Fine-tuning receptor–G protein activation and signaling

Guillaume Lebon

Perspective title:

Fine-tuning receptor–G protein activation and signaling

One sentence summary: Structural basis of GPCR G protein selectivity and G protein activation kinetics

Guillaume Lebon
Institut de Génomique Fonctionnelle (IGF),
University of Montpellier, CNRS, INSERM, Montpellier, France.

G protein coupled receptors (GPCR) are eukaryotic plasma membrane receptors, that are organized into 5 classes in humans, A, B, C, E and Frizzled. They internalize extracellular stimuli by activating a common pool of intracellular signaling partners that subsequently induce an appropriate cellular response. Recent advances in cryo-electron microscopy (cryoEM) enables challenging structures of GPCRs signaling complexes to be solved, providing unprecedented insights into the molecular basis of their signal transduction (*1*). Indeed, Qiao *et al*., recently reported two cryoEM structures of the human glucagon receptor G_s and G_i protein complexes, GCGR-G_s and GCGR-G_i, which provides novel information about the molecular basis of GCGR G protein selectivity(*2*). In page XXXX, Hilger *et al*. report a cryoEM structure of GCGR-G_s complex that, together with a thorough biophysical analysis of receptor conformational changes and G_s protein activation kinetics, reveals the impact of structural changes on GCGR signaling properties. Importantly, the structural determinants of GCGR G protein selectivity together with kinetic properties of GCGR G_s activation supports a common and conserved activation mechanism for class B receptors.

GCGR physiology and signaling are challenging our knowledge of GPCR activation mechanisms. For example, circulating glucagon generated by pancreatic α-cells, activates the GCGR and controls glucose homeostasis in the liver(*3*). Once glucagon activates the GCRC-G_s signaling complex, the G_s G protein interacts with adenylate cyclase and induces cAMP production. It is a common situation that a GPCR interacts with several G proteins, such as G_s, G_i and G_q, GCGR is no exception (*4*). One intriguing question is how do GPCRs

achieve the functional selectivity required for performing the appropriate signal transduction? It is an attractive angle to first look at this from a structural biology perspective. The two cryoEM structures of two GCGR G protein signaling complexes, GCGR-G_s and GCGR-G_i solved respectively at 3.7 Å and 3.9 Å, shed light on the G_s binding selectivity(*2*). As a main feature previously reported for other class B G protein signaling complexes(*5*), the intracellular tip of the transmembrane helix 6 (TM6) is tilted outward, away from the receptor core by approximately 19 Å as are TM5 and TM7 to a lower extent, by 8 Å and 2 Å respectively. As a consequence, this creates a large site for the G protein to bind to. Conformational changes leading GCGR activation occur in a different manner than for class A receptors. The conserved residues of the PXXG motif in TM6 are repositioned, and TM6 locally unwinds, which results in a sharp kink which tilts the straight intracellular tip of TM6 away from the receptor core(*2*)(Hilger et al 2020). Such a kink was reported for several class B GPCRs(*5*), and the superposition of the corresponding receptor signaling complexes clearly illustrates this conserved feature compared to class A receptors, for which there is no kink and instead TM6 simply bends over, with some degree of variability depending on the receptor(*1*).

The G protein binding site compares well to class A GCPRs coupled to G_s, although it is significantly larger, and accommodates a C terminal extremity of a α5 helix from both G_s and G_i. There are significant differences in the sequence α5 helices (position G.H.5.23 and G.H.524), indicative of the requirement of a larger binding site for G_s. The overall contact surface for Gα subunit also differs between G_s and G_i. This molecular interface is mainly mediated by the α5 helix C-terminal extremity and is larger for G_s, 802 Å^2, representing 60 % of the total G_s protein/receptor interface compared to 80% for G_i, with a contact surface of only 551 Å^2. A second difference is the contribution of the intracellular loop 2 (ICL2) loop in the GCGR-G protein complexes. The positioning of the G_i αN helix relative to the receptor stresses the ICL2 conformation, making its contribution to the interaction less significant for the GCGR-G_i complex, whereas ICL2 makes extended molecular interactions with the αN helix, the β1 strand and the α5 helix of G_s. The resulting difference in total contact surfaces established between GCGR with

the G_s an G_i provides an explanation for the lower efficiency of G_i coupling but also for the G_s selectivity over G_i. Disrupting these interfaces by site-directed mutagenesis further highlights the importance of the shape and the size of the G protein binding cavity. Introducing a tryptophan residue has a larger impact on G_s than G_i whereas mutating residues in ICL3 mainly disrupts G_i signaling and glucagon potency. Importantly, mutating ICL2 residues confirms its important role in the activation of G_s when G_i is more sensitive to residues localized in the more discrete molecular contact with ICL3.

Once stimulated by glucagon, the GCGR-G_s complex induces a sustained production of cAMP over time compared to β2AR-G_s, suggesting a difference in the kinetics of cAMP accumulation and G protein activation(*6*). Although the structure of the receptor and receptor signaling complexes provide a snapshot of the receptor in action they do not indicate how the receptor conformation influences G protein activation kinetics. In the second study, the authors compare cryoEM structures of GCGR-G_s to previous β2AR-G_s complex and present a very elegant fluorescence, DEER spectroscopy, and nucleotide exchange analysis for GCGR-G_s and β_2AR-G_s, that connects together structural differences with differential rates of G protein activation. The 3.1 Å resolution cryoEM structure of GCGR bound to the engineered glucagon derivative, ZP3780, first sheds light on the molecular intricacies involved in the agonist-induced structural changes of TM6 which lead to its intracellular outward shift, and finally the opening of the cavity which will allow the G protein to bind. However, ZP3780 is not sufficient to induce the same outward shift of TM6 as observed for β_2AR and class A GPCRs agonists, but rather triggers discrete conformational changes in the environment of TM6, stabilizing a GCGR-GDP-bound G_s intermediate active state. This observation correlates with the weak interaction of G_S with GCGR ICL2 compared to the β_2AR-G_s complex, which likely slows down the GDP release and prevents the formation of the fully activated state and nucleotide free G protein conformation. In addition, biophysical characterization of purified receptors reconstituted in HDL particles shows a large difference in guanine nucleotide exchange factor activity, that is 70 times slower for GCGR. Förster resonance energy transfer of labeled G protein and GCGR highlights that the rate of G protein

association to the receptor is a 1.7 times slower, whilst radiolabeled GDP released is impaired by approximately 21-fold. Finally, they report that GTP binding is 3-times slower which confirms the reduced rate of GCGR G protein activation and nucleotide hydrolysis. A surprising observation lies in the long standing conformation of the TM6 tilt characteristic of the GCGR active conformation that maintains the G protein pocket in an open conformation, even though the G protein has dissociated. This novel finding differs from the fast relaxation of the bent TM6 associated with class A receptors which allows them to return to a more energetically stable conformation after G protein activation. As an explanation, the authors propose that a higher energy barrier separates the GCGR inactive conformation from the intermediate and fully activated states that potentially accounts for the slower kinetic rate of activation and also for the sustained production of cAMP over time. In addition to conserved structural features of the activated state, 3 other class B receptors display a slower G protein dissociation rate compared to class A receptors, indicative of a conserved activation mechanism for class B GPCRs.

We are still at the beginning of our understanding of the complexity and diversity of GPCR activation mechanisms, their coupling selectivity, and all the parameters that may govern their signaling in time and space. GPCR conformational diversity is likely to translate into subtle tuning of intracellular signaling partners such as G proteins, reinforcing the multiplicity of possible biological outputs, in regards to the limited numbers of intracellular partners. In this respect, the composition of the heterotrimeric G protein, namely the β and γ subunits, and other additional regulatory proteins or signaling partners, may also impact the G protein activation rate and will require further investigation. We will also need more structural information, from different receptor classes, to achieve a deeper understanding of their signaling activation mechanism. GPCRs are challenging allosteric machines, and are incredibly important drug targets. In the future it will be necessary to combine and integrate structural information with the dynamic properties of receptor conformation and kinetic parameters of receptor signal transduction when engaging in the development of novel pharmaceuticals.

Acknowledgements. This work was supported by the University of Montpellier, CNRS and INSERM.

References

1. J. García-Nafría, C. G. Tate, *Mol. Cell. Endocrinol.* **488**, 1–13 (2019).
2. A. Qiao *et al.*, *Science*. **367**, 1346–1352 (2020).
3. K. E. Mayo *et al.*, *Pharmacol. Rev.* **55**, 167–194 (2003).
4. D. Wootten *et al.*, *Chem. Rev.* **117**, 111–138 (2017).
5. Y.-L. Liang *et al.*, *Mol. Cell*. **77**, 656–668.e5 (2020).
6. J. H. Exton *et al.*, *J. Biol. Chem.* **246**, 6166–6177 (1971).

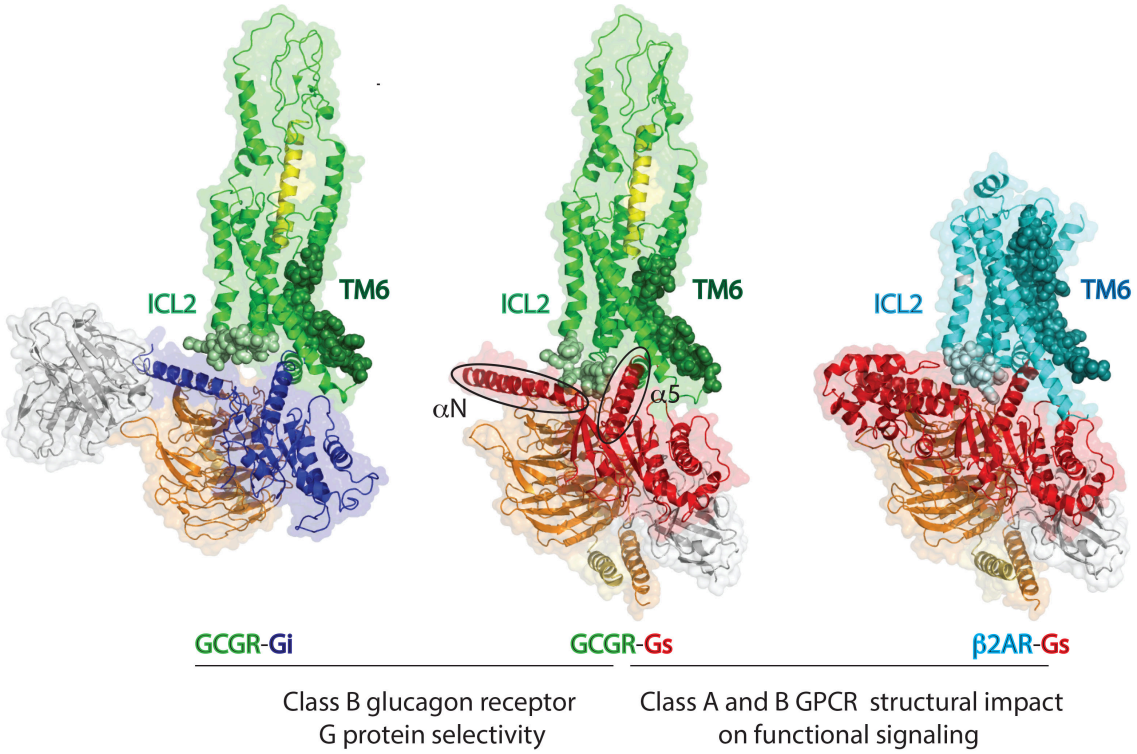

Fig. 1. GPCR structural features of G protein activation and selectivity. Structural analysis of class B glucagon receptor (GCGR) reveals the contribution of α5, αN, TM6 helices and ICL2 loop conformation to the structural mechanism of G protein selectivity. Structural features of class B GPCRs active conformation best illustrated by the sharp kink in TM6 and differential rate of G protein activation at GCGR-G_s compared to β_2AR-G_s signaling complexes define a common activation mechanism for class B receptors. GCGR is represented in green, β_2AR in cyan, G_i G protein in dark blue, G_s in red, beta and gamma G protein subunit respectively in orange and olive. TM6 conformation in represented as dark green sphere balls for GCGR, in dark cyan for β_2AR. ICL2 is represented in light green and light blue , respectively for GCGR and β_2AR.