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

Title of Project : Molecular mechanism of ABC proteins involved in cholesterol homeostasis

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Research Area : Agricultural chemistry, Applied Biological Chemistry
Keyword : Transporter, Atherosclerosis, Alzheimer disease, Membrane meso-domain

【Purpose and Background of the Research】

The breakdown of cholesterol homeostasis is a cause of atherosclerosis and vascular diseases, the leading cause of death in Japan. Cholesterol homeostasis is maintained at the levels of biosynthesis, uptake, and storage. Recently, it was found that cholesterol efflux from cells is also important and that several ABC proteins are involved in this pathway. However, their molecular mechanisms are poorly understood. ABC protein is an ATP-dependent transporter family, and 48 ABC proteins are coded on human chromosomes.

In this study, we will reveal the molecular mechanisms of ABC proteins involved in cholesterol homeostasis by integrating biochemical/cell biological studies with single molecule tracking and 3D crystal structure analyzes.

【Research Methods】

① Mechanism of HDL formation by **single molecule imaging** via a TIRF microscope.

We have revealed that ABCA1 forms a dimer on the plasma membrane during HDL formation by the single molecule imaging. We will analyze ABCA1 mutants and reveal the significance of the ABCA1 monomer-dimer conversion and the molecular mechanism of HDL formation. Membrane meso-domain generation/degeneration by ABC proteins will be also visualized.

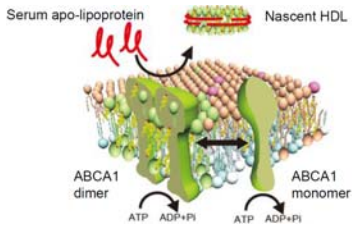

Fig. 1 Model for HDL formation by ABCA1

②Molecular mechanism of ABC proteins by **3D crystal structure analysis**.

We will analyze the 3D crystal structure of ABC protein from single cell eukaryote at the level of world-highest resolution, which allows us to reveal the molecular mechanism of substrate recognition and transport by eukaryote ABC proteins.

③ Mechanism of function of ABC proteins revealed by **biochemical/cell biological studies**.

Important amino acid residues for substrate recognition and transport will be revealed by targeted mutagenesis based on the structure revealed in the project ②. The mechanism of function of ABCA1, ABCA7, ABCA13, ABCB4, ABCG1, ABCG4 etc. involved in cholesterol homeostasis in the body and the brain will be revealed by biochemical/cell biological studies.

【Expected Research Achievements and Scientific Significance】

ABC proteins are not only important for HDL formation, a key for preventing atherosclerosis, but also involved in cholesterol homeostasis in the brain and related to Alzheimer disease and other neuronal diseases. Therefore, understanding of mechanism of ABC proteins is important for the prevention and cure of these diseases.

However, the study of ABC proteins is not so simple because they are large membrane proteins. In this study, we will integrate biochemical/cell biological studies with single molecule tracking and 3D crystal structure analyzes, which allows us to achieve the goal.

【Publications Relevant to the Project】

・Nagata, K., O., Nakada, C.,, Kasai, R. S., Kusumi, A. and Ueda, K. ABCA1 dimer-monomer interconversion during HDL generation revealed by single-molecule imaging. ***Proc. Natl. Acad. Sci. USA***, 110, 5034-5039 (2013)

・Hozoji-Inada, M., Munehira, Y., Nagao, K., Kioka, N., and Ueda, K. LXRβ directly interacts with ABCA1 to promote HDL formation during acute cholesterol accumulation. ***J. Biol. Chem.*** 286, 20117-24 (2011)

【Term of Project】

FY2013-2017

【Budget Allocation】

159, 600 Thousand Yen

【Homepage Address and Other Contact Information】

http:// www.biochemistry.kais.kyoto-u.ac.jp/