2.3.6 Laboratory : Laboratory of Molecular Microbiology

Member :	Professor	Kita, Keiko, Dr. Agric. Sci.
	Associate Professor	Inoue, Yoshiharu, Dr. Agric. Sci.
	Assistant Professor	Arita, Kyohei, Dr. Sci.
	Doctor's program	2
	Master's Program	5
	Undergraduate	3

A. Research Activities (2009.4-2010.3)

A-1. Main Subjects

a) Structural anaylsis of proteins involved in restriction-modification system

We aim to determine the crystal structure of proteins involved in restriction-modification system to understand molecular mechanism of DNA recognition. Crystals of EcoT38I with cognate DNA were successfully obtained by hanging-drop vapor diffusion method. Crystals of EcoO109I mutant enzyme were successfully obtained by hanging-drop vapor diffusion method. Despite the presence of the cognate DNA in the cyrstallization mixture, no electron density for DNA was observed in the crystal. The cyrstal structure of EcoO109I mutant enzyme revealed one protein chain in the asymmetric unit and the overall structure closely resembles to wild type.

b) Regulatory mechanism of the expression of GPX1 in yeast

Saccharomyces cerevisiae has three homologues of the glutathione peroxidase gene, GPX1, GPX2, and GPX3. We found that the expression of GPX1 is induced by glucose starvation and treatment with CaCl2. The induction of GPX1 expression in response to glucose starvation and Ca2+ was dependent on the transcription factors Msn2 and Msn4 and cis-acting elements [stress response element (STRE)] in the GPX1 promoter. The Ras/cAMP pathway is also involved in the expression of GPX1. We found that Snf1, a Ser/Thr protein kinase, is involved in the glucose starvation- and Ca2+-induced expression of GPX1. The activation of Snf1 is accompanied by phosphorylation of Thr210. We found that the Ca2+-treatment as well as glucose starvation causes the phosphorylation of Thr210 of Snf1 in a Tos3, Sak1, and Elm1 protein kinase-dependent manner.

c) Structure biology of epigenetics related factors.

Histone modifications and DNA methylation, epigenetics, regulate the wide range of chromosomal processes in mammals. The patterns of these epigenetic traits modulate chromatin architecture and consequently determine a gene expression pattern. The protein UHRF1 possesses histone binding motifs, a Tudor domain and a PHD finger, followed by the SRA domain responsible for a hemi-methylated DNA binding. Thus, UHRF1 is thought to be a key molecule to link histone modification and DNA methlylation status. We detemined crystal structure of the UHRF1 PHD finger in complex with the N-terminal tail of histone H3. Combined with biochemical data (ITC), the crystal structure has revealed that, unlikely to other PHD fingers, the UHRF1 PHD finger strictly recognizes the modification status of Arg2 rather than Lys4 in H3. Furthermore, biochemical study showed that Tudor-PHD domain (the Tudor domain linked to the PHD finger) recognizes multivalent modification status in the histone H3 tail.

A-2.Publications and presentations

a) Publications

Books

- Keiko Kita: Outline of Applied Enzymology, Koronasha, Tokyo, 2009 (Japanese)

Original Papers

- Lushchak, O. V., N. Z. Nykorak, T. Ohdate, Y. Inoue and V. I. Lushchak: Inactivation of genes encoding superoxide dismutase modifies yeast response to S-nitrosoglutathione-induced stress. Biochemistry (Mosc) 74(4); 445-451, 2009
- Sekiyama N., K. Arita, Y. Ikeda, K. Hashiguchi, M. Ariyoshi, H. Tochio, H. Saitoh and M. Shirakawa: Structural basis fir regulation of poky-SIMO chain by SUMO-like domain of Nip45 Proteins 6; 1491-1502, 2009
- Otani, J., T. Nankumo T, K. Arita, S. Inamoto, M. Ariyoshi and M. Shirakawa: Structural basis for recognition of H3K4 methylation status by the DNMT3A ADD domain. EMBO Rep. 11; 1235-1241, 2009

Reviews

- Izawa, S., and Y. Inoue: Post-transcriptional regulation of gene expression in yeast under ethanol stress. Biotechnol Appl Biochem 53(2); 93-99, 2009

Patents

- Breeding method for yeast strain containing high amount of thioredoxin. Patent number: 4412658. November 27, 2009

b) Conference and seminar papers presented

- The 42nd Meeting of Yeast Genetics and Molecular Biology, Japan: 2 Presentations
- Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2010: 5 Presentations
- The 82nd Meeting of the Japanese Biochemical Society: 1 Presentation
- The 32nd Meeting of the Molecular Biology Society of Japan: 4 Presentation

A-3.Off-campus activities

Membership in academic societies

- Kita, Keiko, Dr. Agric. Sci. : Japan Society for Biosciences, Biotechnology, and Agrochemistry (Director), The Society for Biotechnology, Japan (Coucilor), Japan Bioindustry Association (Editor)
- Inoue, Yoshiharu, Dr. Agric. Sci. : Yeast Society (Committee Member), The Society for Biotechnology, Japan (Committee Member of Kansai Branch)

Membership in Science Council of Japan, etc.

- Inoue, Yoshiharu, Dr. Agric. Sci. : Committee on Redox Life Science, Japan Society for the Promotion of Science (Member)

Research grants

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

- Scientific Research (B) : Yoshiharu Inoue : Metabolic signaling: physiological role and mechanism of signal transduction by glycolytic intermediate.
- Young Scientists（Start-up） : Kyohei Arita : Structure biology of the epigenetics related super macro molecules.

2.Other Research Grants

- Kyoto University Grant-in Aid for Young Scientists (step-up)：Kyohei Arita：Structure biology of the epigenetics related proteins.

B.Educational Activities(2009.4-2010.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level： Introduction of Applied Life Sciences I (Kita), Introduction to Applied Life Sciences II (Kita), Outline of Agricultural Sciences II

(Kita), Applied Life Sciences (Kita), Applied Microbiology II (Kita), Laboratory Course in Biochemistry (Inoue and Arita), Introduction to Applied Life Sciences III (Inoue)

- Graduate level: Cellular Bioenergy Conversion Seminar (Kita, Inoue, and Arita), Experimental Course of Cellular Bioenergy Conversion (Kita, Inoue, and Arita)

B-2.Off-campus teaching etc.

Part-time lecturer

- Inoue, Yoshiharu: University of Shiga Prefecture, Graduate School of Technology, Biogenic and Biofunctional Chemistry (Advanced course)

B-3.Overseas teaching

International students

- International students : Master 1 (China)

C.Other Remarks

- Kita, Keiko: Advisory Committee of Osaka City for Evaluation of Independent Administrative Organization (Member)