STRUCTURAL BIOLOGY

Excitatory receptors in close-up

Glutamate receptors facilitate the transmission of excitatory signals in the brain. A series of structures reveals how the shape of one such receptor alters on activation, providing insight that might aid drug discovery. SEE ARTICLE P.79

KAREN J. GREGORY

Glutamate is the main neurotransmitter molecule in the brain responsible for communicating excitatory signals between brain cells. This communication is mediated through glutamate-activated receptor proteins embedded in the membranes of brain cells. One of these receptors, metabotropic glutamate receptor 5 (mGlu5), is crucial for learning and memory, and is an attractive target for the treatment of several psychiatric and neurological disorders. On page 79, Koehl *et al.*[1] report the first essentially full-length structures of mGlu5, which they obtained by using a combination of X-ray crystallography and cryo-electron microscopy (cryo-EM). These structures reveal how activation of the receptor alters its multidomain structure to initiate cell signalling.

G-protein-coupled receptors (GPCRs) are the largest superfamily of receptors found in cell membranes, and are also the largest group of drug targets[2]. All GPCRs have seven α-helices that span the cell membrane, collectively known as the 7TM domain. Class C GPCRs — the group to which mGlu5 belongs — differ from other types in that they must form a dimeric complex to function, and because they have a large extracellular amino terminus.

The binding site for the naturally occurring activators (agonists) of class C GPCRs is found in the N terminus, and is referred to as the Venus flytrap (VFT) domain because it is formed by two lobes. Most of these receptors also contain a domain that is rich in cysteine amino-acid residues, and this links the VFT to the 7TM domain. How the binding of an agonist in the VFT domain transmits a signal over a long distance (more than 120 ångströms) within the mGlu5 dimer to promote the active conformation of the 7TM domain has been unknown.

Until now, that is. Koehl *et al.* describe two X-ray crystal structures of dimeric mGlu5 VFT domains in complex with a positive allosteric modulator (a molecule, in this case a small antibody known as a nanobody, that stabilizes the binding of agonists) — one with and one without a synthetic agonist. These structures show that the binding of the agonist causes the two lobes of the mGlu5 VFT domain to close. The closed conformation strongly resembles that observed in the crystal structure of the mGlu5 VFT domain bound to its natural agonist, glutamate[3].

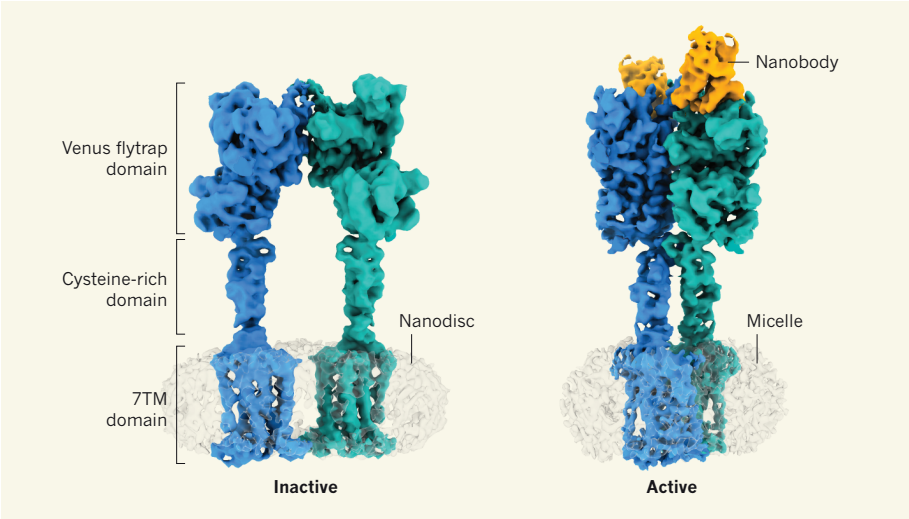

Figure 1 | Structures of the metabotropic glutamate receptor 5 (mGlu5). The dimeric mGlu5 receptor is crucial for learning and memory, and is a promising target for drugs that treat several psychiatric and neurological disorders. Koehl *et al.*[1] used cryo-electron microscopy to obtain structures of mGlu5, which they formed from two essentially full-length mGlu5 proteins (shown in blue and turquoise). Three of the main domains within each monomer are indicated. The structures show that activation of the receptor closes the 'Venus flytrap' domains, and brings the cysteine-rich domain and 7TM domain of each monomer closer together. The nanobody (a small antibody) in the active structure was used to stabilize the active conformation of the receptor. The nanodisc and the micelle mimic the cell membrane in which the 7TM domain is bound in nature. (Adapted from Fig. 3a of ref. 1.)

The authors then obtained cryo-EM structures of dimeric mGlu5 that incorporate all of the receptor's major domains, apart from the intracellular carboxy terminus. In these structures, the agonist-free VFT domains adopt a similar conformation to that seen in the equivalent X-ray crystal structure. The cysteine-rich domain forms a stalk that holds the VFT more than 55 Å above the 7TM domain, and each of the two 7TM domains are separated by more than 20 Å (Fig. 1).

Koehl *et al.* used two methods to reconstitute purified, full-length mGlu5 for their cryo-EM experiments, each generating a different receptor conformation for the mGlu5 dimer. The images of mGlu5 produced using both methods suggest that the agonist-free receptor has minimal or weak interactions between the cysteine-rich and transmembrane domains across the dimer. This agrees with the results of a previous biophysical study[4] of the isolated 7TM domains of mGlu5 and of the related mGlu2 receptor. However, it contrasts with experiments[5] in which proximal amino-acid residues across the dimer in agonist-free mGlu2 were identified on the basis of whether covalent crosslinks could be formed between those residues. Koehl and co-workers also used cryo-EM to visualize the structure of full-length mGlu5 in which the VFT domains were bound to an agonist, using positive allosteric modulators (a nanobody bound to the VFTs, and a small molecule bound to the 7TM domain) to stabilize this conformation of the receptor.

It was already known that the mGlu2 receptor must form a dimeric structure to enable its activation by glutamate, stimulating coupling of the receptor to a G protein[4] and thereby triggering signalling in the cell. A comparison of the full-length mGlu5 structures in which the VFT domains are agonist-bound (activated) and agonist-free (inactivated) provides insight into how the activation of dimeric class C GPCRs transmits structural changes throughout the entire protein.

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The cryo-EM structures show that closure of the VFT domain brings the cysteine-rich stalk and the 7TM domain of each subunit in the dimer closer together (Fig. 1), with the 7TM domains rotating, such that one of the α-helices (known as TM6) of each mGlu5 monomer forms a new interface between the two monomers. It is worth noting that the 7TM domains in the full-length receptors were reconstituted in different media (a nanodisc of lipids or a micelle of detergents) for each structure, and this might have influenced the relative orientation and proximity of the 7TM domains within the dimer. However, the authors carried out crosslinking experiments that provide more evidence of the structural changes proposed to occur on receptor activation, and further support comes from previously published studies[5–7] of other class C GPCRs.

Koehl *et al.* also carried out experiments to examine the effects of mutations to mGlu5 on its activation mechanism. Their results suggest that an interaction between the cysteine-rich stalk and a region of the 7TM domain known as the second extracellular loop (ECL2) governs activation by agonists that bind to the VFT domain, but not activation by agonists that bind to the 7TM domain. The conformation of this loop modelled by the authors is similar to that observed in the X-ray structure[8] of the 7TM domain of the related mGlu1 receptor in complex with an inhibitor. ECL2 is known to influence activation states in other GPCRs[9], but Koehl and colleagues' study provides the first indication that it also has a key role in mediating interdomain communication in class C GPCRs. However, the relatively low resolution of the new structures prohibits meaningful comparisons of the interactions between ECL2 and the cysteine-rich stalk in the inactive and active conformations of mGlu5. Whether these regions constitute targets suitable for drug discovery also remains an open question.

The resolution of the 7TM domains in both conformations is also insufficient to visualize the small-molecule inhibitors or activators that were used in the purification and reconstitution of the agonist-free and agonist-bound receptor structures, respectively. It remains to be seen how the structures of binding pockets in the 7TM domain change in the presence of inhibitors or activators. Indeed, the resolution of the 7TM domains is lower than those previously obtained[10–12] for structures of the 7TM domains of mGlu5 bound to inhibitors. The structure of receptors in complex with an intracellular effector (such as a G protein) will be required to stabilize, and therefore visualize at high resolution, active 7TM conformations, and to understand how allosteric modulators binding to the 7TM domain alter the activation states, to enable the structure-guided design of drugs that target glutamate receptors.

Koehl and colleagues' structures reveal the large-scale conformational changes that occur in a dimeric, full-length, class C GPCR when an agonist binds to the N terminus. The strategies used to stabilize full-length proteins will inform efforts to obtain the structures of other class C GPCRs, including receptors for ions and the inhibitory neurotransmitter GABA, as well as for receptors involved in taste. An appreciation of how these dimeric, multidomain receptors are organized should inform our understanding of how receptor complexes composed of two or more different class C GPCRs, or from different GPCR classes, are formed and activated. These structures might also guide future protein engineering of class C GPCRs to enable the identification of pockets that can be targeted by drugs, and might ultimately open up avenues of research for structure-guided drug discovery. ■

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This article was published online on 23 January 2019.

BIOTECHNOLOGY

On the road to a gene drive in mammals

A method for making a version of a gene more likely to be inherited than normal, generating what is called a gene drive, might be used to control insect populations. It has now been reported to work in mammals, too. SEE LETTER P.105

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When Gregor Mendel tracked pea-plant characteristics over successive generations in the nineteenth century[1], his landmark study revealed key insights into the fundamental mechanisms governing genetic inheritance. Mendel observed consistent patterns of inheritance that corresponded to each descendant receiving one of the two maternal copies of a gene affecting the characteristic and one of the two paternal copies of this gene. In this typical scenario of genetic inheritance, both maternal copies of a gene have an equal probability of being inherited, as do both paternal copies.

However, inheritance does not always proceed so fairly, and in some cases the odds of a particular copy of a gene being transmitted to the next generation can be heavily skewed. One natural example is that of 'jumping genes', which are inherited in a non-Mendelian pattern[2]. Genetic-engineering approaches are being developed to manipulate the inheritance pattern of a gene copy such that it will spread through a population more rapidly than would be expected by normal Mendelian inheritance, generating what is called a gene drive and leading to super-Mendelian inheritance[3,4]. This process generates what is called a gene drive. So far, gene drives have been mainly engineered in insects. Grunwald *et al.*[5] report on page 105 a method for generating a gene drive in mice, offering an option to use this approach in mammals.

Gene drives developed in insects might provide a way to alter mosquito populations to decrease the probability that they transmit diseases such as malaria or dengue fever[3,4]. For example, a gene drive that affects mosquito fertility could be used to specifically eliminate a species of malaria-transmitting mosquito[4], allowing its ecological niche to be filled by other mosquito species that cannot harbour the malaria-causing parasite. Alternatively, gene drives can be designed[6] to confer widespread, species-specific resistance to infection by this parasite, for instance by using a gene drive to spread sequences that encode antimalarial antibodies so that mosquitoes are no longer infected by the parasite[7].

The technology needed for gene drives has been greatly accelerated in insects by harnessing a gene-editing technique called CRISPR[3,4,6]. This system relies on the insects being engineered to express the enzyme Cas9 and a guide RNA that provides gene-targeting specificity. Cas9 generates a cut in a genomic DNA sequence that matches the guide RNA sequence (Fig. 1). If the guided cut generates

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