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

Title of Project : X-ray crystallographic studies of intra- and inter-cellular transport

Tomitake Tsukihara
(University of Hyogo, Graduate School of Life Science, Professor)

Research Area : Biological Sciences (Structural Biochemistry)

Keyword : X-ray crystal structural analysis

【Purpose and Background of the Research】

In higher organisms, various protein complexes are involved in molecular transport function to regulate various biological processes. The aim of our current research is to use X-ray structural analyses of protein complexes to elucidate mechanisms of molecular transport, both within individual cells and between cells.

We have been conducting structural studies on three different protein complexes that function as a channel and carriers. The first of these is a gap junction channel that connects two adjacent cells and allows exchange of small molecules and ions. The second is Vault, a huge complex with a molecular weight of larger than 10 MDa, which is involved in nuclear transport and multi-drug effluence. The third is Exportin, which transfers pre-microRNA from nucleus to cytosol in coordination with GTP-bound Ran.

We will elucidate the molecular mechanisms of these proteins' actions in intra- and inter-cellular transport by solving their X-ray structures at high resolution

【Research Methods】

Connexin-26 (Cx-26) gap junction channel will be crystallized in both open and closed states; we will determine the structure of both states at higher than 3.0 Å resolution. Twenty-one connexins are expressed in human cells. A homotypic channel is an end-to-end dimer of the same hemichannel of connexin hexamer, and a heterotypic channel is a dimer consisting of different hemichannels. We inspect channel formation for any pair of heterotypic channel by predicting a structure of any heterotypic channel using the Cx-26 gap junction channel.

Structure of Vault will be determined at higher than 3.0 Å resolution, in order to elucidate the structural organization of the capsid consisting of 78 major vault proteins (MVPs). In order to identify cholesterol-binding sites, the structure of Vault with cholesterol molecules will be determined at low resolution.

The structure of Exportin:RanGTP: pre-miRNA complex will be determined at 3.0 Å resolution, in order to elucidate the transport mechanism of pre-miRNA from nucleus by Exportin:RanGTP.

【Expected Research Achievements and Scientific Significance】

Determining the structures of Cx-26 gap junction channel in both open and closed states at higher than 3.0 Å resolution will allow us to elucidate the gating mechanism of the Cx-26 gap junction channel. Formation of gap junction heterotypic channel will be elucidated by the structural prediction.

Structural studies of Vault will allow us to elucidate the mechanism of molecular assembly of 78 MVPs and the mechanism of Vault recruitment by lipid rafts.

The structure of Exportin: RanGTP: pre-miRNA will allow us to determine the mechanisms of both recognition and protection of pre-miRNA by the Exportin:RanGTP complex.

【Publications Relevant to the Project】

S. Maeda, S. Nakagawa, M. Suga, E. Yamashita, A. Oshima, Y. Fujiyoshi and T. Tsukihara, Structure of the connexin-26 gap junction channel at 3.5 Å resolution. *Nature*, **458**, 597-602 (2009).

H. Tanaka, K. Kato, E. Yamashita, T. Sumizawa, Y. Zhou, M. Yao, K. Iwasaki, M. Yoshimura and T. Tsukihara, The structure of rat liver vault at 3.5 angstrom resolution. *Science*, **323**, 384-388 (2009).

【Term of Project】 FY2009-2013

【Budget Allocation】 180,900 Thousand Yen

【 Homepage Address and Other Contact Information】

http://www.sci.u-hyogo.ac.jp/life/GCOE/japanese/pico_intro/tsukihara/index.html