

## Chair                      Molecular and Cellular Sciences

### 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	1
	Master's Program	6
	Undergraduate	3
	Other	1
	Researcher	1

#### **A. Research Activities (2010.4-2011.3)**

##### **A-1. Main Subjects**

a) Structures and mechanisms of restriction-modification enzymes from *E. coli*

EcoO109I methyltransferase from *E. coli* H709c recognizes RGGNCCY and transfers a methyl group to the inner cytosines. To reveal the mechanism of substrate recognition, the DNA-free and DNA-bound forms of the enzyme were successfully crystallized and X-ray diffraction data were collected. EcoT38I restriction endonuclease from *E. coli* TH38 recognizes and cleaves GRGCY ↓ C. X-ray diffraction study of the DNA-bound form of the enzyme revealed that the two active sites face away from each other; one site is located at the scissile phosphate moiety while the other site does not contact the DNA. The structure implies that the enzyme forms a dimer and cleaves both DNA strands asymmetrically and sequentially.

b) Ca<sup>2+</sup>/calcineurin-induced degradation of Msn2/Msn4 transcription factor in the nucleus in yeast

Msn2 and Msn4 are C2H2-type zinc finger transcription factors in yeast. We found that Msn2 and Msn4 are concentrated in the nucleus following treatment with Ca<sup>2+</sup>. Intriguingly, the Ca<sup>2+</sup>-induced expression of Msn2/Msn4's target gene was enhanced in the presence of FK506, a potent inhibitor of calcineurin. Consequently, the Ca<sup>2+</sup>-induced expression of Msn2/Msn4's target gene in a mutant that is defective in calcineurin or Crz1, the sole transcription factor downstream of calcineurin, was much greater than that in the wild-type strain even without FK506. This phenomenon was dependent upon a cis-element, the STRE (stress-response element), in the promoter that is able to mediate the response to Ca<sup>2+</sup> signaling together with Hog1 p38 MAP kinase and Msn2/Msn4. We also found that the levels of Msn2 and Msn4 proteins in Ca<sup>2+</sup>-treated cells decreased, and that FK506 blocked the degradation of Msn2/Msn4. We propose that Crz1 destabilizes Msn2/Msn4 in the nuclei of cells in response to Ca<sup>2+</sup> signaling.

#### c) Structural and biochemical analysis of phosphorylated UHRF1.

UHRF1 is an essential factor for the recognition and inheritance of an epigenetic information. We found that the phosphorylation of inter module linker switches the function and structure of UHRF1. UHRF1 has two histone reader modules, which cooperatively recognize the histone H3 containing tri-methylated K9 (H3K9me3). Our ITC and NMR studies showed that the phosphorylation of the linker region between the reader modules caused the disruption of the specific recognition of the H3K9me3. Furthermore, this phosphorylation likely altered the sub-cellular localization of UHRF1.

## A-2.Publications and presentations

### a) Publications

#### Original Papers(including book-reviews)

- Ohdate, T., S. Izawa, K. Kita and Y. Inoue: Regulatory mechanism for expression of GPX1 in response to glucose starvation and Ca<sup>2+</sup> in *Saccharomyces cerevisiae*: Involvement of Snf1 and Ras/cAMP pathway in Ca<sup>2+</sup> signaling. *Genes Cells* 15, 59-75, 2010

- Takatsume, Y., T. Ohdate, K. Maeta, W. Nomura, S. Izawa and Y. Inoue: Calcineurin/Crz1 destabilizes Msn2 and Msn4 in the nucleus in response to Ca<sup>2+</sup> in *Saccharomyces cerevisiae*. *Biochem. J.* 427, 275-287, 2010

- Nomura, W., K. Maeta, K. Kita, S. Izawa and Y. Inoue: Methylglyoxal activates Gcn2 to phosphorylate eIF2 $\alpha$  independently of the TOR pathway in *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 86, 1887-1894, 2010

- Ohdate, T., K. Kita and Y. Inoue: Kinetics and redox regulation of Gpx1, an atypical 2-Cys peroxiredoxin, in *Saccharomyces cerevisiae*. FEMS Yeast Res. 10, 787-790, 2010
  
- Lushchak, O. V., Y. Inoue and V. I. Lushchak: Regulatory protein Yap1 is involved in response of yeast *Saccharomyces cerevisiae* to nitrosative stress. Biochemistry (Mosc). 75, 629-635, 2010
  
- Izawa, S., K. Ikeda, T. Miki, Y. Wakai and Y. Inoue: Vacuolar morphology of *Saccharomyces cerevisiae* during the process of wine making and Japanese sake brewing. Appl. Microbiol. Biotechnol. 88, 277-282, 2010
  
- Sekiyama N., Arita K., Ikeda Y., Hashiguchi K., Ariyoshi M., Tochio H., Saitoh H. and Shirakawa M. Structural basis for regulation of poky-SIMO chain by SUMO-like domain of Nip45. Proteins 78, 1491-1502, 2010
  
- Iwamoto M., Hishiki A., Shimada T., Imasaki T., Tsuda J., Kita K., Shimizu T., Sato M., and Hashimoto H.: Crystallization and X-ray diffraction studies of DNA-free and DNA-bound forms of EcoO109I DNA methyltransferase. Acta Cryst. F66, 1528-1530, 2010

#### b) Conference and seminar papers presented

- The 43rd Meeting of Yeast Genetics and Molecular Biology, Japan: 1 Presentation
  
- Joint Meeting of The 83rd Meeting of the Japanese Biochemical Society and The 33rd Meeting of the Molecular Biology Society of Japan: 6 Presentation
  
- Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2011:3 Presentations
  
- The 4th Annual Meeting of The Japan Society for Epigenetics: 1 Presentation

- 6th NEB Meeting of DNA Restriction and Modification: 2 Presentations

### **A-3.Off-campus activities 1**

#### Membership in academic societies

- Kita, Keiko, Dr. Agric. Sci. : Japan Society for Bioscience, Biotechnology, and Agrochemistry (Director), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Council Member of Kansai Branch), The Society for Biotechnology, Japan (Council Member)

- 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 Innovation, Japan Society for the Promotion of Science (Member)

### **A-3.Off-campus activities 2**

#### Research grants

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

- Grant-in-Aid for Scientific Research (B) : Yoshiharu Inoue : Metabolic signaling: physiological role and mechanism of signal transduction by glycolytic intermediate.

- Grant-in-Aid for Scientific Research on Innovative Areas : Kyohei Arita : Structural and functional analysis of phosphorylated UHRF1.

- Grant-in-Aid for Young Scientists (A) : Kyohei Arita : Structural basis for the regulation of epigenetics by protein interactions.

## **B.Educational Activities(2010.4-2011.3)**

### **B-1.On-campus teaching**

a) Courses given

- Undergraduate level:      Applied Life Sciences (Kita), Introduction to Applied Life Sciences II (Kita), Introduction to Applied Life Sciences III (Inoue), Applied Microbiology I (Inoue), Applied Microbiology II (Kita), Applied Microbiology III (Inoue), Applied Microbiology IV (Kita), Laboratory Course in Biochemistry (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 1**

#### International students

- International students :    Master    1    (China)    Research Students    1    (China)

## **C.Other Remarks**

- Kita, Keiko : Evaluation Committee of Incorporated Administrative Agency of Osaka City (Committee member)