A receptor unlocked

Stephen R. Sprang

G-protein-coupled receptors govern many biological functions, yet little is known about the molecular basis of their activity. The structure of a prominent example of these receptors is now revealed.

The pounding heart and speeding metabolism that accompany fear and fury are the work of the adrenaline molecule, which is produced by the adrenal gland at times of stress. Adrenaline is an agonist — an activator — of β2-adrenergic receptors (β2ARs). Embedded in cell membranes in many tissues, these receptors control diverse effects, such as rapid pulse, pupil dilation and constriction of the blood vessels. But the β2AR has even broader relevance to biologists, because it is the archetypal member of a family of more than a thousand G-protein-coupled receptors (GPCRs)\(^1\). When stimulated by agonists, GPCRs activate the eponymous G proteins that are tethered to the inner surface of the cell membrane. The G proteins in turn initiate cascades of biological signals that control such processes as gene expression, metabolic activity and cell movement.

The remarkable flexibility of GPCRs allows them to adopt several different states of activity\(^2\), but also makes it difficult to determine their three-dimensional structures. To date, only the structure of rhodopsin — a receptor involved in the perception of light, and the most stable GPCR known — has been available\(^3\). Reporting in this issue (Rasmussen et al.\(^4\); page 383) and in Science (Cherezov et al.\(^3\) and Rosenbaum et al.\(^4\)), several collaborating research groups now describe the structure of β2AR.

Like rhodopsin, β2AR consists of a protein chain that threads through the cell membrane seven times, forming a bundle of transmembrane helices (TM1–7, Fig. 1). Peptide loops connect the helices; those exposed on the outside of the cell membrane enfold the agonist binding site, whereas those on the inside of the cell form the binding site for G proteins. The G-protein binding site is formed in part from the peptide chain (known as IC3) that connects TM5 and TM6. It is thought that agonist binding induces subtle bending and twisting of the TM helices, particularly of TM3 and TM6, so exposing the G protein binding site.

Without light to stimulate it, rhodopsin becomes completely dormant. But β2ARs — in common with many other GPCRs — retain some activity in the absence of their agonists. This ‘constitutive’ activity can be partly repressed by so-called inverse-agonist molecules, such as carazolol. Moreover, not all GPCR agonists are equal, as some elicit higher activity levels than others. This variable receptor activity might occur because different molecules interact with the agonist binding site in different ways.

Figure 1 Structure of a G-protein-coupled receptor. The cartoon shows the partial crystal structure of a modified β2-adrenergic receptor (β2AR), as reported by Cherezov et al.\(^3\). Seven helices (TM1–7) cross a cell membrane, although TM5 is not shown as it would obscure the other helices. Ligand molecules bind at a site between TM3 (blue) and TM6 (green); carazolol was used to stabilize the site in order to obtain this structure. The amino acids just below carazolol form a ‘toggle’ that stabilizes the inactive state of the receptor. The amino acids indicated at the bottom can form an ‘ionic lock’ that performs the same function, but this crystal structure and that of Rasmussen et al.\(^4\) show that the lock is broken in β2AR. Water molecules occupy a loosely packed channel; this may provide room for movement of the TM helices upon activation of the receptor, and allow binding of a G protein near the base of TM5 and TM6.

The various molecular binding modes seem to be coupled to specific ‘triggers’ within GPCRs\(^5\). One such trigger is the ‘ionic lock’ — an electrostatic interaction between a triad of charged amino acids that tether TM3 and TM6 together. The ionic lock stabilizes the inactive state of the receptor (Fig. 1). The specific amino acids that form the lock are similar in most GPCRs; mutations that change this sequence can disrupt the lock and increase the constitutive activity of β2AR. A second trigger, called the toggle, is also known, and is thought to involve a cluster of amino acids that are in contact with the agonist binding site. The toggle also seems to constrain the receptor in an inactive state; it is thought to be released when strong agonists bind to the receptor.

The molecules that bind to β2AR can induce distinct conformational changes in the receptor, depending on the nature of their interactions. There is growing evidence that these different conformational states might have signalling functions in the cell other than just G-protein activation. But to understand how agonist binding couples to different conformational states, it is essential to determine the structure of the receptor at atomic resolution using X-ray crystallography.

Unfortunately, GPCRs are difficult to crystallize, because their ‘greasy’ hydrophobic surfaces do not readily make the regular intermolecular contacts required for crystal formation. Moreover, naturally occurring GPCRs are strongly coupled to cell membranes, which probably perturb their receptors’ structures. This means that GPCRs crystallized in the absence of membranes don’t necessarily have the same structure as those in situ. The authors of the current papers\(^4\) all used the inverse agonist carazolol to stabilize the receptor structure, but two different methods were used to obtain a receptor complex that could be crystallized.

Rasmussen et al.\(^4\) embedded β2ARs in nanometre-sized lozenges called bicelles, which are made from bilayers of fatty molecules similar to those that constitute cell membranes. To provide a large, polar surface suitable for crystal-lattice formation, the β2AR molecules were obtained as complexes with an antibody fragment that specifically binds to the IC3 loop. The antibody fragment does not interfere with

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agonist binding or with the conformational changes that accompany receptor activation1. Intense, micro-focused X-ray beams produced by synchrotrons were required to tease out the diffraction data from which the crystal structures were derived.

Cherezov et al.5 used an alternative approach. They replaced the IC3 loop of β2AR with a small, stable protein known as T4 phage lysozyme (T4L). The T4L protein promotes crystal-lattice formation in the same way as the antibody fragment used by Rasmussen et al.4. The modified receptor, bound to carazolol, was crystallized in a semi-solid lipid medium that provided an artificial, membrane-like environment for the receptor. Synchrotron X-rays were also required in this case to obtain good-quality diffraction data. The results of these investigations are described by Rosenbaum et al.6.

A surprise finding from the crystal structures1–3 is that the ionic lock is broken (as expected in an active state) despite the presence of an inverse agonist (which promotes the inactive state). Had only one crystal form of β2AR been available, it could have been argued that this was an artefact of the experimental conditions — either a consequence of the artificial lipid environments, or because of perturbation of IC3. But because the lipid supports and IC3 region are different in each structure, it is unlikely that the same misleading result could have occurred in both cases. Moreover, the TM regions in both structures align well with each other, further corroborating the results.

Thus, the weak constitutive activity of β2AR might be attributed to the breaking of the ionic lock; this can be compared with rhodopsin, which retains the lock and shows no such activity. In contrast, the toggle is intact in the two β2AR structures, just as it is in inactivated rhodopsin, despite considerable differences in the binding modes adopted by the ligand molecules for these two GPCRs. Perhaps stronger inverse agonists than carazolol would stabilize the ionic lock and fully inactivate β2AR, whereas agonists would release both lock and toggle. But these speculations have yet to be confirmed.

The crystal structures1–3 reveal several other interesting clues to β2AR behaviour. For example, many of the amino acids that produce constitutive activity are known to respond to agonist binding, but are not directly connected to the agonist binding site. It can now be seen that these residues are linked to the binding site via packing interactions2, so that movement of amino acids in the binding site could affect the packing of others throughout the structure (Fig. 1). Furthermore, a water-filled channel in the core of the receptor offers space for structural rearrangement, which could enable the receptor to adopt several different active states. Finally, the capacious agonist binding site is compatible with the existence of several molecular binding modes, each with the potential to trigger a different state in the receptor.

To learn how structural changes are conveyed from the agonist pocket to the G-protein binding site, the structure of the fully active, agonist-bound β2AR must be determined. The instability of the activated receptor will make this difficult — in fact, it may be possible to resolve the structure of a receptor only if it is bound to a G protein. Nevertheless, with the structures4–6 of β2AR in hand, we can expect those of other GPCRs to follow, so that the conformational complexities common to the members of this family of receptors might finally be revealed.

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**Isotopic lunacy**

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The Moon could have been derived from a well-mixed disk of rock vapour that was produced after the early Earth collided with another planet. This persuasive idea offers a fresh perspective on the history of both bodies.

“It is the very error of the moon; She comes more nearer earth than she was wont; And makes men mad.” Thus spoke Othello, who was talking of a murderous madness. For 30 years scientists have been gripped, albeit with less deadly consequences, by a maddening paradox to do with the Moon: how near its oxygen-isotope composition is to that of Earth.

It is generally agreed that the Moon formed in a giant impact between Earth and another, smaller body. But previous simulations of that event show that, dynamically, the Moon should be largely derived from the smaller body, in which case its oxygen isotopic composition should be different from that of Earth. Writing in *Earth and Planetary Science Letters*, Paheleyn and Stevenson7 put forward an explanation for the oxygen-isotope paradox: they propose that the giant impact produced a disk of rock vapour within which the atoms were able to mix before the lunar component condensed out.

The scientific context of this story goes back to the discovery of large variations in the isotopic composition of oxygen in meteorites8, which occurred at about the same time as lunar samples were being returned by Apollo astronauts. These variations are so large that they can readily be used to identify which meteorites come from the same planet or asteroid. Lunar samples were found to have a terrestrial isotopic composition9, which was taken as evidence that the Moon was derived from Earth8.

But the evidence from dynamic simulations is that most of the mass of the Moon came from another planet, Theia, that hit the early Earth with a glancing blow10.

The isotopic composition of every element studied so far is the same in lunar samples as it is in samples from Earth, apart from elements that would change because of radioactive decay, or through the effects of cosmic rays or the charged particles of the solar wind. But the background Solar System heterogeneity is far bigger for oxygen than for most other elements8. So, with the development of more precise techniques, a further analysis of lunar samples aimed to resolve an Earth–Moon oxygen-isotope difference that was thought must surely exist at some finite level11. Far from doing that, however, samples from the two bodies were found to be identical to within better than five parts per million. One explanation was that Theia must have formed from the same mix of material as Earth.

Paheleyn and Stevenson’s dynamic simulations10 of Solar System formation build on previous work in assuming that, based on the measured difference between Earth and Mars, an oxygen-isotope gradient existed within the early planetesimals of the circumstellar disk. This is a reasonable assumption, but might not be easy to test at the required precision until samples are returned from sizeable bodies such as Mercury and Venus. The obvious test at present involves a class of meteorites that were probably derived from the asteroid 4 Vesta. The oxygen-isotope data for these meteorites do not fit with the proposed radial-gradient model. But this can be explained if, as seems likely, Vesta has migrated outwards from the innermost Solar System.

Based on this putative gradient, the new calculations’ show that it is unlikely for two bodies such as Theia and Earth to have had the same bulk isotopic composition. Indeed, it is hard to imagine how they could have formed from the same mix of primordial matter unless they grew at exactly the same distance from the Sun, which in turn raises the question of why it took them so long to collide. Tungsten-