to ligand binding, carazolol is located deep within the transmembrane helices, at a site that is consistent with the retinal binding pocket, and some key interactions are consistent with findings in the rhodopsin structure. For example, the inactive state of rhodopsin maintained by 11-*cis*-retinal is thought to be stabilized in part by direct conformational restriction of a conserved tryptophan side chain (*13*). The analogous tryptophan in the β_2AR is similarly restrained (although indirectly) by carazolol. This finding provides a structural basis for interpreting prior mutation studies, which showed that signal propagation mechanisms are largely conserved in members of the GPCR family.

However, the data also indicate variation that may permit specialized responses to specific ligands. A helical structure in the second extracellular loop (ECL2) of β_2AR-T4L makes direct contact with carazolol. This feature is not conserved in rhodopsin. Cherezov *et al.* and Rosenbaum *et al.* suggest that the novel structure in ECL2 and disorder in the N-terminal region of β_2AR may provide a path for diffusible ligands to the binding pocket and contribute to ligand selectivity. Thus, although conformational changes associated with GPCR activation might be conserved in the family, specific kinetic and thermodynamic details of ligand recognition might be specified through modular variation of extracellular loop regions.

A particularly interesting feature is found in the intracellular part of the β_2AR structures. In the inactive state of rhodopsin, a network of hydrogen-bonding interactions links the cytoplasmic end of helix III with a residue in helix VI in a so-called "ionic lock" (*8*). This interaction is broken in both β_2AR structures. This ionic lock–deficient state may represent stabilization of one molecular configuration in the inactive-state ensemble by carazolol, and may explain why this ligand only partially shuts off

The author is at the Green Center for Systems Biology and Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA. E-mail: rama.ranganathan@utsouthwestern.edu

Published by AAAS

basal β_2AR activity. In this view, the ionic-locked state of rhodopsin is an extreme case—a specialized, more fully inactivated state that provides for the remarkable level of silencing of receptor activity required to suppress noise in the dark-adapted state of photoreceptor neurons. Structure determination of additional ligand-receptor complexes may help test the model of a conformationally heterogeneous inactive-state ensemble in β_2AR.

Rosenbaum *et al.* report a systematic functional analysis of β_2AR bound to T4L to establish its physiological relevance. The engineered receptor displays wild-type binding to antagonists and inverse agonists but increased affinity for agonists, a profile similar to that of constitutively active mutants. Thus, although the receptor chimera is similar to the wild-type β_2AR in many ways, this finding also illustrates potential complexities of working with engineered proteins. In this regard, the functional characterization by Rosenbaum *et al.* plays an important role in the interpretation of the atomic structure.

A major next goal is a structure of the agonist-bound active state of the receptor, work that may require formation of a ternary complex with the cognate G protein or with the inhibitory protein arrestin. The modifications made in the β_2AR-T4L complex preclude interaction with these target proteins. Thus, new strategies will be necessary that will require more of the kind of creativity and dedication that led to the present structures. This work would pave the way for a deeper mechanistic understanding of the GPCR family.

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10.1126/science.1151656

APPLIED PHYSICS

Filling the Terahertz Gap

A device made from a layered superconductor emits electromagnetic waves in a frequency range for which good radiation sources had been lacking.

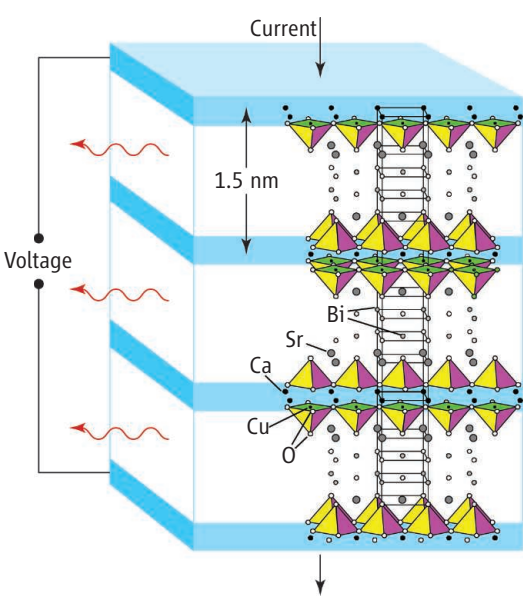
Reinhold Kleiner

At almost every frequency, we have good methods to generate and detect electromagnetic radiation. One crucial exception is the low terahertz range, where despite intensive research there is a severe lack of devices such as oscillators and detectors. With better terahertz technology, researchers could develop new kinds of nondestructive imaging for materials testing and medical diagnosis, and carry out novel spectroscopic studies of materials and molecules. On page 1291 of this issue, Ozyuzer *et al.* (*1*) report an important step toward filling this "terahertz gap." The authors detected relatively strong continuous-wave terahertz radiation emitted by devices made from a cuprate superconductor. In the future, such structures may serve as useful micrometer-sized terahertz devices.

These devices, called Josephson junctions (formed by two superconducting electrodes separated by a thin nonsuperconducting barrier), generate current oscillations when a static voltage is applied between the electrodes. For example, Josephson tunnel junctions with niobium as the superconductor and aluminum oxide as the insulating barrier can generate radiation up to 600 GHz (*2*). In this case, vortices of magnetic flux moving along the junction excite internal resonances (cavity modes). Although the operation frequency can be tuned only moderately on such a resonance, there are many cavity modes, such that the device can be operated over a wide frequency range.

Josephson tunnel junctions based on conventional superconductors like niobium are restricted to sub-terahertz operation. In addition, the output power of a single Josephson junction is typically below 1 μW close to the device and well below 1 nW farther away. One way to increase the power is to use coherently oscillating junction arrays. Just as a laser has increased brightness from coherent oscillation, coherent Josephson arrays can emit much more powerful terahertz radiation. Such arrays have been intensively studied with both low-temperature and high-temperature superconductors (*3*); however, it turned out to be hard to synchronize a large number of junctions at high frequencies.

Recently, a different kind of device called the intrinsic Josephson junction (*4*, *5*) has offered solutions to limitations on power and frequency. Intrinsic Josephson junctions form naturally between the superconducting CuO_2 layers in cuprate materials such as $Bi_2Sr_2CaCu_2O_8$ (BSCCO), with the



Working together. Sketch of intrinsic Josephson junctions in BSCCO. Blue layers indicate the superconducting sheets (CuO_2 layers), and transparent layers in between are the insulating barriers. The crystal structure is superimposed on the diagram. The junctions forming the stack can be individually switched between the zero voltage state and the resistive state where the Josephson current oscillates. In the experiments by Ozyuzer *et al.*, strong THz emission was found at voltages where hundreds of junctions were resistive.

The author is at the Physikalisches Institut, Universität Tübingen, D-72076 Tübingen, Germany. E-mail: kleiner@uni-tuebingen.de