WHAT'S HOT IN... BIOLOGY , March/April 2009

Nailing Down the Structure of the ß2 Adrenoreceptor

by Jeremy Cherfas

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In 1986, Brian Kobilka, of Stanford University School of Medicine, was a member of the team that cloned the $ß_2$-adrenergic receptor, a molecule that spans the cell membrane and that responds to the presence of adrenaline by triggering diverse components of the "fight or flight" response. More than 20 years later, he is one of the lead authors on two Top Ten papers that describe the detailed molecular structure of $ß_2$AR, as the receptor is known. Getting from there to here required a succession of technical and conceptual breakthroughs that have paved the way to a far greater understanding of the most common family of trans-membrane signal receptors.

There are about 1,000 G protein-coupled receptors (GPCRs) that respond to a huge range of stimuli, from the light that activates the visual pigment rhodopsin to hormones and small molecules such as

adrenaline. As Kobilka and his colleagues note in the authors' summary of one of the highly cited papers (#2), "drugs that act on GPCRs command more than 50% of the current market for human therapeutics, with annual revenues in excess of $40 billion." But those drugs often have untoward side effects; asthma drugs, for example, can make the heart beat too fast if the dose is not carefully controlled. Part of the reason is that drug design is difficult because the structure and function of the receptors are not well understood. And that is not surprising.

Biology Top Ten Papers

Rank	Papers	Cites Sep-Oct 08	Rank Jul-Aug 08
1	K. Takahashi, *et al.*, "Induction of pluripotent stem cells from adult human fibroblasts by defined factors," *Cell*, 131(5): 861-72, 30 November 2007. [Kyoto U., Japan; CREST, Kawaguchi, Japan; Gladstone Inst. Cardio. Dis., San Francisco, CA] *243MG	51	1
2	V. Cherezov, *et al.*, "High-resolution crystal structure of an engineered human $ß_2$-adrenergic G protein-coupled receptor," *Science*, 318(5854): 1258-65, 23 November 2007. [Scripps Res. Inst., La Jolla, CA; Stanford U., CA] *233JG	43	†
3	Intl. HapMap Consortium (K.A. Frazer, *et al.*), "A second generation human haplotype map of over 3.1 million SNPs," *Nature*, 449(7164): 854-61, 18 October 2007. [72 institutions worldwide] *221LY	38	†
4	The ENCODE Project Consortium (E. Birney, *et al.*), "Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project," *Nature*, 447(7146): 799-816, 14 June 2007. [80 institutions worldwide] *178FV	35	3

Rhodopsin, which was characterized around a decade ago, is unusual in being physically quite stable, which means it is relatively easy to make the crystals needed to determine its structure. $ß_2$AR is much "wobblier" and is in any case hard to crystallize because the surface of the molecule that sits within the membrane tends to be hydrophobic and thus to steer clear of the close molecular contacts that are essential to crystal formation. It also seems likely that $ß_2$AR, being a trans-membrane protein, needs to be within a membrane to exhibit its true shape.

Kobilka's team adopted two approaches to the problem. In both, they stabilized the outside of the receptor by binding it with the beta-blocker carazolol. For the inside, one group bound one of the intracellular loops to a monoclonal antibody. The other genetically engineered the $ß_2$AR molecule, replacing the same intracellular loop with a small protein derived from T4 bacteriophage. The antibody and the T4 lysozyme both encouraged the formation of a crystal lattice. Of course there is more to mapping the molecular structure than just having the crystals, but without the crystals nothing is possible. To have two different sorts of crystal is fortunate indeed.

"We didn't know which [method] was going to work, so we tried both," Kobilka recently told *The Scientist* (23[2]: 51, 2009). "And they ended up both working at about the same time."

The paper by Rasmussen *et al.*, at #8, reported the structure based on the monoclonal antibody and was published in *Nature* a week before Cherezov *et al.*, at #2, published in *Science* with a higher-resolution version derived from the engineered $ß_2$AR. (A third paper by the team, in the same issue of *Science* [D.M. Rosenbaum, *et al.*, 318(5854): 1266-73, 2007], just missed the current Top Ten with 22 citations this period.) The two structures are all but identical. Perhaps the biggest surprise is that an ionic lock, which holds the intracellular parts of the molecule together in inactivated rhodopsin (and which may be partly responsible for that molecule's stability) is broken in $ß_2$AR, even though the agonist carazolol is blocking the receptor and thus might be expected to have locked the structure. If just one of the structures had demonstrated a broken lock, it might have been dismissed as an artefact, but given that it shows in both structures, even though they had different intracellular components and different lipid supports, the suggestion is that this is an important aspect of $ß_2$AR's functioning.

There are other aspects of the structure that suggest ways in which the receptor actually works. For example, there is a channel through the middle of the molecule that seems to be filled with water. This could provide space for the components of the receptor to move around, reacting to different signal molecules by adopting different positions and shapes and thus possibly helping to trigger subtly different responses within the cell.

5	I.I. Ivanov, *et al.*, "The orphan nuclear receptor ROR[gamma]t directs the differentiation program of proinflammatory IL-17+ T helper cells, *Cell*, 126(6): 1121-33, 22 September 2006. [Howard Hughes Med. Inst., New York U., NY; Schering-Plough BioPharma, Palo Alto, CA] *089RF	35	6
6	D.F. Easton, *et al.*, "Genome-wide association study identifies novel breast cancer susceptibility loci," *Nature*, 447 (7148): 1087-93, 28 June 2007. [87 institutions worldwide] *183HT	33	†
7	A. Barski, *et al.*, "High-resolution profiling of histone methylations in the human genome," *Cell*, 129(4): 823-37, 18 May 2007. [NHLBI, NIH, Bethesda, MD; U. Calif., Los Angeles] *172FA	29	7
8	S.G.F. Rasmussen, *et al.*, "Crystal structure of the human $ß_2$ G-protein-coupled receptor," *Nature*, 450(7168): 383-8, 15 November 2007. [Stanford U., CA; MRC Lab. Molec. Bio., Cambridge, U.K.; Europ. Synchroton Radiat. Fac., Grenoble, France; Argonne Natl. Lab., IL] *231AM	29	†
9	M. Wernig, *et al.*, "*In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state," *Nature*, 448(7151): 318-24, 19 July 2007. [5 U.S. institutions] *191GC	28	4
10	T.S. Mikkelsen, *et al.*, "Genome-wide maps of chromatin state in pluripotent and lineage-committed cells," *Nature*, 448 (7153): 553-60, 2 August 2007. [6 U.S. institutions] *195XV	26	†

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Other researchers have already made use of the $ß_2$AR structure, and even more so the insights that went into determining it, to pursue their own GPCRs with considerable success. Kobilka's group is in hot pursuit of the structure of active $ß_2$AR, bound not by a blocker but by its correct agonist adrenaline. That may reveal details of how exactly it activates the G protein, and will probably require the crystallization of an even more complex three-part molecule—signal, receptor, and G protein responder. That is unlikely to take another 20 years.

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