【Grant-in-Aid for Scientific Research (S)】

Broad Section G

Title of Project : Structural dynamics of GPCR in cells

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Keyword : GPCR, Cryo-EM, nano-disc, liposome, Cryo electron tomography

【Purpose and Background of the Research】

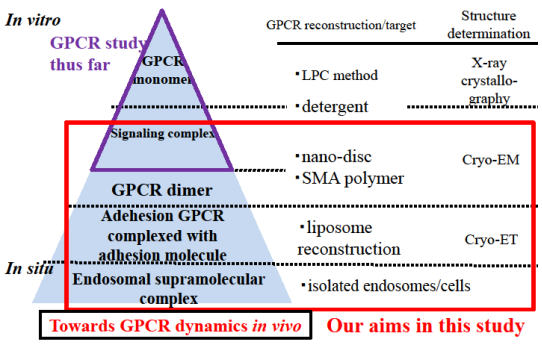
GPCRs are encoded by 10% genes of higher eukaryotes and are essential for living organisms to adjust the surrounding circumstance by triggering enzymatic reactions etc. upon perception of external stimuli such as binding with chemical compounds and light. These 20 years structural biological studies have uncovered the structural basis for the mechanism of selective recognition of their cognate ligands, and their activation and G-protein coupling mechanisms, which greatly help our understanding of physiological phenomena involving GPCRs and significantly promote drug designs and clinical applications for treatment of relevant diseases. However, series of structural studies thus far have targeted GPCRs reconstructed in detergent micelle, which is largely distinct from physiological condition, raising a question that these structures cannot capture their *in vivo* behaviors. Therefore, the objective of this study is to elucidate dynamic molecular mechanisms of GPCR in the condition much closer to *in vivo*.

【Research Methods】

Our aim in this study is to comprehensively elucidate the structural dynamics of GPCRs in *in vivo* environment from protein to cellular level, by reconstructing GPCRs in physicochemical conditions close to natural membrane. For this purpose, we will realize

1. Structure determinations of GPCR signaling complex and GPCR oligomer reconstituted in nano-disc, which well mimics *in vivo* environment
2. Elucidation of structural basis for adhesive interactions of adhesion GPCRs reconstituted in liposome
3. Cryo-ET of endosomal supramolecular complex comprising GPCR, coupling GTPase and β-arrestin

In particular, we will promote structural and functional analyses of signaling complex in lipids of parathyroid hormone (PTH) receptor, somatostatin receptor, lyso-phospholipid (LPS1) receptor, and mechanosensitive GPR68 receptor. Furthermore, we will target heterodimeric MT1/MT2 (melatonin) receptors complexed with Gq and heterodimeric LH/FSH sexual hormone receptors complexed with Gq to elucidate how hetero-oligomerization converts the G-protein specificity and downstream signaling. Then, we will develop our project towards more physiological complexes such as adhesion GPCR complex reconstituted in liposome and endosomal supramolecular complex comprising PTHR, Gs and β-arrestin, which will be analyzed in liposome or cells by Cryo-ET. These studies may allow us to uncover the dynamic molecular mechanism of GPCRs in physiological conditions.



【Expected Research Achievements and Scientific Significance】

These researches on dynamic molecular mechanism of GPCRs *in vivo* may pave the way for drug design and clinical applications for treatment of cancer, osteoporosis, neurodegenerative diseases and sleep.

【Publications Relevant to the Project】

1. "Activation Mechanism of Endothelin ET_B Receptor by Endothelin-1" W. Shihoya *et al*. ***Nature***, **537**, 363-368 (2016). doi: 10.1038/nature19319.
2. "Structural insights into ligand recognition by the lysophosphatidic acid receptor LPA_6" R. Taniguchi *et al*. ***Nature*** **548**, 356-360 (2017).
3. "Cryo-EM structure of the human PAC1 receptor coupled to an engineered heterotrimeric G protein" K. Kobayashi *et al*. ***Nat. Struct. Mol. Biol.*** **27**:274-280 (2020).
4. "X-ray structures of endothelin ET_B receptor bound to clinical antagonist bosentan and its analog" W. Shihoya *et al*. ***Nat. Struct. Mol. Biol.*** **24**, 758-764 (2017).
5. "Crystal structures of human ET_B receptor provide mechanistic insight into receptor activation and partial activation" W. Shihoya *et al*. ***Nat. Commun.*** **9**, 4711 (2018)
6. "Cryo-EM structure of the human MT1-Gi signaling complex." H. H. Okamoto *et al*. ***Nat. Struct. Mol. Biol.*** **28**:694-701 (2021).
7. "Cryo-EM structure of the β3-adrenergic receptor reveals the molecular basis of subtype selectivity." Nagiri C *et al*. ***Mol. Cell*** **81**, 3205-3215 (20221)

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