

# Division of Multidisciplinary Chemistry - Supramolecular Biology -

<http://www.scl.kyoto-u.ac.jp/~umeda/index.htm>



Prof  
UMEDA, Masato  
(D Pharm Sc)



Assist Prof  
TAKEUCHI, Ken-ichi  
(D Pharm Sc)



Assist Prof  
KATO, Utako  
(D Sc)



Res Associate  
INADOME, Hironori  
(D Agr)



PD (JSPS)  
HAMASAKI, Maho  
(D Sc)

## Research Associates (pt)

YAMAGUCHI, Yukiko  
NISHIKAWA, Miwako

## Students

TAKAHARA, Keigo (D1)  
TANIUCHI, Kentaro (M2)

## 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 2004)

### Presentations

Transbilayer lipid movements: A role in cell division and cell polarity formation. Umeda, M. The 3rd Japanese Biochemical Society Biofrontier Symposium. New Aspect of Phospholipid Biology 2004. 10 - 12 May, Kamakura.

Regulation of membrane lipid dynamics in cell polarization. Umeda, M. 45th International Conference on the Bioscience of Lipids. 25 - 29 May, Ioannina Greece.

Role of membrane lipids in regulating the thermoregulatory behavior of *Drosophila*. Umeda, M. Advanced Research Symposium on *Drosophila*. 31 May, Kyoto.

Phylogenetic distribution of sphingomyelin in animals. Umeda, M. Aizu, M. Kobayashi, H. Gordon Research Conference, Glycolipid & Sphingolipid Biology, 25 - 30 July, Harima.

Role of ROS3 proteins, a regulator of membrane phospholipids organization, in cell morphogenesis. Kato U., Murata M., Umeda M. The 77th Annual Meeting of the Japanese Biochemical Society. 13 - 16 October, Yokohama.

Removal of dystroglycan causes locomotor dysfunction

in *Drosophila*. Takeuchi K., Yamamoto D., Umeda M. The 77th Annual Meeting of the Japanese Biochemical Society. 13 - 16 October, Yokohama.

Analysis of expression and function of *Drosophila* ROS3. Taniuchi K., Takeuchi K., Kato U., Umeda M. The 27th Annual Meeting of the Molecular Biology Society of Japan. 8 - 11 December, Kobe.

### 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 2006.

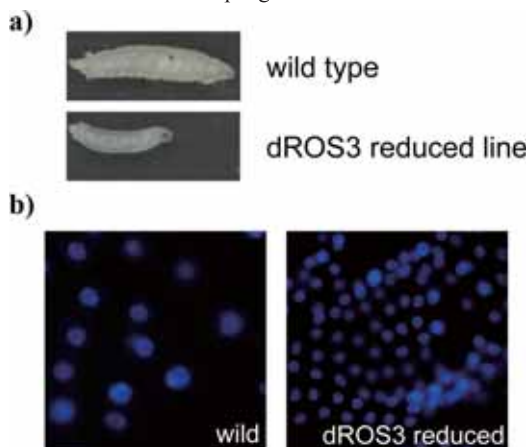
Umeda M, Identification of genes involved in thermoregulatory behavior of insects. Special Cooperation Funds for Promoting Science and Technology from the Ministry of Education, Sports, Science and Technology Agency of Japan. 1 April 2002 - 31 March 2005.

Takeuchi K, Development of a new *Drosophila* model for studying muscular dystrophy. Grant-in-Aid for Exploratory Research, 1 April 2004 - 31 March 2006.

## Regulation of Membrane Phospholipid Dynamics and its Role in Cell Morphogenesis

Biomembranes consist of a lipid bilayer whose major components are phospholipids. Although it is well known that phospholipids are distributed between the inner and outer leaflets of the bilayer membrane, mechanisms underlying the regulation and/or maintenance of this asymmetry are still poorly understood. To investigate the molecular mechanisms, we have isolated a series of yeast mutants with disordered organization of membrane phospholipids. By analyzing the genes defective in these mutants, we have identified a novel membrane protein, Ros3p, required for the translocation of phospholipids across the yeast plasma membrane. Studies using genetic manipulations of the Ros3p expression revealed that Ros3p protein is also involved in the regulation of cell morphogenesis via organizing actin cytoskeleton and cell polarity.

Ros3p is highly conserved among various organisms including worm, fly and mammals. Recently we have cloned a fly Ros3p homolog gene, dROS3, and created loss-of-function flies using RNA-mediated gene interference (RNAi) technique. The reduced expression of dROS3 protein caused marked reduction in body size and lethality at the pupal stage. Further analyses showed that the suppression of dROS3 protein expression also caused remarkable reduction in the size of fat body cells, implying a role of dROS3 in the regulation of cell size (Figure 1). These results suggest that ROS3 family acts as a multifunctional protein regulating both the membrane phospholipid organization and the cell morphogenesis.



**Figure 1.** Suppression of dROS3 expression resulted in decreased cell and body sizes.

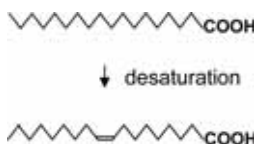
a) Body size of third instar larvae of the wild type and the dROS3 reduced line.

b) Nuclei of fat body cells in the wild type and the dROS3 reduced line were stained with DAPI. Sizes of each cell and nucleus in the dROS3 reduced line are smaller than those in the wild type.

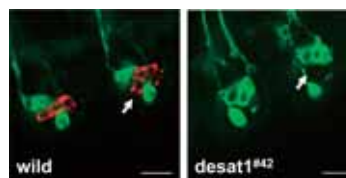
## Stearoyl-CoA Desaturase in *Drosophila*: Thermoregulation and Energy Metabolism

Stearoyl-CoA desaturase is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids. It catalyzes the introduction of the *cis* double bond in the  $\Delta 9$  position of fatty acyl-CoA substrates (Figure 2). The ratio of unsaturated/saturated fatty acids is one of factors influencing cell membrane fluidity, which determines temperature responses of ectothermal animals.

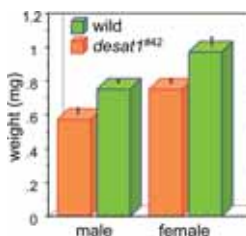
To investigate the action of *desat1*, which is the ortholog of mammalian stearoyl-CoA desaturase in thermoregulation of *Drosophila*, we generated the hypomorphic allele *desat1#42* by mobilizing P element in *desat1* locus. Genetic analyses revealed that *desat1#42* allele lacks both of the third and fourth exons in 5'-flanking region of the *desat1* gene. Immunohistochemical analyses showed that *desat1* was widely expressed in brain, gut, fat body, and oenocyte of the wild-type flies. Although we detected no difference of the expression profile of *desat1* in brain, gut, and fat body between wild type and the *desat1#42* mutant, the expression of *desat1* in oenocyte was severely reduced in the *desat1#42* mutant compared with wild type (Figure 3). The *desat1#42* mutant cultured on a fatty acid-free defined medium also showed a dramatic reduction in body weight and reduced triglyceride storage in fat body (Figure 4). The *desat1#42* mutant will provide a unique model for studying the physiological functions of *desat1* in energy metabolism as well as thermoregulation of *Drosophila*.



**Figure 2.** Oxidative desaturation of fatty acids by stearoyl-CoA desaturase.



**Figure 3.** Patterns of expression of *desat1* in embryonic oenocyte. Oenocyte (arrows) in wild type and the *desat1#42* mutant were immunostained with polyclonal antibodies raised against *desat1* protein (red) and neuron-specific monoclonal antibody (green). Scale bars: 10  $\mu$ m.



**Figure 4.** Body weight of male and female wild-type and the *desat1#42* mutant flies cultured on a fatty-acid-free defined medium.