NEWS IN FOCUS

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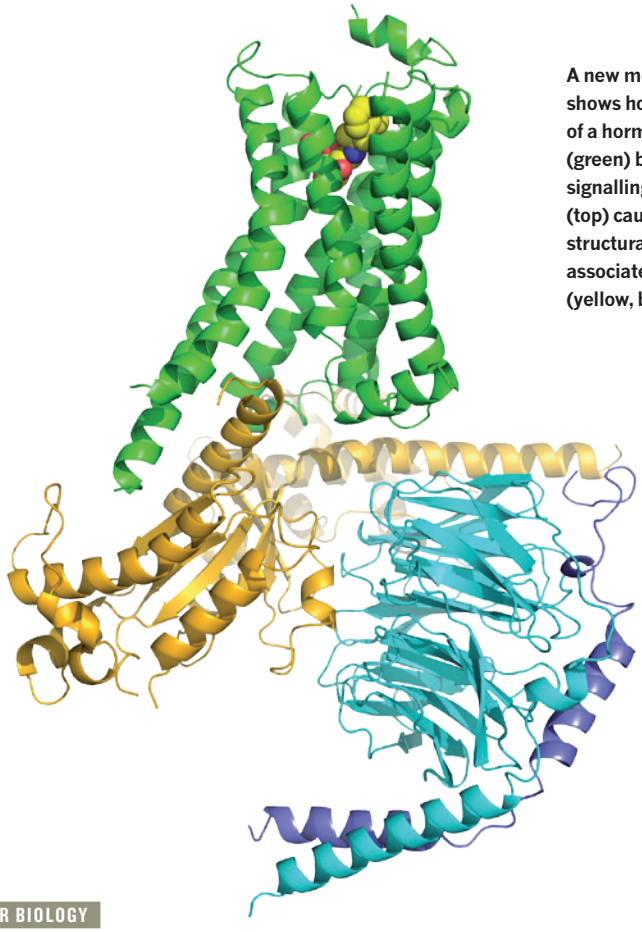

A new molecular portrait shows how the activation of a hormone receptor (green) by a small signalling molecule (top) causes a dramatic structural shift in its associated G protein (yellow, blue and mauve).

MOLECULAR BIOLOGY

Cell signalling caught in the act

Receptor imaged in embrace with its G protein.

BY LIZZIE BUCHEN

Brian Kobilka knew that his postdocs didn't like him peeking at their experiments until they were finished. But he couldn't resist a quick look — after all, he and his entire field had been waiting for this result for more than 20 years.

As Kobilka peered through the microscope, the dream finally came into focus. Nestled in a drop of viscous liquid were tiny crystals, each trapping millions of copies of a fragile protein complex. The structure of this complex could finally reveal how one of biology's most important signalling mechanisms, G-protein-coupled receptors (GPCRs), do their job. This structure, published online in *Nature*[1] by a team led by Kobilka at Stanford University in California and Roger Sunahara at the University of Michigan in Ann Arbor, now reveals the complete three-dimensional atomic structure of an activated GPCR — the β_2 adrenergic receptor (β_2AR) — in a complex with its G protein.

GPCRs sit in the membranes of cells throughout the body, where they detect signals from the outside world — such as light, odours and flavours — and signals from within the body, such as hormones and neurotransmitters. These signals are transmitted to the inside of the cell where they activate intracellular G proteins, which then trigger a variety of biochemical pathways.

The β_2AR is activated by the hormones adrenaline and noradrenaline, and kicks off the body's fight-or-flight response by speeding up the heart and opening airways. It is a key target for anti-asthma drugs. Kobilka's X-ray crystallographic snapshot of β_2AR associated with its G protein reveals some surprises, and could help in the design of more effective medicines — GPCRs are targeted by between one-third and one-half of all drugs on the market, including most of the best-sellers.

Before any protein can be imaged, it has to be crystallized. That is notoriously difficult for GPCRs, which need to be coaxed out of the cell membrane and kept stable in a fatty medium. The structure of the light-detecting GPCR rhodopsin was worked out in 2000 (ref. 2), but the GPCRs activated by hormones and neurotransmitters proved more intransigent. The first of these 'ligand-activated' GPCRs to yield to crystallization was β_2AR, which didn't give up its structural secrets until 2007, after decades of effort by Kobilka's group and others[3–5]. That opened the floodgates: the crystallographic structures of four other GPCRs have been solved in the past year[6–9].

But understanding how GPCRs relay their signal meant crystallizing a complex of a receptor coupled to a G protein, an even harder task. The G protein, made up of three different subunits, is prone to detaching from the receptor and breaking apart, and the complex is about twice the size of β_2AR alone. Getting the structure of the β_2AR–G protein complex entailed developing new techniques to purify and stabilize it, including binding it to an antibody, and the testing of thousands of different crystallization conditions.

"This is a real breakthrough paper," says biochemist Stephen Sprang at the University of Montana in Missoula. "For a long time, many folks in the field have considered this the hoped-for structure that would ultimately provide a real understanding of how the receptors actually work."

Krzysztof Palczewski at Case Western Reserve University in Cleveland, Ohio, ▶

who was the first to crystallize rhodopsin[2], agrees that the work is "a tremendous accomplishment". But he is concerned that the engineered and antibody-stabilized proteins used in Kobilka's study might not be a perfect match for the structure found in nature. Kobilka, however, says that his functional assays show that the engineered proteins behave like the natural proteins.

Researchers already knew that inactive G proteins are bound to a molecule of guanosine diphosphate (GDP) — a complex that Sunahara likens to a Pac-Man with something in its mouth. When a GPCR receives a signal, the receptor forces the G protein to spit out the GDP, allowing a molecule of guanosine triphosphate to swoop in and switch the G protein on.

The structure now reveals how the activated receptor contorts to make this happen. Most surprisingly, it also shows that the G protein's mouth splays wide open when the GDP departs. X-ray crystallography provides static images, so the exact sequence of events is unclear. "But now that we know it happens, it's something we can study," says Kobilka.

The discovery could provide unexpected clues to the molecular mechanism of the cholera toxin. The toxin forces G proteins to stay on all the time and continuously activate signalling pathways in intestinal cells. The affected cells release much of their water, leading to diarrhoea and vomiting. But the site that the toxin modifies is buried deep inside the G protein, which was "sort of puzzling", says Sunahara. "How does it get to that buried site? Our structure showed us that the Pac-Man opens wide enough that it exposes the site. And if that's the way cholera works, it's probably the way a lot of things interact with G proteins."

"Brian's struggled for this for such a long time," says structural biologist Tracy Handel at the University of California in San Diego. "Thank God he got it, because, boy, he deserved it." ■

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1. Rasmussen, S. G. F. *et al. Nature* http://dx.doi.org/10.1038/nature10361 (2011).
2. Palczewski, K. *et al. Science* **289,** 739–745 (2000).
3. Rasmussen, S. G. F. *et al. Nature* **450,** 383–387 (2007).
4. Rosenbaum, D. M. *et al. Science* **318,** 1266–1273 (2007).
5. Cherezov, V. *et al. Science* **318,** 1258–1265 (2007).
6. Shimamura, T. *et al. Nature* **475,** 65–70 (2011).
7. Xu, F. *et al. Science* **332,** 322–327 (2011).
8. Wu, B. *et al. Science* **330,** 1066–1071 (2010).
9. Chien, E. Y. *et al. Science* **330,** 1091–1095 (2010).

SCOTTS MIRACLE-GRO COMPANY

Glyphosate-resistant Kentucky bluegrass has outgrown US rules on genetically modified crops.

BIOTECHNOLOGY

Transgenic grass skirts regulators

Technological advances remove basis for government oversight of genetically modified crops.

BY HEIDI LEDFORD

When the US Department of Agriculture (USDA) announced this month that it did not have the authority to oversee a new variety of genetically modified (GM) Kentucky bluegrass, it exposed a serious weakness in the regulations governing GM crops. These are based not on a plant's GM nature but on the techniques used for its genetic modification. With changing technologies, the department says that it lacks the authority to regulate newly created transgenic crops.

The grass, a GM variety of *Poa pratensis*, is still in the early stages of development by Scotts Miracle-Gro, a lawn-care company based in Marysville, Ohio. The grass has been genetically altered to tolerate the herbicide glyphosate, which would make it easier to keep a lawn weed-free. On 1 July, secretary of agriculture Tom Vilsack wrote to the company to say that the variety "is not subject" to the same regulations that govern other GM crops. The decision allows Scotts to bypass the years of environmental testing and consultation typically required by the regulators for GM plants, although the company says there are no plans to market this particular variety.

The grass can evade control because the regulations for GM plants derive from the Federal Plant Pest Act, a decades-old law intended to safeguard against plant pathogens from overseas. Previous types of GM plants are covered because they they were made using plant pathogens. The bacterium *Agrobacterium tumefaciens* — which can cause tumours on plants — shuttled foreign genes into plant genomes. Developers then used genetic control elements derived from pathogenic plant viruses such as the cauliflower mosaic virus to switch on the genes.

By revealing similar elements in plants' DNA, genome sequencing has liberated developers from having to borrow the viral sequences. And *Agrobacterium* is not essential either; foreign genes can be fired into plant cells on metal particles shot from a 'gene gun'. Scotts took advantage of both techniques to construct the herbicide-resistant Kentucky bluegrass that put the USDA's regulatory powers to the test.