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Journal of Clinical Cardiology Commentary

Commentary on "Structural Basis of the Activation of Heterotrimeric Gs-Protein by Isoproterenol-Bound β1-Adrenergic Receptor"

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G protein coupled receptors (GPCRs) comprise a large superfamily of transmembrane proteins containing more than 800 unique family members [1,2]. Critically, GPCRs mediate most of the cell responses to extracellular stimuli including hormones, neurotransmitters, light, taste, etc. Adrenergic receptors (ARs) bind with norepinephrine and epinephrine and are expressed in many organs, especially in heart [3,4]. Nonetheless, heart failure is one of the major contributions to mortality in the developed countries and the downregulation of ARs is commonly observed in the heart failure patients [5]. Therefore, ARs are significant regulators in cardiac activities. There are 12 subtypes of ARs, beta-1 adrenergic receptor (β_1AR) and beta-2 adrenergic receptor (β_2AR) are the most important subtypes, which both coupled with Gs protein. Previous mutagenesis studies disclosed a few key residues of ARs that interact with the agonist, which provide piece of information for drug discovery [6,7]. To understand the whole picture of ligand binding and interaction with G proteins, structural determination of ARs is necessary. The β_2AR-Gs complex structure published in 2011 shined the light on the protein interaction and the conformational changes upon activation [8]. However, β_1AR is the predominant receptor, accounting for approximately 80% in the adult human heart [3]. The structure determination of β_1AR-Gs complex is more desirable in the drug design of heart diseases. The recent published paper " Structural Basis of the Activation of Heterotrimeric Gs-Protein by Isoproterenol-Bound β1-Adrenergic Receptor" reported the high-resolution structure of β_1AR in complex with Gs protein using cryo-EM method and investigated the molecular mechanism of Gs protein activation induced by β_1AR, which unveil more structural information to facilitate the drug discovery of human heart diseases [9].

Su et al., described molecular mechanism of Gs activation by β_1AR. Briefly, the process of GPCR mediated signaling pathway initiates with the formation of ligand-GPCR complex, in this case, the full agonist isoproterenol binds with β_1AR and fully activates the receptor for further coupling with Gs protein. By comparing with the previous reported inactive state β_1AR structures [10], dramatic differences on the cytoplasm side were noticed including the helix extension of transmembrane helix 5 (TM5), outward ~14 Å shift of the TM6 and the inward ~5 Å rotation of TM7. Particularly, Arg139 within the highly conserved D(E)RY motif forms salt bridge with Glu285 in the inactive state β_1AR structure [9]. The salt bridge plays significant role in the stabilization of the inactive state conformation. The absence of salt bridge in some inactive state GPCRs might be the explanation of the high basal activity [11,12]. In the active state structure, the salt bridge is broken, the Glu285 shifted outward ~ 14 Å and the space is occupied by the C- terminal end of the α5-helix Gs protein. The outward shift creates more space to host G protein, and the interaction with G protein further stabilizes the GPCR. It is worth mentioning that similar conformational changes were observed in other active state class A GPCR structures, which indicates these conformational changes might be universal molecular hallmarks for the active state class A GPCRs [13,14]. The complex structure reported in Su et al., provided a solid evidence of this hypothesis.

In terms of the conformational changes of G protein,

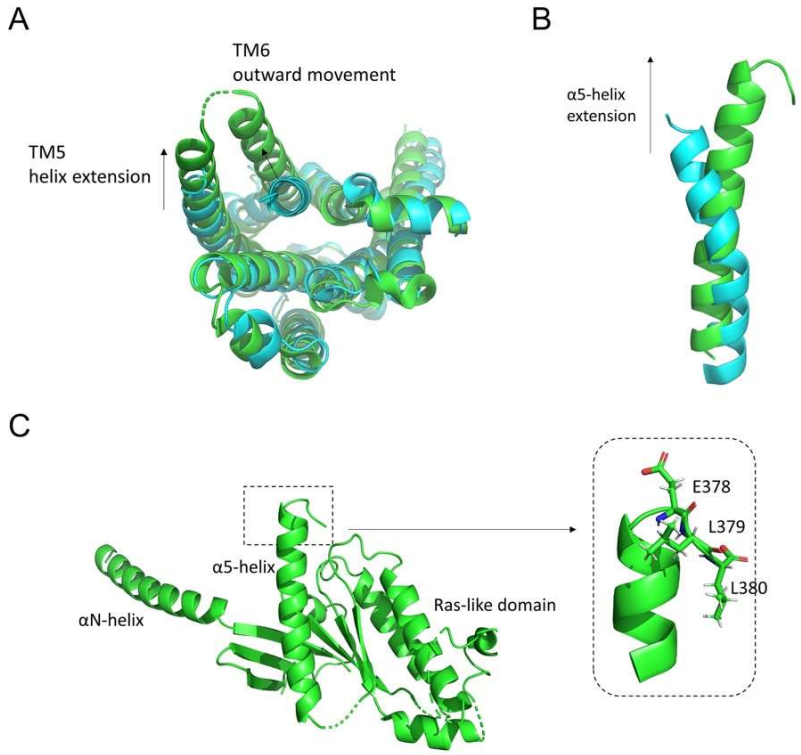

Figure 1: (A) Comparison of the active state (green) as seen in the β_1AR-Gs complex and the inactive state from the structure (cyan PDB ID: 4GPO) from the cytoplasmic view. The outward movement of TM6 and the helix extension of TM5 were presented. **(B)** Comparison of α5-helix of β_1AR-Gs complex (green) and Gs alone (Cyan, PDB ID:1AZT). Helix extension and rotation were presented. **(C)** α_L capping motif formed at the C-terminal of α5-helix.

it mostly happens on the G_α subunit. Early studies have highlighted the importance of the C-terminal tail of the α5-helix [15], the high resolution structure in Su et al., demonstrated molecular basis of this region. According to the G_αs alone structure determined by X-ray crystallography [16], the three amino acids (E378 to L380) of the C-terminal α5-helix were disordered. In comparison, in the β_1AR-Gs complex structure, these corresponding amino acids form a α_L capping motif, which interact with β_1AR-Gs extensively. In addition, the adjacent amino acids (L374 to Q376) form a helix extension that extends the α5-helix by ~6 Å (Figure 1). The α-helical domain rotationally opens away from its Ras-like domain and the distance between mass centers extends to ~38 Å, which were distinguished from the active structure of β_2AR-Gs complex [17]. With all the observed structural changes, GDP release from Gs protein was accelerated in this isoproterenol-bound β_1AR-Gs complex.

The structure of β_2AR-Gs complex also provided molecular insights of the Gs activation induced by β_2AR. Since β_1AR and β_2AR share 44.67% identity of amino acid sequence, the structural conformation of the receptor region in both complex structures display high homogeneity. The notable difference between these two complexes is located at the G protein heterotrimeric region, especially the α-helical domain. The α-helical domain of the β_2AR-Gs complex rotated closer to the receptor. Since the β_2AR-Gs complex structure was determined by X-ray crystallography, the difference of α-helical domain might be due to the crystal lattice contact. Moreover, in the β_1AR-Gs complex structure, the electron density map of α5-helix region of Gs was weaker than the other region which reflected the highly dynamic nature of α5-helix at the GTP free state.

Su et al., revealed the molecular basis by analyzing the high resolution structure of the β_1AR-Gs complex. In this work, cryo-EM was employed to determine the high-resolution complex structure, it shows the advantage of cryo-EM in the structural studies of protein complex structures. The traditional protein crystallography technology requires diffraction-quality crystals. It is always challenging to crystallize the target proteins while crystallization of large complexes is even more difficult. In the modern structural biology study, the core question is not only determining the structure of protein but understanding

the molecular basis of biochemical activities. Therefore, the structural determination of protein complexes or other Marco biomolecules is critical for seeking the molecular insights. The activation of GPCR and G protein is just an initiation of G protein signaling cascade [18]. With more complex structures available in the future, the molecular mechanism of G protein signaling can be further characterized, which may create a new path of drug design and discovery [19]. However, there are still challenges to solve protein structures by cryo-EM. Currently, antibody or nanobody has been commonly applied to stabilize the complex. Notably, scFV16(Fab) is the only one antibody that can stabilize both Gs and Gi. Antibodies and nanobodies generated from immune response have specificity and ability of stabilizing the target protein. In the foreseeable future, the development of antibodies will show more talent in the protein complex structure determination. Manufacturing a new antibody of a specific protein could be time-consuming and low efficiently. Hence, methods for stabilizing the protein complexes in a high-throughput and universal manner are preferred. Chemical biology may have a great potential of achieving this goal. A great number of synthetic molecules have been found to stabilize the protein-protein interactions [20,21]. The addressed protein complexes play significant roles in persistent challenges such as cancer, amyloidosis and hypertension. The discovery of these synthetic molecules could also benefit the drug discovery.

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