Division of Multidisciplinary Chemistry
- Supramolecular Biology -

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Scope of Research

We have undertaken the molecular biology, cell biology and behavioral genetics approaches to study the role of biological membrane systems in controlling animal morphogenesis and behavior. The membrane is a complex supramolecular complex formed by a noncovalent self-assembly of proteins, lipids, and carbohydrates. Our long term objective is to understand the fundamental principles underlying the dynamism of complex membrane systems and to provide a clue to reconstruct an artificial supramolecular membrane complex. Current research topics are as follows:

(1) Identification of a series of proteins that regulate molecular motion of lipid molecules and elucidation of their role in cellular and animal morphogenesis.

(2) Establishment of a series of *Drosophila* mutants with aberrant temperature preference (*atsugari, samugari*, etc) and elucidation of the molecular relationship between the temperature-responding membrane systems and animal behaviors.

Research Activities (Year 2007)

Publication

Saito K, Fujimura-Kamada K, Hanamatsu H, Kato U, Umeda M, Kozminski KG, Tanaka K: Transbilayer Phospholipid Flipping Regulates Cdc42p Signaling during Polarized Cell Growth via Rga GTPase-Activating Proteins, *Dev Cell.*, **13**, 743-751 (2007).

Presentations

Membrane Phospholipid Flip-flop and Its Role in Cell Motility. Kato U. The 10th Membrane Research Forum. 27 February–1 March 2007, Kyoto.

Regulation of Membrane Phospholipid Dynamics and Its Role in Control of Cell Migration. Kato U, Inadome H, Umeda M. The 112th Annual Meeting of the Japanese Association of Anatomists. 27–29 March 2007, Osaka.

The Present Bottom-up System of Grant-in-Aid for Scientific Research and Its Future. Umeda M. Special Symposium. 127th Annual Meeting of the Pharmaceutical Society of Japan, 28–30 March 2007, Toyama.

Requirement of ZO-1 for the Formation of Belt-like Adherens Junction and Tight Junction during Epithelial Cell Polarization. Ikenouchi J, Umeda K, Tsukita S, Furuse M, Tsukita S. The 59th Annual Meeting of the Japan Society for Cell Biology 28–30 May 2007, Fukuoka.

Defective Expression of Dystroglycan Causes Abnormal Energy Homeostasis via Ca^{2+} Handling in a *Drosophila* Cryophilic Mutant, *atsugari*. Takeuchi K, Takahara K, Kiyonaka S, Mori Y, Yamamoto D, Umeda M. BMB2007. 11–15 December 2007, Yokohama.

Membrane Phospholipid Flip-flop and Its Role in Cell Migration. Kato U, Inadome H, Umeda M. BMB2007. 11–15 December 2007, Yokohama.

Grants

Umeda M, Development of Two-dimensional Imaging Systems of Membrane Lipids Using Intense Femtosecond Laser Desorption/ionization Mass Spectrometory. Grant-in-Aid for Exploratory Research, 1 April 2006–31 March 2008.

Ikenouchi J, Grant-in-Aid for Scientific Research for JSPS Fellow, 1 April 2007–30 September 2007.

Ikenouchi J, Elucidation of Molecular Mechanisms Which Generate and Maintain Discrete Membrane Domains in Polarized Cells. PRESTO, Japan Science and Technology Agency, 1 October 2007–31 March 2011.

Regulation of Membrane Phospholipid Dynamics and Its Role in Control of Cell Motility

The basic structure of biological membranes is the lipid bilayer in which phospholipids distribute asymmetrically between the two leaflets of the bilayer. Although this asymmetry is regulated by the transbilayer movement of phospholipids, its physiological significance and molecular mechanisms are largely unknown. Previously we have identified a novel membrane protein, designated Ros3p, which is required for the transbilayer movement of phospholipids across the yeast plasma membrane. To investigate its biological functions, we have cloned mROS3, a mammalian homolog of Ros3p. In mammalian cells, mROS3 interacted with P-type ATPase (ATP8A1), a candidate enzyme responsible for the inward movement of aminophospholipids, and is essential for the recruitment of ATP8A1 to the plasma membrane. mROS3 knockdown cells were defective in inward movement of fluorescence-labeled analogs of aminophospholipids across the plasma membrane and exhibit decreased cell motility, while overproduction of mROS3 facilitated the membrane ruffling and cell migration in CHO cells. Cell migration was also inhibited by knockdown of ATP8A1 and the expression of dominant negative ATP8A1 (Figure 1 a). Furthermore, ATP8A1 localized at the leading edge of the serum-stimulated migrating cells and colocalized with actin cytoskleleton. These results suggest that organized movement of phospholipids plays an important role in regulation of cell motility by regulating actin reorganization and membrane ruffling.

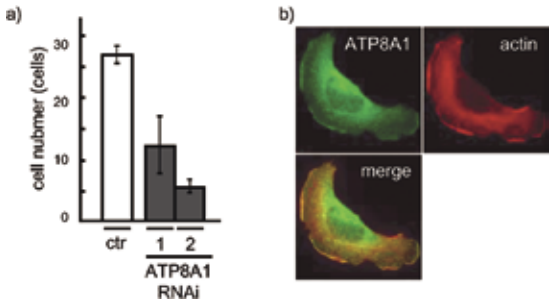

Figure 1. Aminophospholipid translocase ATP8A1 is required for the regulation of cell motility. a) Migration of ATP8A1 knockdown cells was studied by transwell assay and was quantitated by counting cells migrating across the membrane after 3h. b) Colocalization of ATP8A1 and actin cytoskeleton in the serum-stimulated migrating cell.

Elucidation of Molecular Mechanisms which Generate and Maintain Discrete Membrane Domains in Polarized Cells

The plasma membranes of cells are fundamental components of our body. They are composed of discrete membrane domains in which membrane proteins and lipids are differentially partitioned. Compared to plasma membrane proteins which have been investigated by many researchers, plasma membrane lipids are less well understood, even though they are the other main component of membranes. Using epithelial cells as an experimental model, we aim to clarify what kind of lipids and lipid metabolites are enriched in the apical as well as the basolateral membrane, and how these asymmetric membrane domains are maintained to be separated (Figure 2).

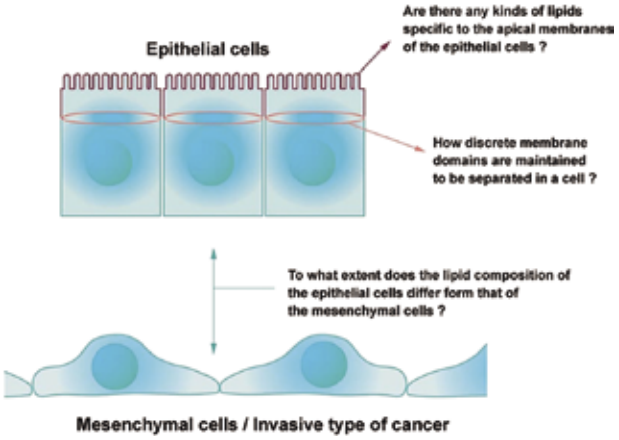

Figure 2.

Generation of the Transgenic Flies Expressing Delta 12 Fatty Acid Desaturase

Polynsaturated fatty acids (PUFAs) play an essential role in the biophysical characteristics of cell membrane. The proportion of PUFAs present in cell membrane, which regulates membrane fluidity and determines the proper function of membrane proteins, has been suggested to contribute to temperature adaptation in poikilothermic organisms. However, the precise mechanisms underlying the effect of PUFAs on thermal traits such as temperature preference and resistance of poikilotherms remain to be understood. To assess this problem, we have made a series of transgenic flies overexpressing fatty acid desaturases, which catalyze the fatty acid desaturation. Many eukaryotic organisms can synthesize dienoic fatty acid, but *Drosophila* can introduce only a single double bond at the delta 9 position. Here we generated the transgenic flies that express *C. elegans* delta 12 fatty acid desaturase, which is involved in biosynthesis of linoleic acid (C18:2). The analyses for fatty acid composition of phospholipids showed the content of linoleic acid was drastically increased in the transgenic flies. The transgenic flies expressing delta 12 fatty acid desaturase will provide important models understanding the molecular mechanisms of temperature preference and resistance of poikilothermic organisms.