2.3.13 Laboratory: Molecular Microbial Science

Associate Professor	Kurihara, Tatsuo, Dr. Eng.
Assistant Professor	Kawamoto, Jun, Dr. Agric. Sci.
Doctor's program	8
Master's Program	8
Other	1
Post-Doctoral fellow	2
Researcher	1
	Associate Professor Assistant Professor Doctor's program Master's Program Other Post-Doctoral fellow Researcher

A. Research Activities (2009.4-2010.3)

A-1. Main Subjects

a) Physiological role of eicosapentaenoic acid-containing phospholipids in the folding of a cold-inducible porin

Shewanella livingstonensis Ac10 isolated from Antarctic seawater is a psychrotrophic bacterium, which can grow at low temperatures close to 0°C. The bacterium produces eicosapentaenoic acid (EPA), a polyunsaturated fatty acid, as a component of membrane phospholipids at 4°C. EPA is found in fish oils and has various physiological effects on membrane properties. OmpC74, an outer membrane porin inducibly produced at low temperatures, has two different conformations in the wild-type cells grown at 4°C, but not in the EPA-less mutant. In order to analyze the interaction between OmpC74 and EPA, we performed in vitro reconstitution experiments with chemically synthesized phospholipids containing EPA. We found that OmpC74 was more rapidly inserted into the EPA-containing liposomes than into the liposomes not containing EPA. We also found that the conformational change in its C-terminal region was dependent on the presence of EPA-containing phospholipids.

b) Analysis of membrane dynamics of eicosapentaenoic acid-containing phospholipids with synthetic probes

Eicosapentaenoic acid (EPA) is a polyunsaturated fatty acid that acts as a precursor for various bioacitve substances, such as prostaglandin and thromboxane. Shewanella livingstonensis Ac10, a cold-adapted bacterium, inducibly produces EPA-containing phospholipids as a membrane component at low temperatures. When the EPA synthesis

genes were disrupted, growth retardation, formation of filamentous cells, and formation of abnormal intracellular membranes were observed at 4°C, but not at 18°C. These results suggest that EPA has specific effects on membrane proteins for their normal function at low temperatures. To elucidate the membrane dynamics of EPA, we synthesized ether phospholipids containing EPA and a fluorescent group and added this probe to the EPA-less mutant. As a result, fluorescent signals were localized in the mid-region of the cells, suggesting that EPA-containing phospholipids are localized at the cell division site of this bacterium and interact with cell-division proteins.

A-2.Publications and presentations

a) Publications

<u>Books</u>

- Kurihara, T., H. Mihara, and N. Esaki: Mechanistic analysis of selenium incorporation into proteins and its application. Metal-biotechnology for Environmental Conservation and Resource Recycling. 241-246, 2009

Original Papers

Omi, R., S. Kurokawa, H. Mihara, H. Hayashi, M. Goto, I. Miyahara, T. Kurihara, K. Hirotsu, and N. Esaki: Reaction mechanism and molecular basis for selenium/sulfur discrimination of selenocysteine lyase. J. Biol. Chem. 285; 12133-12139, 2010
Toyoda, M., K. Jitsumori, B. Mikami, L. P.Wackett, T. Kurihara, and N. Esaki: Crystallization and preliminary X-ray analysis of L-azetidine-2-carboxylate hydrolase from Pseudomonas sp. strain A2C. Acta Crystallogr Sect F Struct Biol Cryst Commun in press, 2010

- Zhang, W., A. Urban, H. Mihara, S. Leimku"hler, T. Kurihara, and N. Esaki: IscS functions as a primary sulfur-donating enzyme by interacting specifically with MoeB and MoaD in the biosynthesis of molybdopterin in Escherichia coli. J. Biol. Chem. 285; 2302-2308, 2010

- Goto, M., T. Yamauchi, N. Kamiya, I. Miyahara, T. Yoshimura, H. Mihara, T. Kurihara, K. Hirotsu, and N. Esaki: Crystal structure of a homolog of mammalian serine racemase from Schizosaccharomyces pombe. J. Biol. Chem. 284; 25944-25952, 2009

- Imai, T., H. Mihara, T. Kurihara, and N. Esaki: Selenocysteine is selectively taken up by red blood cells. Biosci Biotechnol Biochem. 73; 2746-2748, 2009

- Imai, T., H. Mihara, T. Kurihara, and N. Esaki: Possible role of red blood cells in selenocysteine metabolism. Trace Nutri. Res. 26; 22-25, 2009

- Jitsumori, K., R. Omi, T. Kurihara, H. Mihara, I. Miyahara, K. Hirotsu, and N. Esaki: X-ray crystallographic and mutational studies of fluoroacetate dehalogenase from Burkholderia sp. FA1. J. Bacteriol. 191; 2630-2637, 2009

- Kamachi, T., T. Nakayama, O. Shitamichi, K. Jitsumori, T. Kurihara, N. Esaki, and K. Yoshizawa: The catalytic mechanism of fluoroacetate dehalogenase: A computational exploration of biological dehalogenation. Chemistry 15; 7394-7403, 2009
- Kawamoto, J., T. Kurihara, K. Yamamoto, M. Nagayasu, Y. Tani, H. Mihara, M. Hosokawa, T. Baba, S. B. Sato, and N. Esaki: Eicosapentaenoic acid plays a beneficial

role in membrane organization and cell division of a cold-adapted bacterium,

Shewanella livingstonensis Ac10. J. Bacteriol. 191; 632-640, 2009

Nakamura, T., A. Yamaguchi, H. Kondo, H. Watanabe, T. Kurihara, N. Esaki, S.
 Hirono, and S. Tanaka: Roles of K151 and D180 in L-2-haloacid dehalogenase from
 Pseudomonas sp. YL: Analysis by molecular dynamics and ab initio fragment
 molecular orbital calculations. J. Comput. Chem. 30; 2625-2634, 2009

- Omori, T., H. Mihara, T. Kurihara, and N. Esaki: Occurrence of phosphatidyl-D-serine in the rat cerebrum. Biochem. Biophys. Res. Commun. 382; 415-418, 2009

- Tobe, R., H. Mihara, T. Kurihara, and N. Esaki: Identification of proteins interacting with selenocysteine lyase. Biosci. Biotechnol. Biochem. 73; 1230-1232, 2009

- Yamauchi, T., M. Goto, H.-Y. Wu, T. Uo, T. Yoshimura, H. Mihara, T. Kurihara, I. Miyahara, K. Hirotsu, and N. Esaki: Serine racemase with catalytically active lysinoalanyl residue. J. Biochem. 145; 421-424, 2009

Reviews

- Kurihara, T., J. Kawamoto, and N. Esaki: Biosynthesis and function of long-chain polyunsaturated fatty acids in cold-adapted bacteria. Tanpakushitsu Kakusan Koso (Protein, Nucleic Acid and Enzyme). 55; 94-99, 2010

- Kurihara, T: Function of phospholipids containing polyunsaturated fatty acids in cold-adapted bacteria. Seikagaku. 81; 716-719, 2009

- Kurihara, T: Production of a chiral organohalogen compound by using a novel enzyme catalyzing asymmetric reduction. Bioscience & Industry. 67; 161-163, 2009

- Kurihara, T., J. Kawamoto, and N. Esaki: Proteins and phospholipids involved in cold-adaptation of psychrotrophic bacteria. Seikagaku. 81; 1072-1079, 2009

- Kurihara, T., and N. Esaki: Enzymes that function at low temperatures. Kagakukogaku (Chemical Engineering of Japan). 73; 324-327, 2009

b) Conference and seminar papers presented

- The 20th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements: 1 Presentation

- The 61th Annual Meeting of the Society for Biotechnology, Japan 2008: 1 Presentation

- The Fifth International Conference on Iron-Sulfur Cluster Biogenesis and Regulation :1 Presentation

- The 82nd Annual Meeting of the Japanese Biochemical Society: 6 Presentations

- The 10th Annual Meeting of the Japanese Society for Extremophiles: 1 Presentation

- The 61th Annual Meeting of the Vitamin Society of Japan: 1 Presentation

- Xth SCAR International Biology Symposium: 2 Presentations

- NAIST Global COE International Symposium 2009 Environmental Adaptation: 1 Presentation

- Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2010: 5 Presentations

A-3.Off-campus activities

Membership in academic societies

 Kurihara, Tatsuo, Dr. Eng. : The Society for Biotechnology, Japan (Editorial Board, Vice-Chairperson), The Japanese Biochemical Society (Kinki Branch Councilor, Secretary, Educational Committee for Biochemistry)

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Scientific Research (B) : Kurihara, Tatsuo, Dr. Eng. : Molecular Basis of Cold Adaptation of Psychrotrophic Bacteria

- Scientific Research (B) : Kawamoto, Jun, Dr. Agric. Sci. : Development of a System for the Bioremediation of Rare Metal Pollution and Rare Metal Recovery Using Novel Metal-Metabolizing Bacteria

2. Other Research Grants

- A Grant for Research for Promoting Technological Seeds from JST: Kurihara, Tatsuo, Dr. Eng.: Development of New Enzymes That Catalyze the Conversion of Organohalogen Compounds for Environmental Remediation and Production of Useful Chemicals

- A Grant for Research for Promoting Technological Seeds from JST: Kawamoto, Jun, Dr.

Agric. Sci.: Development of a Protein Production System at Low Temperatures

B.Educational Activities(2009.4-2010.3)

B-1.On-campus teaching

a) Courses given

- Graduate level: Molecular Microbial Science Seminar (Kurihara and Kawamoto), Experimental Course of Molecular Microbial Science (Kurihara and Kawamoto), Molecular Microbial Science (Kurihara)

B-2.Off-campus teaching etc.

Part-time lecturer

- Kurihara, Tatsuo, Dr. Eng.: Nara Women's University, Faculty of Science(Biochemistry)

B-3.Overseas teaching

International students

- International students : Master 1 (China) Doctral 5 (Egypt 1, China 2, Korea 1, India 1) Research Students 1 (China)