A Structural Understanding of Class B GPCR Selectivity and Activation Revealed

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Ma et al. (2020) and Liang et al. (2020) describe the cryo-EM structures of three class B G protein-coupled receptors (GPCRs) in complex with native peptides and Gs. Their work establishes the structural basis of peptide specificity and a conserved mechanism of receptor activation and G protein coupling for class B GPCRs.

G protein-coupled receptors (GPCRs) are the largest class of membrane proteins in the human genome. There are more than 800 human GPCRs, which can be classified into five major families: Rhodopsin (class A); Secretin and Adhesion (class B); Glutamate (class C); and Frizzled/taste receptor 2 (TAS2) (Wacker et al., 2017). These families can be further divided into subfamilies on the basis of sequence similarity. The term ''7TM receptor'' is a commonly used term that is interchangeable with ''GPCR.'' These receptors share a common architecture consisting of a single polypeptide with an extracellular N terminus, an intracellular C terminus, and seven hydrophobic transmembrane helices (TM1–TM7) linked by three extracellular loops (ECL1–ECL3) and three intracellular loops (ICL1–ICL3). Approximately half of the known GPCRs are important for mediating olfaction, taste, light perception, and pheromone signaling (Wacker et al., 2017), while the remaining (~350 non-sensory GPCRs) facilitate intracellular signaling through ligands that range in size from small molecules to peptides to large proteins (Roth, 2019). In the recent issue of *Molecular Cell*, Liang et al. (2020) and Ma et al. (2020) extend the structural knowledge of class B1 receptors through previously unknown peptide-bound structures of corticotropin-releasing factor receptors, CRF1R and CRFR2, and pituitary adenylate cyclase-activating polypeptide receptor, PAC1R, with their cognate G protein transducer (Liang et al., 2020; Ma et al., 2020). Their work provides new insights into class B1 GPCR peptide discrimination and receptor activation (Figure 1A).

Class B1 GPCRs (a subfamily of the Secretin and Adhesion family) respond to peptides that modulate key physiological functions including appetite and glucose handling, amino acid metabolism, cardiovascular tone, cardiovascular and gastrointestinal development and repair, bone metabolism, and immune responses. In contrast to the most frequently studied GPCRs of class A (Rhodopsin-like), class B GPCRs have a large extracellular domain (ECD) at their N terminus whose inherent flexibility has contributed to the difficulty in getting complete receptor structures. Partial receptor domain structures have been available for some time in a ''divide and conquer'' approach in which individual components (ECD with peptide [Underwood et al., 2010] or transmembrane with peptide or small molecule [Siu et al., 2013]) have been resolved. These fragmentary structures leave unanswered important questions such as how and to what extend the ECD modulates ligand binding and what its role in receptor activation might be.

Breakthroughs in the development of biologics such as nanobodies and single-chain fragment antibodies (Zhang et al., 2017) together with dominant-negative G proteins (Liang et al., 2017) have enabled the relative ease by which GPCR complexes can be formed and stabilized for structural determination. Simultaneously, advances in single-particle cryo-electron microscopy (cryo-EM) facilitate the visualization of these intact GPCRs complexes bound to agonists and their cognate transducer proteins (Liang et al., 2017; Zhang et al., 2017). In addition to the assortment of class A GPCR-G protein complexes, current class B structures determined by cryo-EM include calcitonin receptor (CTR) (Liang et al., 2017), calcitonin gene-related peptide receptor (CGRPR), glucagon-like peptide 1 receptor (GLP-1R) (Zhang et al., 2017), and parathyroid hormone receptor 1 (PTH1R).

Liang et al. (2020) and Ma et al. (2020) show with their respective structures that, with the exception of the calcitonin family peptides, all peptide agonists of the class B1 GPCRs penetrate into the receptor core to a similar extent and sit above a central polar network. In part due to high-resolution cryo-EM maps, particularly in the corticotropin-releasing factor (CRF) bound CRF1R structure, Liang et al. (2020) unravel the presence of structural waters and suggest they play a key role in receptor conformation and ligand binding. Additionally, they identified a conserved interaction with position 5.40 in TM5 that was within H-bond distance of all peptides and is, therefore, suggested to be dynamic. Previous mutagenesis studies have indicated that this is a key interaction for peptide-mediated Gs coupling and critical for receptor signaling. With the exception of TM4, class B peptides interact to different extents with all TM segments and the extracellular loops. The unstructured N terminus of the peptides inserts themselves between TM5 and TM6, and in some cases the peptides fold back upon themselves and nearly exit the extracellular vestibule. The C termini of the bound peptides extend from the respective receptor core and, upon exiting the 7TM bundle, interact with the proximal portion of the ECD. The peptides further interact with

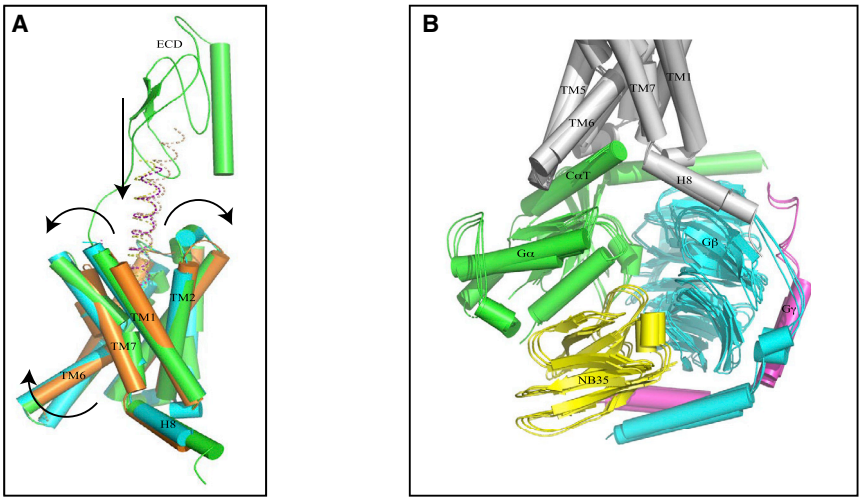

Figure 1. Structural Comparison of Class B GPCRs Reveal Conserved Features

(A) Peptide agonists of class B1 GPCRs penetrate into the receptor core and sit above a central polar network. Peptides diverge upon exit from the extracellular vestibule due in part to differences in peptide sequences and interaction with the ECD. Structural changes to 7TM as a result of peptide binding include the outward movement of the 7TM segments at both extracellular and intracellular domains. Notably, the "kink" of TM6 allows the insertion of G protein.

(B) The interaction of C-terminal tail of G protein subunit with the GPCR core is well conserved, with both subtle and distinct angles of engagement for other subunits of the Gs-heterotrimer which can be seen when receptor backbones are aligned. This despite the fact that the overall conformations of the G protein remain relatively similar between complexes.

(A) Receptor color scheme: PAC1R, orange; PACAP, yellow; CRF1R, cyan; CRF, purple; CRF2R, green; UCN1, sand. (B) G protein color scheme: GαS, green; β, cyan; γ, magenta; NB35, yellow. Peptides are drawn as dashed lines, and 7TM and ECD helical are drawn as cylinders. For (B), all aligned receptors are colored in gray. Putative membrane demarcation lines are colored in red.

the ECD residing in a preformed binding groove formed by the three-layer α-β-β/α fold of the ECD as seen in previous crystal structures of the ECD peptide complexes. Ma et al. (2020) suggest that peptide-receptor discrimination between CRF1R and CRF2R (and presumably other class B receptors) is achieved not only through specific receptor-peptide ligand interactions but also through charge-to-charge interactions between the respective peptides and their orthosteric binding sites. They also suggest that hormone binding is controlled at two levels with an initial fast recognition by the ECD and a second slow step of receptor TMD recognition and subsequent receptor activation. They also show that the CRF1R and CRF2R ECD are highly divergent despite adopting similar architectures. The orthosteric sites between the two receptors share ~55% sequence identity but have comparable affinity for the peptide urocortin-1 (UCN1). In contrast, CRF1R and CRF2R show an approximately 10-fold difference in their affinity for CRF.

Markedly, the ECD in the active-state cryo-EM structures of CRFR1, CRFR2, and PAC1 receptors is of lower resolution relative to the 7TM core and G proteins and reflects the dynamic nature of this domain. Nonetheless, the "metastable" position of the ECD varies among the individual receptors, leading to distinct angles with which the peptides enter the receptor core. These observations led to Liang et al.'s suggestion that the metastable ECD position is primarily driven by the interaction between the peptide N terminus and the receptor core. In contrast, the ECD structure of the CGRP receptor is much more ordered than those of CRFR1, CRFR2, and PAC1. This might in part be explained by the stabilization of ECD in CGRP receptors by Receptor Activity-Modifying Protein 1 (RAMP1). It is known that RAMPs can modulate the activity of multiple class B GPCRs, though there is still debate as to whether they are functionally required for all class B GPCRs. Thus, the "metastable" position of CRFR1/CRFR2 and PAC1 ECD might be an inherent property of these receptors and/or domains.

The activation of class A GPCRs going from an inactive (apo) to active ligand-bound, G protein-coupled state involves structural rearrangements of key signature motifs such as E/DRY and NPxxY, collapse of the orthosteric and sodium ion binding sites, rearrangement of microswitches and ICLs, and outward movements of TM5 and TM6 (Wacker et al., 2017). In class B GPCRs, many of these class A structural motifs are absent, and thus activation of class B GPCR appears to be different from class A GPCRs. In the class B GPCR structures to date, peptide binding causes reorganization of ECLs and an opening of the extracellular vestibule (instead of contraction of extracellular vestibule as seen in class A). Similar to class A GPCR activation, TM5 and TM6 undergo an outward movement from the receptor core; however, TM6 then undergoes an approximate 90° kink or unraveling of the TM at the PxxG motif. Liang et al. compared multiple peptide-bound structures of the class B family GPCRs and identified a reorganization of ECL2 in active-state receptors, with an upward translation of TM4 and TM5 and the repositioning of both ECL2 and ICL2. They further suggest that the peptide interaction with ECL2 influences the conformation of ICL2 and downstream receptor signaling. For comparison, previous studies have shown that not only the correct orientation of ICL2 but also the conserved residues in both class A and class B receptors are important for the engagement of the Gαs subunit. Ma et al. further suggest that in the class B1 receptors, receptor-associated conformational changes in the G protein are required for nucleotide exchange and show that the ICL2 conformation is altered in a ligand-specific manner on a receptor-by-receptor basis.

From the cryo-EM structures presented by Ma et al. (2020) and Liang et al. (2020) (CRF1R, CRF2R, and PAC1), the binding of the Gαs protein (except as discussed above) appears to be fairly conserved among these receptors, with many charged and hydrophobic interactions maintained among the receptors. As expected, the Gαs C-terminal tail inserts into the cavity created by the outward movements of the TMs, and the superposition of the complexes against the receptor backbone reveals they are almost identical. However, Liang et al. suggest that in comparison to other known class B structures there exist subtle differences

in angles of engagement for the Gαs-heterotrimer with the respective receptors, which can be attributed to the distinct ligand:receptor interactions. This is despite the fact that the overall conformation of the G protein remains relatively similar between the presented complexes (Figure 1B).

In summary, work by Ma et al. and Liang et al. nicely complements previous work on the structures of class B GPCRs; all major GPCRs of the class B family now have representative structures. These manuscripts provide key insights into how peptide ligands are discriminated among class B GPCRs and expand our understanding of class B GPCR-G protein engagement and receptor activation. Additional structural studies spanning both inactive and active states of class B members will further help delineate the mechanism by which these receptors are activated. Finally, these results should provide useful templates for structure-guided discovery of novel therapeutics targeting class B GPCRs (Lyu et al., 2019).

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