Preparation and Crystal Structure Determination of Eukaryotic Membrane Proteins

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Keywords: *Membrane protein, crystal structure, cell-free protein synthesis, lipidic mesophase, expanded genetic code.*

Abstract

We developed useful techniques for membrane protein preparation, crystallization, and interaction analysis, and studied structures and functions of eukaryotic membrane proteins.

We determined crystal structures of the human adiponectin receptors AdipoR1 and AdipoR2 (*1,2*). Stimulation of AdipoR1 and AdipoR2 by adiponectin increases the activities of AMPK and PPAR, respectively, and thereby contributes to healthy longevity. AdipoR1 and AdipoR2 were supposed to have seven transmembrane helices with the opposite topology to GPCRs. In this study, we determined the 2.9 and 2.4 Å resolution crystal structures of human AdipoR1 and AdipoR2, respectively. They are quite similar to each other, and represent a novel class of receptor structure, as the seven-transmembrane helices with different conformations from those of GPCRs enclose a large cavity in which a zinc ion is coordinated by the three conserved His residues. The zinc-coordinating structure appears to be important for the adiponectin-stimulated AMPK phosphorylation and UCP2 upregulation. The adiponectin-binding site of AdipoR1/AdipoR2 may exist broadly on the extracellular side.

We have established membrane protein production techniques, using the *Escherichia coli* cell-free protein synthesis system supplemented with lipids/detergents, suitable for structure determination by X-ray crystallography. The quantity and the quality of the cell-free expressed integral membrane proteins were generally better than those of the proteins expressed in conventional cell-based systems. Actually, a plant rhodopsin, which could not prepare by the conventional methods, was successfully prepared by the cell-free method and crystallized by the lipidic mesophase method, and its crystal structure was determined (*3*).

We have developed the expanded genetic code technologies to incorporate a variety of non-natural amino acids site-specifically into proteins. By using a photocrosslinking amino acid, we identified the interface between membrane proteins expressed on the surface of mammalian cells. This method is complementary to the crystallographic structure determination in understanding the functional mechanisms of the membrane proteins.

References

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