Structural basis of signal recognition and regulation at the full-length glucagon receptor

Class B G-protein-coupled receptors (GPCRs), which are therapeutic targets for treatment of various diseases, consist of an extracellular domain (ECD) and a transmembrane domain (TMD) [1]. The human glucagon receptor (GCGR), a member of class B GPCR family, is activated by the peptide hormone glucagon and plays critical roles in glucose homeostasis. Thus, GCGR has been considered as an attractive drug target for type 2 diabetes [2] (Fig. 1). Due to conformational flexibility and diversity, structural determination of full-length class B GCGR structure remains challenging. To better understand the ligand recognition and signal transduction mechanisms of class B GPCRs, we have determined the crystal structures of the full-length GCGR in complex with different ligands [3,4].

To obtain crystals of the full-length GCGR, the fusion partner T4L was inserted into the second intracellular loop (ICL2) of GCGR, and the receptor C terminus was truncated by 45 residues. To further improve protein stability, the antigen-binding fragment (Fab) of an inhibitory antibody, mAb1, and a negative allosteric modulator NNC0640 were used to purify and co-crystallize with the receptor. The GCGR-NNC0640-mAb1 complex structure was determined at 3.0 Å resolution by collecting data at SPring-8 **BL41XU** and the Linac Coherent Light Source (LCLS) in the SLAC National Accelerator Laboratory [3]. To further solve the peptide-bound structure of GCGR, we designed a partial agonist NNC1702 by introducing four point mutations in glucagon. Collecting data at BL41XU, the structure of the GCGR-NNC1702 complex was also determined at 3.0 Å [4].

The overall architecture of GCGR comprises an α-β-β-α fold of the ECD and seven transmembrane α-helices (Fig. 2(a,b)). In the GCGR-NNC0640-mAb1 structure, NNC0640 binds to the receptor on the external surface of the TMD, forming hydrogen-bond interactions with $S350^{6.41b}$, $T353^{6.44b}$ and $N404^{7.61b}$ in helices VI and VII (Fig. 2(c)). The binding mode of NNC0640 suggests that this negative allosteric modulator most likely inhibits receptor function by blocking the conformational changes of helices VI and VII.

In the peptide-bound GCGR structure, the C terminus of the peptide NNC1702 binds to the receptor ECD, and its N terminus penetrates into the ligand-binding pocket within the TMD, consistent with the previously proposed two-domain peptide binding model of class B GPCRs [1] (Fig. 2(b)). Additionally, the middle region of NNC1702 makes extensive contacts with the first extracellular loop (ECL1), the second extracellular loop (ECL2) and the stalk region that connects the ECD and TMD (Fig. 2(d)). The GCGR-NNC1702 structure, for the first time, provides a detailed molecular map for the interactions between a class B GPCR and its peptide ligand, which would benefit drug design and development targeting GCGR.

Comparing the two GCGR structures, there is a 90° orientation change of the ECD relative to the TMD (Fig. 3(a,b)). This introduces questions about inter-domain conformational flexibility required for

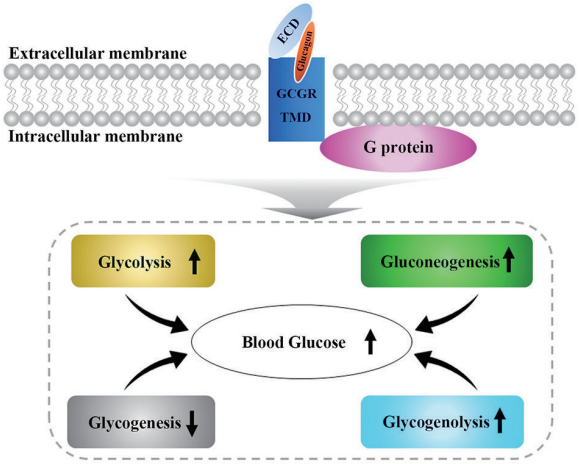

Fig. 1. Schematic illustration of the roles of GCGR in glucose regulation. Once activated by its endogenous ligand glucagon, GCGR increases blood glucose level, and is involved in modulating glucose homeostasis in human body.

the receptor binding to different ligands. Remarkably, the stalk and ECL1 adopt completely different conformations in the two structures. In the GCGR-NNC0640-mAb1 structure, the stalk and ECL1 form a compact β-sheet structure, stacking on top of the ligand-binding pocket of the TMD (Fig. 3(c)). In contrast, these two regions depart from each other and form an α-helix and a loop-α-helix-loop conformation respectively in the peptide-bound structure, acting as two 'arms' holding the peptide ligand tightly (Fig. 3(d)). These differences suggest that the structure rearrangement of the stalk and ECL1 may play a role in modulating peptide binding and receptor activation, which was further supported by our extensive studies of hydrogen–deuterium exchange, disulfide crosslinking, ligand binding, cell signaling and molecular dynamics simulations. Based on these findings, we proposed a dual-binding-site trigger model of GCGR activation. This model extends the previously established two-domain peptide binding model of class B GPCRs by including the interactions between the middle region of the peptide and the stalk and ECL1 as another trigger of receptor activation in addition to the binding of the peptide N terminus to the receptor TMD.

In summary, we reported two crystal structures of the full-length GCGR at different conformational states. These structures reveal molecular details of the receptor binding to different types of ligands, providing structural basis for drug development of diabetes. Additionally, the insights into the full-length GCGR structures shed light on the molecular mechanisms of receptor activation modulation, and thereby greatly expand our understanding about signal transduction of class B GPCRs.

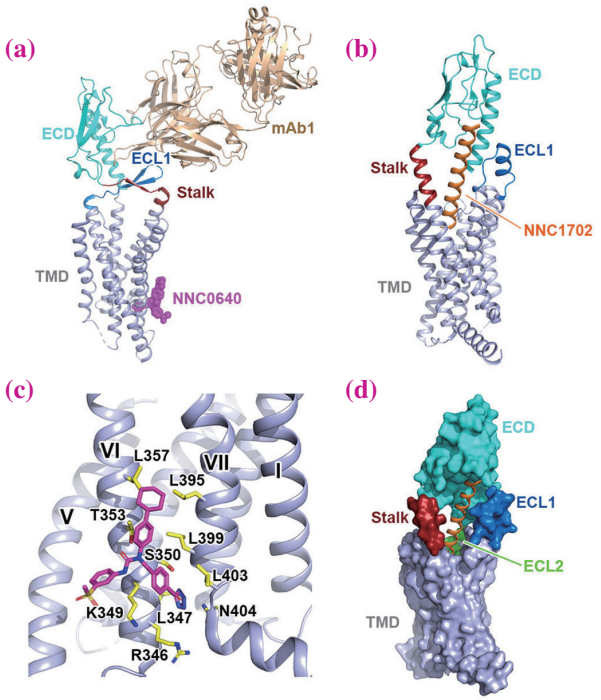

Fig. 2. Structures and ligand binding modes of GCGR. **(a)** Structure of the GCGR–NNC0640–mAb1 complex. **(b)** Structure of the GCGR-NNC1702 complex. **(c)** Binding pocket of NNC0640. **(d)** Binding mode of NNC1702.

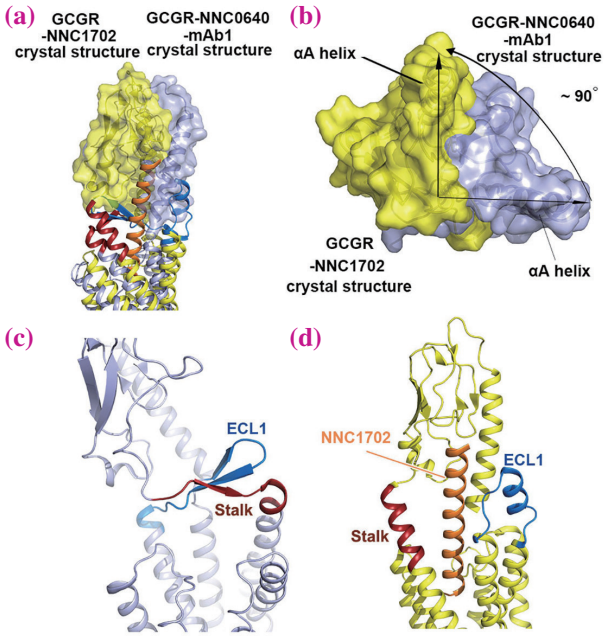

Fig. 3. Conformational changes of the stalk and ECL1. **(a)** Comparison between the two GCGR structures. **(b)** Extracellular view of the ECDs. **(c)** Conformation of the stalk and ECL1 in the GCGR-NNC0640-mAb1 structure. **(d)** Conformation of the stalk and ECL1 in the GCGR-NNC1702 structure.

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