Molecular Biology and Information
- Biopolymer Structure -

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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 2003)

Presentations

Transbilayer lipid movements: A role in cell division and cell polarity formation. Umeda M. Gordon Research Conference on Molecular and Cellular Biology of Lipids, 20-25 July, Meriden, NH. USA.

Identification of Ros3p, a novel membrane protein required for phospholipid translocation across the plasma membrane in Saccharomyces cerevisiae. Kato U., Emoto K., Umeda M. Gordon Research Conference on Molecular and Cellular Biology of Lipids, 20 - 25 July, Meriden, NH. USA.

Membrane lipid control of Cytokinesis. Umeda M., Emoto K., Kato U. Symposium on A break-through in researches on functional glycerolipids. The 76th Annual Meeting of the Japanese Biochemical Society. 15 - 18 October, Yokohama.

Regulation of membrane phospholipid dynamics and its role in cell polarization. Kato U., Umeda M. Symposium on dynamics of the intracellular molecular networks. The 76th Annual Meeting of the Japanese Biochemical Society. 15 - 18 October, Yokohama.

Lipid Analisis in *Drosophila* cryophilic mutant, *atsugari*, Takeuchi T., Umeda M., *et al*. The 76th Annual Meeting of the Japanese Biochemical Society. 15 - 18 October, Yokohama.

Local change in membrane lipid composition during cytokinesis. Umeda M. The 25th Symposium on Biomembrane-Drug Interaction of the Pharmaceutical Society, 13 - 14 November, Kanazawa.

Development of phospholipid-specific probes and their application to cell biology. Umeda M. Symposium of Japanese Consociation for Phosphatidylserine. 21 November, Tokyo.

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.

Regulation of membrane phospholipid dynamics and its role in cell polarity formation

Polarity formation is important for biological phenomena such as cell morphogenesis, movement, proliferation and organism development. Although recent studies have revealed that cytoskeletal and membrane systems participate in this process, it is unclear how they cooperate with each other. We have identified a novel membrane protein, Ros3p, that is required for the transbilayer movement (flip-flop) of phospholipids across the yeast plasma membrane (Fig.1). Ros3p deficient cells exhibited abnormal morphology (Fig.2b) and disorganized cortical actin patches. Overproduction of Ros3p caused multibudded cells (Fig.2c). These results suggest that Ros3p is involved in both the regulation of phospholipid movement and the actin organization in yeast.

Ros3p is highly conserved among various organisms including worm, fly and mammals. In mammalian cells, overproduction of Ros3p also resulted in multinucleus. Moreover, mouse homolog is localized at the apical side of the epithelial tissues, implying a role of Ros3p in polarized vesicular transport. We propose that Ros3p may function as a coordinator between membrane phospholipids organization and cell polarization via polarized vesicular transport.

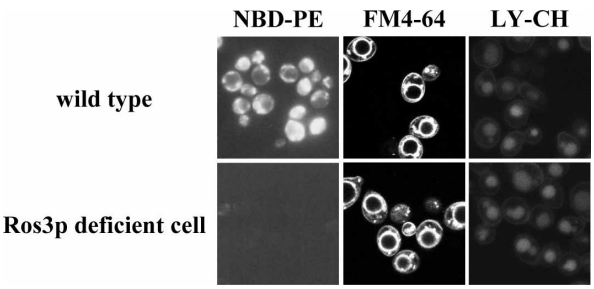

Fig. 1 Internalization of fluorescence-labeled phospholipid and endocytic markers.
In Ros3p deficient cells, the uptake of fluorescence-labeled phosphatidylethanolamine (NBD-PE) was markedly decreased but endocytic markers (FM4-64 and LY-CH ; a lipophilic or soluble dye respectively) were not affected.

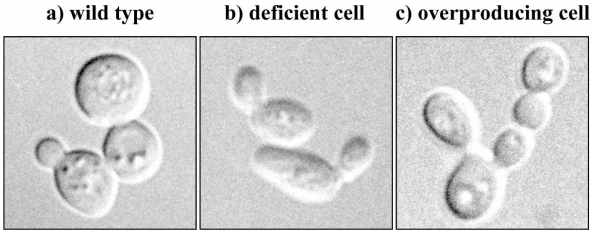

Fig. 2 Abnormal morphology of Ros3p -deficient or -overproducing cells

Defective expression of a *Drosophila* homologue of dystroglycan in a cryophilic mutant, *atsugari*

Behavior plays a major role in thermoregulation in both ectothermic and endothermic species. A wide variety of animals has been shown to move towards and remain preferentially in a thermally comfortable environment. Moreover the preferred temperature differs among species as well as among individuals within a species. Progress has been made in the identification of molecules involved in the sensation of peripheral temperature, but molecular mechanisms underlying temperature preference remain poorly understood. In an effort to identify genes involved in behavioral thermoregulation, we searched for mutations that affect temperature preference of *Drosophila melanogaster*. We identified a new *Drosophila* mutant with a preference for unusually low temperatures and we designated the mutant *atsugari* (Fig. 3). We found that the atsugari phenotype was caused by the reduced expression of the *Drosophila* homologue of dystroglycan (DmDG; Fig 4), a membrane glycoprotein that forms the core of the dystrophin-glycoprotein complex. Reduced expression of DmDG induced a significant increase in the fluidity of membrane lipids that was associated with the cryophilic phenotype. Our studies demonstrate a novel role for dystroglycan in thermoregulation of *Drosophila*. The *atsugari* mutant provides a unique model for studies of the physiological functions of dystroglycan.

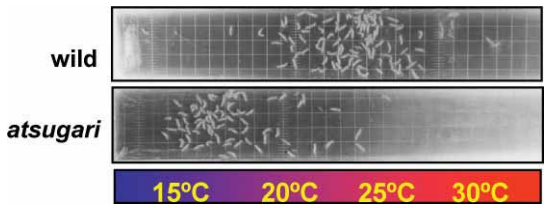

Fig. 3 Representative results of temperature-preference assays.
Third-instar wandering larvae were placed on a linear thermal gradient and the distribution of the larvae after 20 min of wandering was recorded.

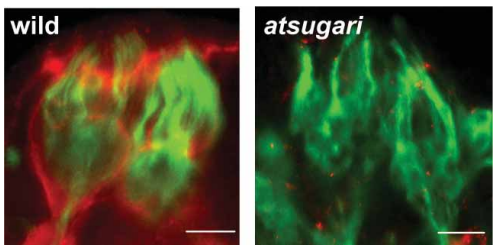

Fig. 4 Patterns of expression of DmDG in embryonic neural tissues.
Sensory organs in the wild type and the *atsugari* mutant were immunostained with DmDG-spscific antibodies (red) and neuron-specific monoclonal antibody (green). Scale bars: 10 mm