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 2006)

Presentations

Regulation of Membrane Phospholipid Dynamics and Its Role in Cell Size Control. Kato U. The 9th Membrane Research Forum. 15–17 March, Kyoto.

Dynamics of Membrane Phospholipids and Its Role in Cytoskeletal Reorganization. Inadome H., Kubo A., Kato U., Umeda M. 28th Symposium on Biomembrane-Drug Interaction. 9–10 November, Shizuoka.

Role of Phospholipid Flip-flop in Cell Polarization. Umeda M. The 46th Annual Meeting of the American Society for Cell Biology. 9–13 December, San Diego, USA.

Grants

Umeda M, Cellular Morphogenesis Based on the Positional Information of Membrane Phospholipids. Grant-in-Aid for Scientific Research (A) (2), 1 April 2003–31 March 2007.

Umeda M, Identification of Genes Involved in Thermoregulatory Behavior of Insects. Special Cooperation Funds for Promoting Science and Technology from the Ministry of Education, Sports, Science and Technology Agency of Japan. 1 April 2002–31 March 2006.

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.

Takeuchi K, Development of a New *Drosophila* Model for Studying Muscular Dystrophy. Grant-in-Aid for Exploratory Research, 1 April 2004–31 March 2007.

Inadome H, Analysis of the Asymmetric Distribution of the Phospholipids in the Golgi Apparatus in Yeast. Grant-in-Aid for Scientific Research for Young Scientists (B), 1 April 2005–31 March 2007.

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 occurred by a protein-mediated process, its physiological significance and molecular mechanisms are largely unknown. To identify the molecules that regulate the movements of membrane phospholipids, we established a series of yeast mutants with disordered organization of membrane phospholipids. By analyzing these mutants, we have identified a novel membrane protein, designated Ros3p, which is required for the transbilayer movement of phospholipids across the yeast plasma membrane. Ros3p is highly conserved in various organisms, implying a general role for cellular functions. To investigate its biological functions, we have cloned mROS3, a mammalian homolog of Ros3p. Overproduction of mROS3 facilitated the membrane ruffling and cell motility in CHO cells, while knockdown of mROS3 expression resulted in the decreased rate of cell migration (Figure 1). Immunoprecipitation and immunocytochemical analysis revealed that mROS3 interacted with P-type ATPase, a candidate enzyme responsible for the inward movement of aminophospholipids. mROS3 knockdown cells caused mislocalization of P-type ATPase and were defective in inward movement of fluorescence-labeled analogs of aminophospholipids across the plasma membrane. These results suggest that one of the cellular functions of mROS3 is serving as an escort protein that is required for the proper localization of P-type ATPase, and that organized movement of phospholipids plays an important role in regulation of cell motility.

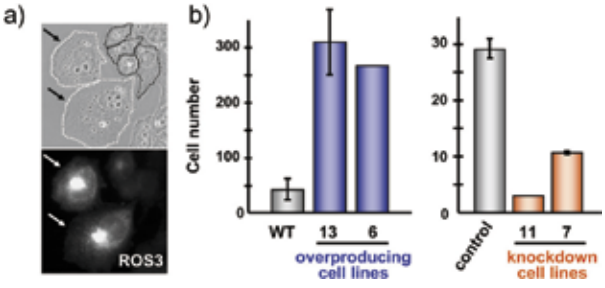

Figure 1. Expression levels of mROS3 protein affected cell morphology and cell motility in CHO cells.
a) Wild CHO cells (a black line) and mROS3 overproducing cells (arrows, a white line).
b) Migration of mROS3 overproducing or knockdown cells was studied by transwell assay and was quantitated by counting cells migrating across the membrane after 3h.

Role for Dystroglycan in Ca^{2+}-mediated Regulation of Mitochondrial Oxidative Phosphorylation and Behavioral Thermoregulation of *Drosophila*

Both ecothermic and endothermic animals move towards thermally comfortable zones, spending most of time at their preferred environmental temperatures. Considerable progress has been made in the identification of molecules involved in the peripheral thermal sensation, but the molecular mechanisms underlying temperature preference remain poorly understood. Here we identify a new *Drosophila* mutant that exhibits a preference for extremely low temperatures, named *atsugari (atu)*. We show that the cryophilic phenotype of the *atu* mutant is caused by the reduced expression of the *Drosophila* orthologue of dystroglycan (DmDG), a membrane glycoprotein that forms the core of dystrophin-glycoprotein complex. The cryophilic phenotype is rescued by ectopic expression of DmDG and is reproduced by the RNA interference-mediated suppression of the DmDG expression in wild-type flies. The reduced expression of DmDG causes sustained increase in the concentration of intracellular Ca^{2+} and activation of pyruvate dehydrogenase, a key enzyme involved in mitochondrial energy metabolism, resulting in a marked increase in metabolic rate and ATP synthesis (Figure 2). The cryophilic phenotype of the *atu* mutant is reversed completely by brief exposure to hyperoxic conditions, suggesting that the insufficient supply of oxygen for the activated mitochondrial oxidative phosphorylation lowers the set point for temperature preference. This study reveals a novel role for dystroglycan in the control of energy homeostasis and behavioral thermoregulation of *Drosophila*, which is critical for the adaptability of ectothermic animals to their respective thermal environments.

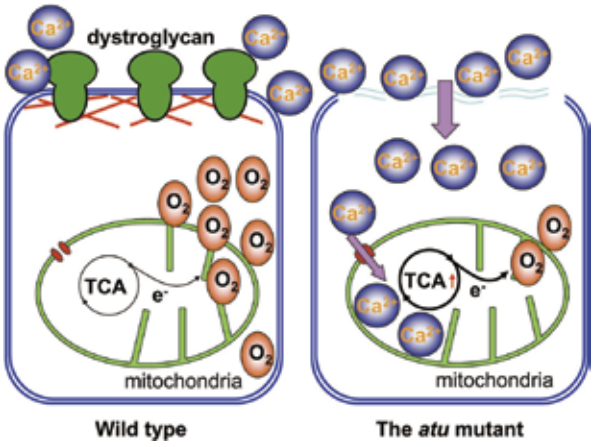

Figure 2. Dystroglycan plays a crucial role in the maintenance of energy homeostasis via intracellular Ca^{2+} handling, which is closely linked to thermoregulation in *Drosophila*.