



Figure 1 | Structural features of class B GPCRs. Hollenstein *et al.*³ and Siu *et al.*⁴ present the crystal structures of two class B G-protein-coupled receptors: CRF₁R (orange ribbons) and GCGR (blue ribbons), respectively. **a**, The structures reveal the locations of conserved amino-acid residues that form similar interactions in the two receptors, including between the transmembrane helices TM2, TM3 and TM4 (cyan), TM2 and TM3 (purple), TM1, TM2 and TM7 (beige), and TM2 and TM6 with the intracellular helix 8 (blue). **b**, The view of the proteins from outside the cell highlights the differences between the two structures at their extracellular faces, particularly in TM6 and TM7.

osteoporosis and type 2 diabetes, and are being actively pursued as targets for treating many more, from obesity and migraine to depression and chronic obstructive pulmonary disease.

Hollenstein and colleagues present a 3.0-ångström-resolution structure of the corticotrophin-releasing factor-1 receptor (CRF₁R) in complex with a small-molecule inhibitor. They arrived at this structure by introducing 12 thermostabilizing mutations into this GPCR and inserting the protein T4 lysozyme into its second intracellular loop. Siu and co-workers produced their 3.4-Å-resolution structure of the glucagon receptor (GCGR) using a version of the protein that was largely unmodified, except that its amino-terminal domain had been replaced with a thermally stabilized protein. The native N-terminal domain of class B GPCRs is crucial for peptide binding, but both teams removed this region to aid crystallization of the proteins.

As predicted, the core of both structures features seven transmembrane helices (TM1–TM7). However, although the relative positions of these helices at the intracellular face of the proteins overlap with those in class A GPCRs, there is substantial deviation between the two classes at the extracellular face. In both class B proteins, there are differences in the positioning of TM6 and TM7 that result in TM6 being shifted away from TM5, with TM1 seeming to move in parallel with TM7. This results in a wider and deeper extracellular cavity in the receptor core of the class B proteins that presumably forms part of the peptide-binding site. In addition, there are differences between the CRF₁R and GCGR structures themselves, in the upper segments of TM6 and

TM7 (Fig. 1). Although it is unclear whether these differences were influenced by the crystallization process, they indicate that the solution of transmembrane-core structures for other class B receptors will be required to help us understand how ligands bind and activate these proteins.

A major obstacle for the therapeutic targeting of class B receptors has been their notorious intractability for the identification of small-molecule ligands, in particular, small-molecule activators. The new structures shed light on why this is so: the openness of the receptors' binding pocket makes it difficult for a small ligand to engage sufficient key amino-acid residues to initiate activation of the receptor. Nonetheless, the solved structures show distinct subpockets that could represent sites for structure-based drug design.

Intriguingly, Hollenstein and colleagues' structure shows that the small-molecule inhibitor binds to a very deep pocket in the intracellular half of the CRF₁R core. This ligand forms extensive contacts with residues in TM3, TM5 and TM6, and presumably inhibits receptor activation by tethering the cytoplasmic half of TM6 to TM3 and TM5, thereby restricting conformational rearrangement of the intracellular face. This represents a new target for the design of small-molecule ligands. However, the amino-acid side chains in the equivalent region in the GCGR structure are more compact and would require reorganization to allow similarly sized ligands to bind.

The evolutionarily conserved amino-acid motifs in class A receptors have an important role in maintaining the receptors in an inactive (or weakly active) state. Although the

intracellular face of the class B receptors is similar to that seen for class A proteins (with the exception of an inward shift of TM7), some of the interactions that maintain the inactive class A conformations (including the ionic lock that tethers the cytoplasmic half of TM3 to TM6, the CWXP motif in TM6 and the NPXXY motif in TM7) are not present in the two class B receptors studied.

Class B receptors also have a distinct pattern of conserved amino-acid motifs that are important for maintenance of the inactive conformation and/or for conformational transitions required for activation. The CRF₁R and GCGR structures suggest conserved interactions between some of these key residues (Fig. 1). In addition, similar regions of contact are present between TM1 and TM2, TM1 and TM7, TM3 and TM4, and TM3 and TM6 in structures of both class A and B, although these interactions are mediated by different patterns of residues in each class. Thus, the new structures suggest that the two classes of proteins use distinct mechanisms for conformational control.

Although these reports represent a tremendous breakthrough in GPCR biology, as with all crystal structures, the intramembranous class B structures provide only a snapshot of the receptors, which in reality are known (from cysteine-trapping studies⁵) to be highly dynamic proteins. Important questions remain about the final orientations of the N-terminal domains and transmembrane helices of the receptors, and about how natural activator molecules engage with both domains to activate the receptors. Answering these questions will require both crystallization of an intact ligand–receptor–G-protein complex and studies of receptor dynamics. ■

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CORRECTION

In the News & Views article 'Cloaking of heat' by Ulf Leonhardt (*Nature* **498**, 440–441; 2013), it was stated that "Einstein received his Nobel prize for his theory of diffusion, not for relativity." In fact, he received the prize "for his services to Theoretical Physics, and especially for his discovery of the law of the photoelectric effect".