2010 KAIST - Osaka University Graduate Students Symposium In Biotechnology

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2010 KAIST - Osaka University Graduate Students Symposium

July 22nd 15:30 ~ 18:40

Opening remark (Prof. Kanaya)	15:30 ~ 15:35
Chair person : Jin Hyun Kim	15:35 ~ 15:55
1. Yoshihiro Ida	Construction of adh deletion strains of *Saccharomyces cerevisiae* for industrial production
2. Hiroshi Sawada	Analysis of cellular adhesion between lactic acid bacteria and yeasts
Chair person : Keisuke Tomiyama	15:55 ~ 16:15
3. Young Dok Son	Improvement of Recombinant EPO Sialylation in Chinese Hamster Ovary Cells by Combinatorial Genetic Engineering
4. Sun Young Park	Selection of a single cell producing a glycoprotein with desired glycosylation by using a microwell array
Chair person : MoonHyeong Seo	16:15 ~ 16:35
5. Yasumune Nakayama	Turnover analysis of Central metabolism by time course of stable isotope dilution rate
6. Hiroshi Tsugawa	Development of Useful Identification and Annotation Tool for Nontargeted GC/MS Based Metabolomics
	Break 16:35 ~ 16:45
Chair person : Kotaro Iwami	16:45 ~ 17:05
7. Changmin Koh	TCTP and its genetic interaction with wingless signaling pathway components
8. Taehyeung Kim	Discovery of novel genes associated with rheumatoid arthritis susceptibility in a Korean population
Chair person : YONG KEOL SHIN	17:05 ~ 17:25
9. Shinya Yamamoto	Development of a nano HPLC-MS/MS method for the specific and sensitive determination of bioactive amines
10. Takatoshi Wakabayashi	Study of oligosaccharide-degrading enzyme Involved in parasitic weed germination
Chair person : Momoko Mune	17:25 ~ 17:45
11. Cheolam Hong	Gel-siRNA for highly enhanced gene silencing
12. Jeong Yu Lee	Paclitaxel-encapsulated albumin for cancer therapy
	Break 17:45 ~ 17:55
Chair person : Seung-Yeol Park	17:55 ~ 18:15
13. Tomohiro Ohmura	Application of live cell imaging to plant cell nuclei
14. Ryuto Nakao	Aggregation of biopharmaceutical antibodies
Chair person : Yoshihiro Ida	18:15 ~ 18:35
15. Jinseok Seo	Effect of Protein Disulfide Isomerase under hypothermic condition
16. Nury Kim	Expression profiles of miRNAs in human embryonic stem cells during differentiation into hepatocytes
Chair person : Namsuk Kim	18:35 ~ 18:55
17. Ken-ichiro Haruna	Clarification of autoregulator-signaling cascades controling secondary metabolism in *Streptomyces avermitilis* - AvaL-cascade -
18. Aiko Morisugi	Activation of secondary metabolism in Entomopathogenic fungi by using genetic engineering

July 23rd 8:30 ~ 11:55

Chair person : Hiroshi Sawada	8:30 ~ 8:50
19. Hyung Gwan Lee	Screening and Cloning of novel Ginsenoside transforming B-glucosidase Gene
20. Da Jung Kim	Development of a novel antimicrobial peptide as a topical agent in skin wound care
Chair person : Young Dok Son	8:50 ~ 9:10
21. Kotaro Iwami	Development of PCR-mediated duplication method for defined chromosomal regions in budding yeast
22. Momoko Mune	Molecular breeding of lactic acid tolerant yeast - Mechanism of tolerance to lactic acid conferred by overexpression of ESBP6 gene in *Saccharomyces cerevisiae* -
Chair person : Yasumune Nakayama	9:10 ~ 9:30
23. Jin Hyun Kim	A genetic circuit system based on Quorum sensing signaling for directed evolution of Quorum-quenching enzymes
24. MoonHyeong Seo	Site-specific labeling of protein using unnatural amino acid for single-molecule FRET analysis
	Break 9:30 ~ 9:40
Chair person : Sun Young Park	9:40 ~ 10:00
25. Aiko Hibino	The whole-cell biocatalysis with hydrophobic bacterium *Rhodococcus Opacus* B-4
26. Lee Kyoung ho	Establishment of Novel Gene amplification platform in CHO cell line for biopharmaceutical production
Chair person : Hiroshi Tsugawa	10:00 ~ 10:20
27. SOOHEE RYU	Epigenetics in Aging : Histone hypoacetylation at repetitive DNA elements represses repeat-associate transcripts in aged mice brain
28. Sungsu Lim	Proteasome mediates mRNA export through SAGA/TREX-2 complex
Chair person : NURY Kim	10:20 ~ 10:30
29. Keisuke Tomiyama	Single-cell imaging of intracellular ATP concentration inside *E.coli*
	Break 10:30 ~ 10:40
Chair person : Tomohiro Ohmura	10:40 ~ 11:00
30. YONG KEOL SHIN	Mus81 regulates acetyltrasnferase activity of Tip60
31. Seung-Yeol Park	Aberrant glycosylation of β-Haptoglobin in sera of colon cancer
Chair person : Hyung Gwan Lee	11:00 ~ 11:20
32. Ryo Uehara	Tk-subtilisin stabilization mechanism during maturation
33. Koji Yokoyama	Study on ribonuclease HI from *Halobacterium salinarum* NRC-1
Chair person : Ryuto Nakao	11:20 ~ 11:50
34. Hee Won Yang	Visualizing dynamic interaction between calmodulin and calmodulin-related kinases
35. Namsuk Kim	Intercellular transfer of Vax1 transcription factor
36. Hyejung Won	Selected SALM(Synaptic Adhesion-Like Molecule) family proteins regulate synapse formation
Closing remark (Prof. Kim)	11:50 ~ 11:55

Construction and Analysis of alcohol dehydrogenase-negative yeast based on genome scale metabolic model

Yoshihiro Ida

Graduate School of Information Science and Technology, Osaka University

The production of useful chemicals by using microorganism is of special interest from viewpoint of post-petrochemistry. The purpose of this study is establishment of method to design metabolism for bioproduction. For this purpose, our research use computer model. The model used in our research is called genome scale metabolic model which is constructed based on whole genome data.

The immediate objective of this study is to verify effectiveness of model based metabolic design. First of all, we tried to construct a strain to produce glycerol as a case study. Model simulation estimates that alcohol dehydrogenase (ADH) deletion is effective to make yeast produce glycerol. Then I made *adh1* deletion strain and confirmed this single gene deletion strain produce a large amount of glycerol (Result1). In presentation, I show comparison between computer simulation and experimental result. Next, we checked a role of adh isozymes. Alcohol dehydrogenases have more than 6 isozymes, *adh1*, *adh2, adh3, adh4, adh5* and *sfa1*. We sat up a hypothesis that ethanol production of *adh1* deletion strain would recover by using isozymes. We did the following experiments to confirm this hypothesis. First, we cultivate mutants in batch cultivation, when it entered the stationary phase, cultivation was shifted to continuous culture.

Analysis of cellular adhesion between lactic acid bacteria and yeasts

Hiroshi Sawada

Department of Biotechnology, Graduate School of Engineering, Osaka University

Lactic acid bacteria (LAB) and yeasts coexist and help each other in many fermented foods. Cheirsilp *et al*. [1)] reported that the production of a exopolysaccharide (EPS) by *Lactobacillus kefiranofaciens* JCM6985 was promoted in a co-culture with lactate-assimilating yeast *Saccharomyces cerevisiae* IFO0216, concluding that the contact interaction between them is an important factor for stimulation of the production. This led to a question "What is it in the LAB that recognizes the yeast?" For this question, we clarified previously that *Lactcoccus lactis* IL1403 displays cytosolic proteins such as DnaK, GroEL and GAPDH, which cause the LAB to adhere to the yeast.[2)] This finding made further question "How is the signal transduced?". Thus, our current study approaches transcriptional responses of *Lactobacillus casei* ATCC334 to physical contact with yeast. A DNA microarray analysis revealed that addition of heat-inactivated yeast or yeast mannan upregulated the genes of the LAB concerning biosynthesis of EPS such as glycosyltransferase, whereas functions are unknown for the half of the responded genes.

It is important to analyze responses of microorganisms caused by adhesion to heterogonous cells, whereas most of studies have been on the basis of mono-culture. Our model study about adhesion of LAB to yeast will provide useful knowledge on formation of biofilm, gastric colonization and microbial infections.

1) B. Cheirsilp *et al*.: Interactions between *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae* in mixed culture of kefiran production., J. Biosci. Bioeng. **96**, 279-284 (2003)

Improvement of recombinant EPO sialylation in Chinese hamster ovary cells by combinatorial genetic engineering.

Young-Dok Son

Department of Biological Sciences, KAIST

The terminal sialylation of *N*-linked glycan is most important factor to determine the *in vivo* clearance rate of therapeutic glycoproteins. In this study, we introduced the sialuria-mutated rat GNE/MNK to increase the intracellular CMP-sialic acid by genetic engineering. From *in vitro* activity assays of various sialuria-mutated rat UDP-GlcNAc 2-epimerases, rat GNE/MNK-R263L-R266Q mutant sustained the highest activity among the five mutants, and feedback inhibition was not detected. To improve the sialylatioin pathway of CHO cells, Chinese hamster CMP-SAT, and human α2,3-ST were transfected simultaneously with sialuria-mutated rat GNE/MNK into the rhEPO-producing CHO cells. The intracellular CMP-sialic acid pool of engineered CHO cells was significantly increased up to 10.7 folds compared to control. The sialic acid contents of produced rhEPO were significantly increased up to 43%. The asialo- and mono-sialylated *N*-glycans were decreased by half compared to control, and the tetra-sialylated *N*-glycans were increased up to 32.0%. In this study, we found the effective sialuria-mutated rat GNE/MNK to increase the intracellular CMP-sialic acid pool by genetic engineering. And, the new CHO cell lines were constructed which could produce the more sialylated therapeutic glycoproteins by overexpression of sialuria-mutated GNE/MNK, CMP-SAT, and α2,3-ST.

Seletion of a single cell producing a glycoprotein with desired glycosylation by using a microwell array.

Sunyoung Park

Department of Biological Sciences, KAIST.

The therapeutic efficacy and *in vivo* biological function of a glycoprotein is significantly affected by its glycosylation profile. For the development of glycoproteins with therapeutic applications, selection of cell lines producing a glycoprotein with adequate glycoform is crucial. Here we demonstrate an array-based analysis of secreted glycoproteins for rapid and efficient selection of a single cell producing a glycoprotein with desirable glycosylation. Our approach relies on microengraving and interrogation of glycoproteins produced by individual cells in a microwell array in terms of glycosylation profile as well as the produced amount. Based on statistical analysis of the interrogation, corresponding single cell is selected, retrieved, and expanded. We applied the approach to human recombinant erythropoietin (rhEPO)-producing CHO cells, and verified the selection of a single CHO cell that produces rhEPO with a high sialylation degree. Human erythropoietin (hEPO) bearing highly sialylated oligosaccharide was shown to display a much longer plasma half-life, resulting in high therapeutic efficacy. This method can find widespread use in the clonal selection for the production of other glycoproteins with specific glycosylation as well as analysis of the heterogeneity in cell populations in a high-throughput manner.

Turnover analysis of central metabolism by time course of stable isotope dilution rate

Nakayama Yasumune

Department of Biotechnology, Osaka University

The both of quantity of metabolites and dynamism of metabolic pathway are quite important to understand physiology of inside of cells. Although the strategy of quantification in metabolomics is well established to distinguish biological expression or environmental changes in high resolution, the method to analyze metabolic dynamics is not fully developed. Most analysis of metabolic dynamics has been carried out by fitting of defined differential equation and experimental data. This kind of analysis sometimes causes over fitting when it is applied to a metabolic map which contains unknown metabolic pathways. Therefore, in this research, we developed metabolic turnover analysis which does not need differential equations using time course of stable isotope dilution rate and PCA (principal component analysis). The method was verified using simulation data and experimental data of *Saccharomyces cerevisiae*.

To calculate simulation data, virtual metabolic map which has cycle and branch was built. The metabolites on the map had been completely constituted by certain isotope and a metabolite labeled by other isotope inflowed from outside. This time course of isotope dilution rate was calculated. Obtained data was analyzed by PCA using time course of isotope dilution rate as sample vector. In the result, a dynamics of the virtual metabolic map was expressed on the PCA score plot. For experimental data, isotopes of metabolites on central metabolites were diluted by uptake of isotopically labeled substrate, and time course of isotope dilution rate was obtained by CE/MS (capillary electrophoresis mass spectrometry). The result of PCA analysis of the data shows the difference of metabolic dynamics between Crabtree positive and negative strains. It means differences of metabolic dynamics could be analyzed with the developed method.

Development of Useful Identification and Annotation Tool for Nontargeted GC/MS Based Metabolomics

Hiroshi Tsugawa

Department of Bioengineering, Osaka University

Metabolomics is a useful technology that has been widely applied in many fields including quality evaluation, food chemistry, and biomarker search. GC-MS has become a powerful tool in metabolomics, since many peaks can be obtained (200~400) and identified using an in-house library based on their retention time (RT) and mass spectra. Determination and compound prediction of peaks from a flood of data, however, become laborious and time consuming works in GC-MS data analysis. Moreover, sufficient knowledge and experience are required to get an accurate determination and prediction. Here, a useful tool for precise, quick, and easy GC-MS peak identification and annotation was developed. To this end, two mathematical algorithms were employed: Pearson product-moment correlation coefficient totally-weighted was used to identify the peak based on RT, and Soft Independent Modeling of Class Analogy (SIMCA) was used to annotate unknown amine-, sugar-, acid-, and fatty acid-related compounds. SIMCA provided a Principle Component Analysis (PCA)-based modeling method to predict compound structure by fitting mass spectra pattern of group of compounds to the model. This tool was able to determine and predict a large number of peaks precisely, and enable us to evaluate biological data quickly and easily.

TCTP and its genetic interaction with wingless(wg) signaling pathway components.

Changmin Koh

Department of Biological Sciences, KAIST

TCTP(abbreviation of Translationally Controlled Tumor Protein) is highly conserved protein throughout various eukaryotic organisms. Its relevance to cell proliferation and survival is well identified in tumorigenesis and growth studies, but its genetic and molecular interactions are not so clearly discovered yet. We set a drosophila model for TCTP genetic interaction, have screened several partner genes by utilizing eye-specific Gal4 and UAS-gene system. Here we introduce evidences of genetic interaction between wingless(wg) signaling pathway component genes and TCTP, by showing how downregulation or overexpression of certain genes affects the growth of fly eye. As results, wg shows antagonistic effect against TCTP RNAi induced phenotype and TCTP also shows same effect against wg overexpression induced phenotype. Overexpression Dsh and Arm also shows drastic eye reduction with TCTP RNAi, but severity of phenotype seem to depend on sexual differences. Those data suggest that TCTP-involved signaling have close relationship with wg signaling pathway.

Discovery of novel genes associated with rheumatoid arthritis susceptibility in a Korean population.

Taehyeung Kim

Department of Biological Sciences, KAIST

In this study, we aim to search the new single-nucleotide polymorphisms associated with rheumatoid arthritis in Koreans through candidate gene approach. We collected the distinct 21 gene expression profiles of rheumatoid arthritis patients. And, we choose the reproducible 69 genes among these profiles. These reproducible genes were classified according to whether these genes were located on the susceptible loci which were found by linkage study of rheumatoid arthritis or not. As a result, we found 19 genes which were reproducible and located on susceptible loci. We can select the 49 tag SNPs from the 19 genes. In the first screening step, their associations with RA were tested with a Korean population of 399 RA-patients and 404 non-patient controls, who were genotyped for the 49 SNPs using the MassARRAY® iPLEX system. We could find the 5 potential SNPs (allelic p-Value<0.05). To find the statistically significant SNPs, The additional Koreans of 927 RA and 611 controls were genotyped for the 5 SNPs. The two SNPs (rs189586, rs1596231) of CXCL13 were marginally associated with RA (p-Value=0.013, 0.047, OR=1.18, 1.14) in Koreans.

Development of a nano HPLC-MS/MS method for specific and sensitive determination of bioactive amines

Shinya Yamamoto

Department of Biotechnology, University of Osaka

Bioactive amine compounds are present in cell in very low amount, so it is difficult to detect them. Therefore, it is important to develop a sensitive detection system to analyze them. LC/MS/MS system has many advantages to analyze broad range of compounds. However, conventional LC/MS/MS method is less sensitive to detect bioactive amines compared to other common high sensitive methods. To overcome this problem, two strategies were proposed to increase the sensitivity. First, derivatization of amines with 3-aminopyridyl-*N*-hydroxy- succinimidyl carbamate (APDS)*. This will enhance the efficiency of ionization, so detection intensity will increase, resulting in increased sensitivity. Hydrophobicity of amine compounds will also increase, so the compounds will be retained in a reversed-phase column during chromatographic separation, resulting in better separation. Second, scaling down the system to nano HPLC. This will increase the concentration of sample, as well as provide a better ionization and minimize sample diffusion.

The signal-to-noise (S/N) ratio of an amine compound, serotonin, represented a 25-fold increase by derivatization with APDS in selective ion monitoring (SRM) mode. A mixture containing tryptophan, 5-hydroxy-tryptophan, serotonin, N-acetyl-serotonin, and melatonin was analyzed by nano HPLC-MS/MS system under optimized gradient condition. The limit of detection (LOD) of serotonin was 1.8 fmol, which was achieved at S/N of 3, lower than LOD of that of ELISA method. This system was able to detect serotonin from a single zebrafish embryo. Thus, a high sensitive detection system for bioactive amines was developed successfully.

Reference: * K. Shimbo et al., Rapid Commun. Mass Spectrom., 23, 1483-1492 (2009)

Study of oligosaccharide-degrading enzyme involved in parasitic weed seed germination

Takatoshi Wakabayashi

Grad. Sch. Eng., Osaka Univ.

Parasitic weeds, *Striga* spp. and *Orobanche* spp. cause serious damage to agriculture worldwide. A novel and effective strategy for the parasitic weed control is desired for economical and humanitarian reasons. Since the life cycle of parasitic weeds is significantly different from that of host plants, understanding of the parasite-specific biological events is important for design of selective control strategies. We focused on the germination process of parasitic weeds to find biological events specific to these species and conducted metabolic profiling of *O. minor* seeds. Consequently, we revealed that gentianose was decreased immediately after GR24 treatment. Gentianose is a trisaccharide consisted of two glucoses and a fructose. The amounts of glucose and fructose significantly increased after GR24 treatment indicating these were supplied with the hydrolysis of gentianose. An inhibitor of glycoside hydrolases, nojirimycin, decreased the germination rate and also the amounts of glucose and fructose. From these result, we hypothesize that gentianose metabolism is essential for germination of *O. minor* seed and the key enzyme of the gentianose metabolic pathway could be a novel target for selective control of parasitic weeds. In this study, we extracted crude enzyme from *O. minor* seeds and measured glycoside hydrolysis activity to characterize the enzymes involved in their seed germination.

Gel-siRNA for highly efficient gene silencing.

Cheolam Hong

Department of Biological Sciences, KAIST

Small interfering RNA(siRNA) is known as promising therapeutic agents for the post-transcriptional gene silencing with a highly sequence-specific manner. As a novel strategy to enhance the delivery efficiency of siRNA, we increased the charge densities of siRNA. With a less cytotoxic cationic polymer as a delivery carrier, Gel-siRNA were complexed as a nano- sized particles and delivered in the cancer cells to silence the target gene expression. As a result, Gel-siRNA showed more enhanced target gene inhibition than Multi-siRNA and Naked siRNA at the low siRNA concentration. We have demonstrated here that a new class of siRNA structure and efficient gene-silencing activities owing to their increased charge density.

Paclitaxel-encapsulated albumin for cancer therapy

Jeong Yu Lee

Department of Biological Sciences, KAIST

Paclitaxel is one of the most effective therapeutic agents for cancer therapy. Due to its extremely low aqueous solubility, paclitaxel has been formulated by dissolving in a solution of ethanol mixed cremophor EL (Taxol). However, there are serious side effects resulted from this solvent system. Therefore, many researches have been performed to overcome solvent-related toxicity.

In this study, we developed human serum albumin nanocapsules cross-linked by PEG containing paclitaxel with improved water solubility. Methylene chloride containing paclitaxel and 6 arm PEG-succinimidyl succinate was emulsified by sonication in aqueous solution containing HSA. The resultant particles had a size of 200~300 nm and negative surface charge. Hph-1-PTD-conjugated nanocapsule induced a remarkable cancer cell death and an accumulation in tumor, as compared with unmodified one. Our study demonstrated that HSA nanocapsules hold great promise for broad biomedical applications.

Application of live cell imaging to plant cell nuclei

Tomohiro Ohmura

Dept. Biotech., Grad. Sch. Eng., Osaka Univ.

One of the most striking features of plant growth and development is the massive, yet precisely regulated, accompanying increase in cell size. Yeast and animal cells usually only double their size during their development, whereas plant cells commonly enlarge to hundreds or even thousands of times their original size. One common mechanism by which plants achieve this increase in cell size is through increasing their ploidy level by successive rounds of DNA replication, a process called endoreduplication. The relationship between ploidy and cell size was first described in the early part of the 20th century and gave rise to the 'karyoplasmic ratio' or 'nuclear–cytoplasmic ratio' theory; namely that there is a control mechanism that regulates cytoplasmic volume, adjusting it with respect to the DNA content of the nucleus. Furthermore, it was reported that endoreduplication induces root growth. So, in my study, I have performed to make ploidy map in the basal region of plant root, and tried to elucidate the effect of endoreduplication on plant organogenesis.

I measured fluorescence intensity of *A. thaliana H2B::tdTomato* construct. The histogram which reflects its intensity showed a few peaks of its intensity level. As a result, I could make the ploidy map. This result supported the hypothesis that endoreduplication induces root growth.

Aggregation of biopharmaceutical antibodies

Ryuto Nakao

Department of biotechnology, University of Osaka

In recent years, therapeutic proteins, in particular monoclonal antibodies, have emerged as an important class of protein therapeutics for various types of diseases such as cancer and rheumatism. Of course, antibodies are used as one of the most versatile reagents.

In both applications, the antibodies must be in the native, monomeric state to be stable and functional. During the pre-clinical development, including manufacturing and formulation processes of monoclonal antibodies, aggregation of the proteins are often observed. Antibodies aggregation leads to loss of pharmaceutical efficacy and serious allergic response, so aggregation of antibodies must be minimized for pharmaceutical applications. Recently, investigations of their aggregation mechanisms are being actively pursued and it is showed that there are a number of factors, including temperature, shear stress, pH, ionic strength and surface exposure can lead to aggregation of monoclonal antibodies in solution. However, despite the tremendous efforts, detailed mechanism of antibody aggregation and the nature of aggregates remain unknown. From a formulation development perspective also, it is important to understand the nature of aggregates and the related aggregation pathways in antibody molecules.

So in my presentation, I would like to introduce interesting behavior of antibody aggregates produced by heat stress and I'm going to discuss the mechanism of large aggregates formation.

Effect of PDI under hypothermic condition

Seo Jinseok

Department of Biological Sciences, KAIST

Chinese hamster ovary (CHO) cells are one of the most popular hosts used for therapeutic protein production in industry. Lately, there have been many attempts to improve the production efficiency of recombinant CHO cells. Among them, increasing the specific productivity (q_p) by the addition of Sodium Butyrate (NaBu) and hypothermic culture condition have exhibited positive results. The use of molecular chaperones to relieve the post-translational bottleneck thereby enhancing the q_p has largely yielded mixed results. This study focuses on the effect of the molecular chaperone, PDI, under stressful condition of hypothermic culture.

PDI was inducibly overexpressed in two recombinant CHO cell lines producing therapeutic proteins -TNFR-Fc and Antibody. Batch cultures were performed at 37°C and at 32°C and ELISA for the protein titers and qRT-PCR for the relative transcription level were performed.

PDI overexpression had no effect on the q_p at 37°C. However, PDI overexpression at low culture temperature enhanced the q_p by about 4.2 fold for TNFR-Fc cell line and 1.4 fold for the Ab-producing cell line. In addition, PDI overexpression at low culture temperature enhanced the transcription level by about 1.3 fold for TNFR-Fc cell line and 2.7 fold for the Ab-producing cell line. As hypothesized, PDI helped in further enhancing the q_p at low temperature by probably relieving the cells of the limitation in disulfide bond formation and hence boosting protein folding.

Expression profiles of miRNAs in human embryonic stem cells during differentiation into hepatocytes

Nury Kim

Department of Biological Sciences, KAIST

Human embryonic stem cells (hESCs) are cells which have ability to maintain self-renewal and to differentiate into many cell types. As hESCs can differentiate into all cell lineages of the body, they are used as valuable tools for understanding human development, and are regarded as promising therapeutic reagents for regerative medicine. MicroRNAs are small, non-coding RNAs which repress gene expression at the posttranscriptional level, and are emerging as key regulators of hESC differentiation. We speculated that different kinds of microRNAs may function at specialized differentiation steps during the differentiation of human embryonic stem cells (hESCs). Expression of microRNAs analyzed by microRNA array was compared among hESCs, hESC-derived definitive endoderm (DE) cells, and hESC-derived hepatocytes. As a result, 9, 9, and 77 microRNAs were enriched in hESCs, DE cells, and hepatocytes, respectively. Differential expressions of microRNAs during hepatic differentiation were confirmed by quantitative RT-PCR. In particular, miR-21, miR-214, and miR-216a which are negative regulators of PTEN were enriched in hESC-derived hepatocytes. In the downstream of the PI3K pathway, a target of PTEN was inhibited in hESC-derived DE cells, expressions of hepatocyte marker genes remarkably decreased. We provide not only the overall expression pattern of microRNAs during hepatocyte differentiation, but also the possible relationship between microRNAs and differentiation process of hESCs into a specialized cell type.

Clarification of autoregulator-signaling cascades controling secondary metabolism in *Streptomyces avermitilis* - AvaL-cascade -.

Ken-ichiro Haruna

International Center for Biotechnology, Osaka University, Japan

Streptomycetes are gram-positive, filamentous, soil-dwelling bacteria that undergo a complex program of morphological differentiation. In addition to this singular multicellular morphogenesis, the members of the Streptomycetes are well known for their abilities to produce a variety of secondary metabolites with important uses in medicine and in agriculture. Interestingly, some production of secondary metabolites in this genus is regulated by "autoregulator" and its specific receptor protein. The regulatory system associated with autoregulator is called "autoregulator-signaling cascade" But, there is little knowledge on how antibiotic production is controlled. Therefore, Investigation of the whole regulatory cascades will be helpful for achievement of enhanced production of antibiotics and discovery of new bioactive compounds.

Previously, it was thought that one strain has one autoregulator-signaling cascade, but bioinformatic analysis of the *Streptomyces* genome, some strains have multiple genes encoding autoregulator receptor homologue. Relationships of these genes haven't clarified yet. So we try to elucidate relationship of these receptors. Through this study, we finally aim to elucidate the whole regulatory system by autoregulator in Streptomycetes. To achieve this purpose we select *S. avermitilis*.

S. avermitilis is producer of Avermectin that is highly active against a broad spectrum of nematode and arthropod parasites. For utility of Avermectin, in 2003, the entire genome of *S. avermitilis* were completely determined. *S. avermitilis* possesses 5 genes (*avaR1*, *avaR2*, *avaR3*, *avaL1*, *avaL2*) encoding homologous genes of autoregulator in genome. From transcriptional analysis, we clarified that two autoregulator- signaling cascades (AvaL-cascade, AvaR-cascade) are present in *S. avermitilis*. Multiple autoregulator-signaling cascades haven't reported in Streptomycetes. Therefore, we try to investigate these cascades. In this study, I focused on AvaL-cascade, and clarify this cascade by characterization of AvaL1 & AvaL2 and identification of signaling molecule(s).

Activation of secondary metabolism in entomopathogenic fungi by using genetic engineering.

A. Morisugi

Laboratory of Molecular Microbiology, Department of Biotechnology, University of Osaka

The increasing data of fungal genome sequences have revealed that filamentous fungi possess many secondary metabolism genes which are silent under normal laboratory conditions. Entomopathogenic fungi (EPF) are a group of fungus which shows insect pathogenic properties and can be used as a biological pesticide. During their growth on host insect, EPF have been considered as producing many biologically active compounds by secondary metabolism, including toxin against insect hosts. Moreover some species of them have also been used as herbal medicine for more than 1500 years in China. These facts suggest that EPF also have a lot of genes which responsible for secondary metabolite production, while it is very hard to reproduce their expression under laboratory cultivation condition. So in this study, in order to obtain novel biological active compounds, I tried to awaken the silent genes related to biosynthesis of secondary metabolites by genetic engineering.

Recent researches identified LaeA as a global regulator of secondary metabolisms in several genera of filamentous fungi, which can induce the expression of majority of secondary metabolites genes. Since *laeA* seemed to be a good tool for stimulating the silent gene clusters in EPF, heterologous overexpression of *laeA* was conducted in this study.

One of the entomopathogenic fungi, *Beauveria bassiana* HF730 was selected as the host strain for introducing *laeA* because only a limited number of compounds have been identified from this fungus while the species is used as a biological pesticide with high pathogenicity. Therefore, the successful activation of secondary metabolism in this strain expects the acquisition of novel bioactive compounds.

laeA ORF from *A. nidulan*s was put under the glyceraldehyde-3-phosphate dehydrogenase (GPD) promoter from *B. bassiana* for overexpression, and introduced into the genome of *B. bassiana* by integration. Metabolic profile was analyzed by HPLC by comparison with wild type, revealing that some now peaks appeared only in the transformant. These peaks are currently analyzed.

Screening and Cloning of novel Ginsenoside Transforming β-glucosidase Gene

Hyung-Gwan Lee

Department of Biological Science, KAIST.

Ginsenoside Rb1 is the most predominant ingredient in Panx species(ginseng) and metabolite of Rb1 produce various pharmacological activities, such as anti-tumor properties. Although various chemical and biological methods have been reported for preparation of it, enzymatic production by using a specific β-glucosidase is able to produce metabolite with specific selectivity and high efficiency. The purpose of this study is to isolate, purificate and characterize novel β-glucosidase-producing bacteria converting G-Rb1 to minor ginsenoside. The screening was performed with esculin and X-Glc (5-Bromo-4-chloro-3-indolyl-β-D-glucoside) containing 1/5 R2A agar plate and then observed color development. The selected positive isolates were cultured with 1000ppm G-Rb1 and the reactants were analyzed by TLC and HPLC. Fosmid clone libraries were constructed in the pCC1FOSTM-vector using Epicentry Kit. The sequencing analysis of one fosmid clone was performed using shotgun sequencing method. Strain 3E-5, one of the isolates having strong β-glucosidase activity , converted ginsenoside Rb1 to the active metabolites C-K via Rd, F2. It was novel species candidate of the genus Mucilaginibacter in the Sphingobacteria. BLAST search using two β-glucosidase candidates from analyzed fosmid clone sequence revealed significant homology to family 3 glycoside hydrolase and produced different characters. We isolated a novel bacterial strain, Mucilaginibacter sp. 3E-5, transforming G-Rb1 to C-K, from around soil of ginseng roots in a ginseng field. The strain could transform not only G-Rb1 but also ginsenosides Rb2, Rc and Rd to C-K.

Effect of local application of a synthetic antimicrobial peptide NKC on the wound-healing

Kim, D.J.

Department of Biolgical Sciences, KAIST.

A novel antimicrobial peptide (named NKC) composed of two repeats of helix structures flanked by helix-capping motifs in both N- and C-termini was investigated for its possible application as a new topical agent. This peptide showed broad-spectrum antimicrobial activity without cytotoxicity against Gram-positive, Gram-negative bacteria and fungi in the range of the minimal inhibitory concentration (MIC) values (0.5~2 μg/ml). The other activity has been described for human keratinocyte cells that may contribute to an wound healing response: NKC activated migration of human keratinocyte cells (HaCaT) during the wound healing process, and these effects were maximized at 2 μg/ml with HaCaT. Our results support potential therapeutic applications of NKC for treatment of skin infections and regeneration as a potential topical agent.

Development of PCR-mediated duplication for desired chromosomal regions in budding yeast.

Kotaro Iwami

Department of Biotechnology, Graduated School of Engineering, Osaka University

Chromosome engineering is playing an increasingly important role in the functional analysis of genomes. A simple and efficient technology for manipulating large chromosomal segment is obviously a key to advancing these analyses. We have developed an innovated method to split chromosome in *Saccharomyces cerevisiae*, which we call PCR-mediated chromosome splitting (PCS). Recently, we have also another genome engineering method called PCR-mediated chromosomal deletion (PCD). PCD method made it possible to eliminate a specific chromosomal region, especially an internal region by one-step transformation. In this study, we aim to further develop genome engineering method which is designated as PCDup (PCR-mediated Chromosome segmental Duplication), by which we can duplicate desired chromosomal regions. The PCDup requires only two-step PCR and one transformation per duplication event. Briefly, i) 400 bp of both ends of desired regions for duplication are amplified as target sites for homologous recombination, and then ii) these target fragments are fused with marker fragments consisting of selection marker, centromere (*CEN4*), and telomere seed sequences by overlap extension PCR, respectively. Next, iii) the fused PCR fragments are then introduced into cells by conventional transformation. Finally, iv) karyotype of obtained transformants are analyzed by pulse field gel electrophoresis combined with Southern analysis. So far, I obtained transformants which harbored duplicated chromosomal segment as expected for the duplication of 50 kb ~ 200 kb regions in Chr. I, II, VIII, and X, and frequencies of each duplication were 10 ~ 40 %. These results suggest that the PCDup method is a useful tool for duplicating desired chromosomal regions for analyzing function of genes on it and the defined chromosomal regions.

Molecular breeding of lactic acid tolerant yeast
- Mechanisms of lactic acid tolerance conferred by overexpressed Esbp6 in *Saccharomyces cerevisiae* -

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Plant-based plastics such as the poly L-lactic acid are being developed as carbon neutral resource alternatives to petroleum-based plastics. To spread the use of these carbon neutral plastics widely, reduction in production cost is indispensable. Yeast, *Saccharomyces cerevisiae* is more tolerant to acid than *Lactobacilus* species which are generally used in lactic acid fermentation. Therefore lactic acid fermentation by yeast could be performed under non-neutralizing condition. However, even though *S. cerevisiae* is tolerant to lactic acid, low pH of the culture caused by the accumulation of lactic acid causes decrease in lactic acid productivity. In our earlier study, *ESBP6* was isolated as the gene whose overexpression conferred lactic acid tolerance. *ESBP6* encodes protein with similarity to mammalian monocarboxylate transporters but Esbp6 does not appear to play a significant role in monocarboxylate transport. The mechanisms of lactic acid tolerance conferred by overexpressed Esbp6 are unclear. Therefore, in this study, we aim to investigate the mechanisms of lactic acid resistance conferred by overexpressed Esbp6. We have shown that the content of intracellular amino acids decreased under lactic acid stress. Therefore we focused the involvement of intracellular amino acids in lactic acid tolerance and measured the content of intracellular amino acids in wild-type strain and Esbp6 overexpressed strain with 2h in 4% lactic acid. Level of almost all amino acids in Esbp6 overexpressed strain in 4% lactic acid condition did not decrease compared with wild-type strain. This result suggests that the improvement of intracellular amino acids homeostasis that resists decrease of intracellular amino acids under lactic acid stress results in lactic acid tolerance in Esbp6 overexpressed strain.

A genetic circuit system based on quorum sensing signaling for directed evolution of quorum-quenching enzymes

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Quorum sensing is a cell–cell communication mechanism that is involved in the regulation of biological functions such as luminescence, virulence, and biofilm formation. Quorum-quenching enzymes, which interrupt quorum-sensing signaling through degradation of quorum-sensing molecules, have emerged as a new approach to controlling and preventing bacterial virulence and pathogenesis. In an effort to develop quorum-quenching enzymes with improved catalytic activities, a genetic circuit system based on acylhomoserine-lactone (AHL)-mediated quorum-sensing signaling was constructed. Using the genetic circuit system, we attempted the directed evolution of a quorum-quenching enzyme from *Bacillus sp*. As a result, the best mutant V69L/I190F exhibited 7-fold increased catalytic efficiency (k_{cat}/K_M) toward a quorum-sensing molecule N-hexanoyl-L-homoserine lactone. The mechanistic causes for the improvement were rationalized based on an autodocked substrate model and kinetic analysis.

Site-specific labeling of protein using unnatural amino-acid for single-molecule FRET analysis

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Single-molecule fluorescence resonance energy transfer (smFRET) measurement provides a unique and powerful approach to understand complex biological processes including conformational and structural dynamics of individual biomolecules. For the efficient smFRET analysis of protein, site-specific dual-labeling with two fluorophores as an energy donor and an acceptor has been one of major issues. Here we demonstrate an incorporation of unnatural amino acid into a specific position of protein followed by click-chemistry using alkynyl dye for dual-labeling. As a model study, maltose binding protein (MBP) was dually labeled via incorporation of *ρ*-azido-L-phenylalanine and cysteine at specific positions, immobilized on a surface, and subjected to smFRET analysis for unfolding and refolding processes. Comparison with conventional labeling using double cysteines revealed that the use of unnatural amino acid and click chemistry results in reduced heterogeneity in protein conformations in smFRET experiments. This result emphasizes the importance of site-specific labeling of protein for more effective smFRET analysis.

The whole-cell biocatalysis with hydrophobic bacterium *Rhodococcus Opacus* B-4

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Rhodococcus opacus B-4, a Gram-positive strain isolated from a gasoline-contaminated soil, has a highly hydrophobic cell-surface structure and exhibits a high affinity for water-immiscible hydrocarbons. This feature allows a better accessibility between the bacterial cells and hydrophobic chemicals. In the present study, we demonstrated the catalytic ability of *R. opacus* B-4 cells for the conversion of water-immiscible chemicals in the presence of bulk amount of organic solvents.
Gene encoding a thermophilic alcohol dehydrogenase of *Thermus thermophilus* HB27 (TtADH) was cloned and expressed in *E. coli* and *R. opacus* B-4. The specific activity of TtADH of recombinant *E. coli* was approximately 20 times higher than that of *R. opacus* B-4. Nevertheless, when the stereospecific reduction of trifluoroacetophenone to (*R*)-α-(trifluoromethyl)benzyl alcohol was performed in an organic solvent containing TtADH-expressing cells, *R. opacus* B-4 cells exhibited a markedly higher conversion rate. On the basis of this finding, we are now in attempt to convert γ-aminolevulinic acid to uroporphyrinogen-III, which is a compound of particular interests in molecular electronics, supramolecular building blocks, and supramolecular chemistry, using three recombinant thermophilic enzymes, namely HemB, HemC, and HemD.

Establishment of novel gene amplification platform in CHO cell line for biopharmaceuticals production

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Chinese hamster ovary (CHO) cell line is one of the most important hosts for biopharmaceuticals production. There have been a variety of tries to improve productivity in CHO cell line, for instance, media optimization, highly producible vector development, and also gene amplification. Among them, gene amplification technique has been most widely used to increase the copy number of an exogenous gene. Basically, many chemical reagents including methotrexate (MTX) and methionine sulfoximine (MSX) can induce gene amplification in mammalian cells. However, the mechanism of gene amplification is still unclear. In our research, we had constructed BAC library of *Dhfr* gene amplified CHO cell line, DR1000L-4N. Through the analysis of the BAC clone which contained amplified *Dhfr* gene region, we found a large palindrome structure with a small inverted repeat structure in the junction region. To investigate the effect of the palindrome structure on gene amplification in CHO cell line, we constructed plasmids containing the junction region of the palindrome structure. CHO DG44 cells which were transfected with the constructed plasmid showed better adaptation to high MTX concentration. Moreover, the cells containing the junction region had higher ratio of GFP-positive result during gene amplification. On the basis of the results, we assume that the junction region plays an important role in gene amplification in CHO cells.

Epigenetics in aging : Histone hypoacetylation at repetitive DNA elements represses repeat-associated transcripts in aged mice brain.

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The correlation between the expression of repeat-associated transcripts (RATs) and epigenetic state at repetitive sequences was investigated to explain the age-dependent gene expression. Repeat DNA elements were hypermethylated in old mice brain. Histone hypoacetylation and altered histone trimethylation at repetitive sequences were detected in brain during aging. The expression of RATs was then measured to understand the transcriptional influence by the observed epigenetic alterations. Majority of RATs investigated were down-regulated along with age. Epigenetic modifications at the promoter region of RATs were examined to correlate with the observed transcriptional repression. DNA methylation was stably maintained, whereas histones were deacetylated in aged mice brain. The result indicated that the age-dependent histone hypoacetylation at repeat DNA elements involved in the transcriptional repression of RATs. This study strongly suggests that histone hypoacetylation at repeat sequences is responsible, at least in part, for the altered gene expression profile in old mice brain.

Proteasome mediates mRNA export through SAGA/TREX-2 complex

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Over the years it has been proven that all the steps involved in gene expression, from gene activation to the nuclear export of mRNAs, are tightly linked. Previous results indicated that the 19S regulatory particle of proteasome is involved in transcription through a mechanism that is not dependent on proteolysis. Here, using biochemical and genetic approaches, we show that 19S RP regulates mRNA export through the SAGA component Sgf73p, a yeast homologue of the human Ataxin-7 protein. We also demonstrate that the *rpt2-1* strain, a mutant defective in binding to Sgf73p, exhibits decreased transcription of the *GAL1* gene, impaired localization of mRNA export factor Sac3p, and defects in mRNA export. These findings indicate that 19S RP is important for targeting of the TREX-2 complex to the nuclear pore complex (NPC). Finally, we show that the TREX-2 complex in both *rpt2-1* and the ATPase mutant, *sug1-25*, is retained in the promoter region. These data suggest that 19S RP is involved in mRNA metabolism beyond transcription.

Single-cell imaging of intracellular ATP concentration inside *E.coli*

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Metabolism is a highly complex and organized biological system. Among large numbers of metabolites, ATP is one of the most important molecules in metabolism, playing a role as a universal energy currency of all living organism. But, it is unclear how ATP concentration inside bacteria changes during growth or affected by surrounding environments at single cell level. In this study, we utilized FRET-based ATP indicators, ATeams, to moniter intracellular ATP concentration in living cells . ATeam is composed of the ε-subunit of the bacterial F_oF_1-ATP synthase sandwiched by the cyan- and yellow-fluorescent proteins. ATP Binding to ε-subunit triggers its conformational change, resulting in higher FRET state. Figure1 shows the scheme of ATeam FRET signal(YFP/CFP) change depending on ATP concentration. By expressing ATeam in *E.coli*, we are measuring intracellular ATP concentration from YFP/CFP fluorescent ratio at single cell level. From this measurement, ATP concentration inside *E.coli* was estimated at several mM. And in order to check the confidence of ATP concentration obtained from single cell measurement, bulk measurement using firefly luciferase is conducted. This measurement also suggested the intracellular ATP concentration of *E.coli* was several mM and it doesn't depend on the specific growth rate.

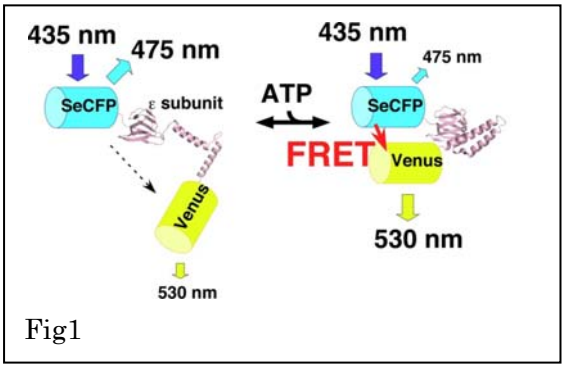

Fig1

Mus81 regulates acetyltransferase activity of Tip60.

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The Mus81-Eme1 complex is highly conserved structure specific endonuclease that can cleave 3' flap substrate, replication fork, and Holliday junction *in vitro*. In this study, we found that histone acetyltransferase enzyme, Tip60, interacts directly with the Mus81-Eme1 complex through Mus81 subunit in a yeast two-hybrid system. This interaction has been confirmed by co-immunoprecipitation using purified proteins.

Tip60 is originally found as a interacting protein of HIV-1-encoded transactivator protein and the interaction of Tat with Tip60 efficiently inhibits the Tip60 histone-acetyltransferase activity. As similar with this result, Mus81 inhibits acetyltrasferase activity of Tip60. Therefore, we speculate that the interaction between Tip60 and Mus81-Eme1 complex is important for regulation of Tip60 activity.

Aberrant glycosylation of β-haptoglobin in sera of colon cancer

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The glycosyl epitope dimeric Le^a (Le^a-on-Le^a), defined by mouse monoclonal antibody NCC-ST-421, was identified previously as tumor-associated antigen, expressed highly in various human cancer tissues and cell lines derived therefrom, but with minimal expression in various normal tissues (Watanabe M, et al, Cancer Res 51: 2199, 1991; Stroud MR, et al, JBC 266: 8439, 1991). In the present study, we observed clearly higher expression of this epitope, defined by ST421, in β-haptoglobin (β-Hap) from sera of patients with colorectal cancer, compared to normal, healthy subjects or patients with chronic inflammatory processes (Crohn's disease, ulcerative colitis). We focused, therefore, on biochemical characterization of glycosyl epitope status expressed in β-Hap. We concluded that the dimeric Le^a epitope is carried by O-linked but not by N-linked structure, based on the following observations: (i) Strong reactivity of Colo205 supernatant with ST421 was reduced clearly by pre-incubation of cells with benzyl-α-GalNAc. (ii) Treatment of β-Hap with α-L-fucosidase reduced its reactivity with ST421, but did not affect its reactivity with anti-Hap antibody. In contrast, treatment of purified β-Hap with PNGase F, which releases N-linked glycans, had no effect on reactivity with ST421, but changed molecular mass from 40 kDa to 30 kDa.

Tk-subtilisin stabilization mechanism during maturation

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Subtilisin is a serine protease widely distributed in various organisms, including bacteria and archaea. According to the studies on bacterial subtilisins, these subtilisins are synthesized as a precursor which consists of a signal peptide (Pre), a propeptide (Pro) and catalytic domain (mature domain) and it is activated through activated processes, 'autoprocessing' and 'degradation of propeptide'.

Tk-subtilisin is a homologue of bacterial subtilisins from hyperthermophilic archaeon *Thermococcus kodakaraensis* KOD1. Like bacterial subtilisins, Tk-subtilisin is also matured upon autoprocessing and degradation of the Tk-propeptide. However, unlike bacterial suctilisins, Tk-subtilisin requires Ca^{2+} ion for the maturation. Compared with an amino acid sequence of bacteria subtilisins, the sequence of Pro-Tk-subtilisin contains three insertion sequences in the mature domain and two of them, first sequence (Gly70~Pro82) and second sequence (Pro207-Asp226), consist of Ca^{2+} ion binding loops. Recently, it was found that the second insertion loop forms four Ca^{2+} binding sites and is required for folding of mature domain. Meanwhile, it is proposed the first insertion loop enable to form Ca^{2+} binding site before autoprocessing, but the function during the maturation is still unknown. To investigate the function of the region during maturation, a deletion mutant of first insertion loop (ΔGly70~Pro82, DM1) was biochemically analyzed.

Pro-from of DM1 (Pro-DM1) matured at 80 degrees and matured DM1 showed as high activity as that of Tk-subtilisin. In addition, an autoprocessed complex of DM1 was stable same as wild type and mostly completed folding. However, unautoprocessed form of Pro-DM1 was quite unstable and sensitive to degradation by chymotrypsin due to exposure of the hydrophobic area to the molecular surface. These results suggest first insertion loop (Gly70~Pro82) stabilizes unautoprocessed form of Pro-Tk-subtilisin to obtain maximum maturation efficiency with resistance to autolysis or denaturation under high temperature.

Study on haloadapted ribonuclease HI from *Halobacterium salinarum* NRC-1

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Enzymes from extremophiles are known to have special ability to adapt extreme environments in which their host organisms grow. Previous studies have shown that enzymes from halophiles (halophilic enzymes) are characterized by high density of acidic residues on their surface to prevent salting-out. The mechanism by which halophilic enzymes maintain their stabilities at highly saline conditions has been well studied. In contrast, the mechanism by which halophilic enzymes maintain their activities at highly saline conditions remains to be understood. To understand this mechanism, ribonuclease HI from *Halobacterium salinarum* NRC-1 (Halo-RNase HI) was chosen as a model protein and its biochemical properties were characterized at highly saline conditions.

Ribonuclease HI (RNase HI) is an enzyme that specifically cleaves the RNA strand of RNA/DNA hybrids. Its catalytic mechanism and substrate recognition mechanism have been proposed and electrostatic interaction is considered to be important for substrate recognition. Since electrostatic interaction is reduced at highly saline conditions, halophilic RNase HI may recognize the substrate with unique mechanism.

The enzymatic activities of Eco-RNase HI (RNase HI from *Eschericia coli*) and Halo-RNase HI were determined in the presence of various concentrations of NaCl using [H^3]-labeld M13 DNA/RNA hybrid as a substrate, to compare their dependencies on salt concentration. Halo-RNase HI possessed >80% of its activity even in the presence of 3.5 M NaCl, while Eco-RNase HI lost more than 95% of its activity in the presence of 0.5 M NaCl. The far-UV CD spectra showed that the secondary structure of Eco-RNase HI is not seriously changed even at 4.0 M NaCl. However, analysis for the interaction between RNA/DNA hybrid and Eco-RNase HI by surface plasmon resonance indicates that the dissociation constant (K_D) of Eco-RNase HI increases from 78 nM to 4.2 M when NaCl concentration increases from 0.1 M to 1.0 M. These results suggest that the difference in halophilicity between Eco-RNase HI and Halo-RNase HI is derived from the difference in K_D value rather than the difference in the stability of secondary structure or salting-out. Halo-RNase HI probably acquires unique substrate recognition mechanism to adapt to highly saline environment.

Visualizing dynamic interaction between calmodulin and calmodulin-related kinases

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A new visualizing method was developed for monitoring protein-protein interactions in live mammalian cells. This method can visualize several kinds of protein-protein interactions by directing the localization of a bait protein to endosomes. Moreover, this method is also robust enough to analyze signal-dependent protein-protein interactions such as a calcium-dependent interaction. Using this method, we have visualized the interaction of activated calmodulin with the calmodulin-binding proteins, to observe interesting oscillatory interactions via time-lapse imaging. In addition, this new method can simultaneously monitor multiple protein-protein interactions in a single live cell to compare the interactions of several prey proteins with a single bait protein. As a result, it was observed that CaMKK1 and CaMKIIα bind calmodulin with distinct binding affinities in live cell, which indicates calcium signaling was fine-tuned by distinct activations of CaM kinases. This method can contribute to understand cellular processes based on dynamic P-P interactions.

Intercellular transfer of Vax1 transcription factor

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Development of multicellular organism is mediated by dynamic communication with their neighboring cells. Especially, the reciprocal communication between neurons and glial cells is essential for their proper development, survival, and migration into specific target sites. The ventral anterior homeobox 1 (Vax1) transcription factor has been known to control the development of ventral forebrain structures not only by facilitating glial fate of ventral optic neuroepithelium through transcriptional regulation of target genes autonomously, but also by regulating the migration of SVZ neurons in a non-autonomous manner. There has been an inconsistency on the distribution of Vax1 transcripts and proteins in developing mouse eyes, and it intrigued me to investigate a potential regulation of eye development by intercellular transfer of Vax1 protein. I found that Vax1 protein could be secreted out from expressed cells, and then move into the neighboring cells. Non-nuclear Vax1 protein was highly enriched in cholesterol-rich fraction in extracellular space as well as in cytoplasm. Moreover, the Vax1 secretion was sensitive to cellular sphingomyelin lipid level, indicating that Vax1 secretion is mediated by sphingomyelin-derived cholesterol-rich lipid-rafts. The results strongly suggest that intercellular transfer of Vax1 protein is mediated by vesicular trafficking. I also found that the directional vesicular trafficking of Vax1 from nucleus to membrane was facilitated by JNK-interacting protein 3 (JIP3), which was isolated by proteomic screening for the Vax1-interacting proteins. Together, my results support the existence of non-autonomous regulation of neuronal development by Vax1 protein.

Selected SALM (Synaptic Adhesion-Like Molecule) family proteins regulate synapse formation

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Synaptic cell adhesion molecules regulate various steps of synapse formation. Despite the great diversity of neuronal synapses, relatively few adhesion molecules with synaptogenic activity have been identified. Synaptic adhesion-like molecules (SALMs) are members of a family of cell adhesion molecules known to regulate neurite outgrowth and synapse maturation; however, the role of SALMs in synapse formation remains unknown. We found that expression of the SALM family proteins SALM3 and SALM5 in nonneural and neural cells induces both excitatory and inhibitory presynaptic differentiation in contacting axons. SALM3 and SALM5 proteins are enriched in synaptic fractions, and form strong (SALM3) or weak (SALM5) complexes with postsynaptic density-95 (PSD-95), an abundant postsynaptic scaffolding protein at excitatory synapses. Aggregation of SALM3, but not SALM5, on dendritic surfaces induces clustering of PSD-95.KnockdownofSALM5reduces thenumberand function of excitatory and inhibitory synapses. These results suggest that selected SALM family proteins regulate synapse formation, and that SALM3 and SALM5 may promote synapse formation through distinct mechanisms.

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