

**8th Osaka University – KAIST
Graduate Students Symposium in
Biotechnology**

2009.02.15(SUN) ~ 2009.02.17(TUE)



Department of Biological Science

KAIST

373-1 Guseong-dong, Yuseong-gu, Daejeon, Korea

Symposium Venue :

Lecture Hall in biomedical research Center(E7 building)



Programs

02/15 (Sunday)

11:00 Arrival at Incheon Airport
11:30 Lunch
13:00 Move to Daejeon
(Daedeok Innopolis, Guest House)
18:00 Dinner

16:30 Closing remark
– Prof. Kwang-Wook Choi
17:20 Dinner

02/17 (Tuesday)

10:00 Departure from Guest House
Touring

02/16 (Monday)

08:40 Move to KAIST
09:00 Opening remark
– Prof. Kobayashi, Akio
09:20 Session 1
09:50 Session 2
10:20 Break
10:30 Session 3
11:00 Session 4
11:30 Session 5
12:00 Lunch
13:30 Poster session

Opening Remark : KOBAYASHI, Akio

Session 1

Chairperson : Woo-Yong Shim

-
- S1-1 Ju Ri Shin
 - S1-2 Irshad Ahmad
 - S1-3 Yeon-Gu Kim
 - S1-4 Jaechan Kwak
 - S1-5 Dongjun Lee
 - S1-6 Nguyen Van Nhuong
 - S1-7 Kyuri Lee

Session 2

Chairperson : Suzuki Toshihiro

-
- S2-1 Cao Yihua
 - S2-2 Kazuteru Takagi
 - S2-3 Kei-ichi Ishidoh
 - S2-4 Koji Tsukada
 - S2-5 Mizue Tanigawara
 - S2-6 Naoki Wada
 - S2-7 Rui Kawahara

Session 3

Chairperson : Sang-Chul Lee

-
- S3-1 Young-Gyun Park
 - S3-2 Woo-Yong Shim
 - S3-3 Nam Suk Kim
 - S3-4 Kang Ju Hyun
 - S3-5 Young Kue Han
 - S3-6 Hyung-Gwan Lee
 - S3-7 Jiwon Park

Session 4

Chairperson : Kazuteru Takagi

-
- S4-1 Ryosuke Kitakado
 - S4-2 Shun-ichi Tanaka
 - S4-3 Sukanda Tianniam
 - S4-4 Suzuki Toshihiro
 - S4-5 Ying Zhou
 - S4-6 Yuki Takeuchi
 - S4-7 Zhou Yan

Session 5

Chairperson : Kyuri Lee

-
- S5-1 Sang-Chul Lee
 - S5-2 Ji Yun Song
 - S5-3 Sung-Tae, Hong
 - S5-4 Yunxian, Piao
 - S5-5 Liang Wang
 - S5-6 Sang-Wook Park

Closing remark : Kwang-Wook Choi

Opening remarks

Prof. KOBAYASHI, Akio

Cell Technology Lab., Dept. Biotechnology, Graduate School of Engineering, Osaka Univ.

Session 1

(Chairperson : Woo-Yong Shim)

S1-1

High level expression of an antimicrobial peptide histonin as a natural form by multimerization and furin-mediated cleavage

Ju Ri Shin (Ph. D course)

Molecular Biotechnology Lab., Dept. of Biological Science, KAIST

S1-2

Constitutive Expression of an Endogenous Xylose Reductase Gene in Candida tropicalis by Using a Promoter of Glyceraldehyde-3-Phosphate Dehydrogenase

Irshad Ahmad (Ph. D course)

Cellular Metabolic Engineering Lab., Dept. of Biological Science, KAIST

S1-3

Effect of Bcl-x_L overexpression on apoptosis and autophagy in recombinant Chinese hamster ovary cells under nutrient-deprived condition

Yeon-Gu Kim (Ph. D course)

Animal Cell Engineering Lab., Dept. of Biological Science, KAIST

S1-4

SAGA targeting stimulation by proteasomal ATPase during transcription initiation: Proteasomal ATPase Rpt2p interacts functionally with Sgf73p in SAGA complex

Jaechan Kwak (Ph. D course)

Chromatin and Epigenomics Lab., Dept. of Biological Science, KAIST

S1-5

ER71 Acts Downstream of BMP, Notch, and Wnt Signaling in Blood and Vessel Progenitor Specification

Dongjun Lee (Ph. D course)

Molecular Genetics Lab., Dept. of Biological Science, KAIST

S1-6

Molecular mechanism of familial Parkinson's disease

Nguyen Van Nhung (Ph. D course)

Molecular Genomics Lab., Dept. of Biological Science, KAIST

S1-7

Magnetic force driven enhanced siRNA-ss-PEG delivery using Pluronic/PEI nanocapsule encapsulating iron oxide nanoparticles

Kyuri Lee (Ph. D course)

Nano Biomaterial Lab., Dept. of Biological Science, KAIST

Session 2

(Chairperson : Suzuki Toshihiro)

S2-1

Construction of a BAC-based chromosome physical map of CHO-DG44 cell

Cao Yihua (Ph. D course)

Biochemical Engineering Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S2-2

Photoresponse analysis of phytochrome A in a non-photosynthetic parasitic plant, Orobanche minor Sm

Kazuteru Takagi (Ph. D course)

Cell Technology Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S2-3

Entomopathogenic Fungi as a Potential Bioresources of Bioactive Compounds

Kei-ichi Ishidoh (M. D course)

International Center for Biotechnology Lab.,

Dept. of Biotechnology Graduate School of Engineering, Osaka University

S2-4

Quantitative analysis of the bacteriophage ϕ infection cycle

Koji Tsukada

Evolution and Life System Science Lab.,

Dept. of Biotechnology Graduate School of Engineering, Osaka University

S2-5

Attempt to see single molecule rotation of sodium transporting ATP synthase

Mizue Tanigawara (M. D course)

Dept. of Biotechnology Graduate School of Engineering, Osaka University

S2-6

Transformation of rice with large DNA fragments using a bio-active beads method

Naoki Wada (Ph. D course)

Dynamic Cell Biology Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S2-7

Prediction of steaming index in green tea industrial process by means of FT-NIR-based Metabolic fingerprinting

Rui KAWAHARA (Ph. D course)

Bioresource Engineering Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

Session 3

(Chairperson : Sang-Chul Lee)

S3-1

CaV3.1 is a Tremor Rhythm Pacemaker in the Inferior Olive

Young-Gyun Park (Ph. D course)

Neuragenetics Lab., Dept. of Biological Science, KAIST

S3-2

Enhancement of Sialylation of Recombinant Erythropoietin in Chinese Hamster Ovary Cells

Woo-Yong Shim (Ph. D course)

Cellular metabolic engineering Lab., Dept. of Biological Science, KAIST

S3-3

Intercellular trafficking of Vax1 homeotic transcription factor

Nam Suk Kim (Ph. D course)

Neural Development Lab., Dept. of Biological Science, KAIST

S3-4

Creating Dogs with Parkinson's diseases

Kang Ju Hyun (Ph. D course)

Molecular physiology Lab., Dept. of Biological Science, KAIST

S3-5

Enhanced interferon- β production by CHO cells through elevated osmolality and reduced culture temperature

Young Kue Han (Ph. D course)

Animal Cell Engineering Lab., Dept. of Biological Science, KAIST

S3-6

Caldibacter thermae gen. nov., sp. nov., Thermophile of a previously uncultured lineage of the Phylum Chloroflexi, Caldibacteria classis nov

Hyung-Gwan Lee (Ph. D course)

Environmental & Molecular Microbiology Lab., Dept. of Biological Science, KAIST

S3-7

Efficient Gene Delivery by PEGylated Adenovirus using Specific Cell-targeting Ligand and Avidin-Biotin Interaction

Jiwon Park (Ph. D course)

NanoBiomaterials Lab., Dept. of Biological Science, KAIST

Session 4

(Chairperson : Kazuteru Takagi)

S4-1

Identification of new genes involved in the PHO pathway in *Saccharomyces cerevisiae*

Ryosuke Kitakado (Ph. D course)

Molecular Genetics Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S4-2

Subtilisin from the hyperthermophilic archaeon with modulated maturation process

Shun-ichi Tanaka (Ph. D course)

Molecular Biotechnology Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S4-3

Quality assessment of commercial *Angelica acutiloba* roots through the application of metabolomics techniques

Sukanda Tianniam (Ph. D course)

Bioresource Engineering Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S4-4

Genome-wide identification of genes necessary for tolerance to lactic acid in yeast *Saccharomyces cerevisiae*

Suzuki Toshihiro (Ph. D course)

Molecular Genetics Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S4-5

***Escherichia coli* single-gene knockout mutants which are able to enhance P450 activity in whole-cell biocatalysis**

Ying Zhou (Ph. D course)

Biochemical Engineering Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S4-6

Roles of calcium ions on folding and stability of Tk-subtilisin

Yuki Takeuchi (M. D course)

Molecular Biotechnology Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

S4-7

Development of a screening system for α -glucosidase-displaying yeasts using alginate beads

Zhou Yan (Ph. D course)

Dynamic Cell Biology Lab., Dept. of Biotechnology Graduate School of Engineering, Osaka University

Session 5

(Chairperson : Kyuri Lee)

S5-1

Designing the substrate specificity of D-hydantoinase using a rational approach

Sang-Chul Lee (Ph.D. course)

Biomolecular Engineering of Lab., Dept. of Biological Science, KAIST

S5-2

Parent of origin specific gene expression changes in 39,XO mice suggest perturbations of common neural systems in Turner's syndrome, autism, and schizophrenia

Ji Yun Song (Ph.D course)

Epigenetics Lab., Dept. of Biological Science, KAIST

S5-3

cAMP signalling in mushroom bodies modulates temperature preference behaviour in Drosophila.

Sung-Tae, Hong

Neural Network of Cognition Lab., Dept. of Biological Science, KAIST

S5-4

Magnetic bead based immunoassay with enzymes immobilized in mesocellular carbon foam.

Yunxian, Piao(Ph.D course)

Biomolecular Engineering Lab., Dept. of Biological Science, KAIST

S5-5

Vadicola ignavus gen. nov., sp. nov., the member of Actinomycetaceae family, first isolated from environment

Liang Wang (Ph.D course)

Environmental & Molecular Microbiology Lab., Dept. of Biological Science, KAIST

S5-6

Modulation of MEK/ERK and BMP4 signaling pathways induces efficiently differentiation of human embryonic stem cells toward hemangioblast-like cells

Sang-Wook Park (Ph.D course)

Development and Differentiation Lab., Dept. of Biological Science, KAIST

Closing remarks

Prof. Kwang-Wook Choi

Developmental Genetics Lab., Dept. Biological Science, KAIST

S1-1: High level expression of an antimicrobial peptide histonin as a natural form by multimerization and furin-mediated cleavage

Laboratory of Molecular Biotechnology

Ju Ri Shin

Direct expression of an antimicrobial peptide (AMP) in *Escherichia coli* causes several problems such as the toxicity of AMP to the host cell and its susceptibility to proteolytic degradation. To overcome these problems and produce a large quantity of a potent AMP histonin (RAGLQFPVGKLLKLLKRLKR) in *E.coli*, an efficient expression system was developed, in which the toxicity of histonin was neutralized by a fusion partner F4 (a truncated fragment of PurF protein) and the productivity was increased by a multimeric expression of a histonin gene. The expression level of the fusion peptide reached a maximum with a 12-mer of a histonin gene. Because of the RLKR residues present at the C-terminus of histonin, Furin cleavage of the multimeric histonin expressed produces an intact, natural histonin. The AMP activity of the histonin produced in *E.coli* was identical to that of a synthetic histonin. With our expression system, 167 mg of histonin was obtained from 1L of *E.coli* culture. These results may lead to a cost-effective solution for the mass production of AMPs that are toxic to a host.

S1-2: Constitutive Expression of an Endogenous Xylose Reductase Gene in *Candida tropicalis* by Using a Promoter of Glyceraldehyde-3-Phosphate Dehydrogenase

*Laboratory of Cellular Metabolic Engineering
Irshad Ahmaa*

Xylose reductase (XR) is a key enzyme in D-xylose metabolism, catalyzing the reduction of D-xylose to xylitol. Expression of XR in *Candida tropicalis* is significantly repressed in cells grown on glucose by catabolic repression. This is one of the reasons that glucose cannot be used as a cosubstrate in xylitol production. A gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was isolated from the genomic DNA of *C. tropicalis* ATCC 20913 to construct a constitutive expression cassette for this strain. The endogenous XR gene (*xyl1*) was placed under the control of GAPDH promoter and integrated into the genome of *C. tropicalis* N43 which is *xyl2*-disrupted and *xyl1*-partially disrupted mutant. Expression of XR was confirmed by determining enzyme activity in cells grown on a medium containing glucose as a carbon source. The resulting recombinant yeast, *C. tropicalis* JA10, showed higher XR activity (668mU/mg of proteins) than that of the parental strain (23mU/mg of proteins).

S1-3: Effect of Bcl-x_L overexpression on apoptosis and autophagy in recombinant Chinese hamster ovary cells under nutrient-deprived condition

*Laboratory of Animal Cell Engineering
Yeon-Gu Kim*

Upon nutrient deprivation during culture, recombinant Chinese hamster ovary (rCHO) cells are subjected to two types of programmed cell death (PCD), apoptosis and autophagy. To investigate the effect of Bcl-x_L overexpression on apoptosis and autophagy in rCHO cells, an erythropoietin (EPO)-producing rCHO cell line with regulated Bcl-x_L overexpression (EPO-off-Bcl-x_L) was established using the Tet-off system. The expression level of Bcl-x_L in EPO-off-Bcl-x_L cells was tightly regulated by doxycycline in a dose-dependent manner. Bcl-x_L overexpression enhanced cell viability and extended culture longevity in batch culture. Upon nutrient depletion in the later stage of batch culture, Bcl-x_L overexpression suppressed apoptosis by inhibiting the activation of caspase-3 and -7. Simultaneously, Bcl-x_L overexpression also delayed autophagy, characterized by LC3-II accumulation. Immunoprecipitation analysis with a Flag-tagged Bcl-x_L revealed that Bcl-x_L interacts with Bax and Bak, essential mediators of caspase-dependent apoptosis, as well as with Beclin-1, an essential mediator of autophagy, and inhibits their function. Taken together, it was found that Bcl-x_L overexpression inhibits both apoptosis and autophagy in rCHO cell culture.

S1-4: SAGA targeting stimulation by proteasomal ATPase during transcription initiation: Proteasomal ATPase Rpt2p interacts functionally with Sgf73p in SAGA complex

*Laboratory of Chromatin and Epigenomics
Jaechan Kwak*

The proteasome is a protein degradation machinery which contains a 26S catalytic core capped by a 19S regulatory particle at either end. The regulatory particle consists of the base and the lid sub-complexes. We have already shown that the 19S proteasome is involved in the SAGA complex functioning. SAGA complex is an essential transcriptional coactivator that facilitates formation of the transcriptional pre-initiation complex. The purified 19S proteasome stimulated interactions between the SAGA complex and transcriptional activators, and increased SAGA complex recruitment to DNA. We used in vitro binding assay to verify specific interaction between 19S proteasome and SAGA complex. The proteasomal ATPase subunit RPT2 interacts with SAGA subunit SGF73. Interestingly, basic patch of Rpt2p interacts with acidic patch of Sgf73p which means this interaction is electrostatic. Finally, *sug1-25* and deleted *sgf73* showed a synthetic sick phenotype, indicating that proteasomal ATPase genetically interacts with Sgf73. We are now focusing on in vivo assays using SAGA mutant and proteasome strains.

S1-5: ER71 Acts Downstream of BMP, Notch, and Wnt Signaling in Blood and Vessel Progenitor Specification

*Laboratory of Molecular Genetics
Dongjun Lee*

FLK1-expressing (FLK1⁺) mesoderm generates blood and vessels. Here, we show that combined BMP, Notch, and Wnt signaling is necessary for efficient FLK1⁺ mesoderm formation from embryonic stem cells (ESCs). Inhibition of BMP, Notch, and Wnt signaling pathways greatly decreased the generation of FLK1⁺ mesoderm and expression of the Ets transcription factor Er71. Enforced expression of ER71 in ESCs resulted in a robust induction of FLK1⁺ mesoderm; rescued the generation of FLK1⁺ mesoderm when blocked by BMP, Notch, and Wnt inhibition; and enhanced hematopoietic and endothelial cell generation. Er71-deficient mice had greatly reduced FLK1 expression, died early in gestation, and displayed severe blood and vessel defects that are highly reminiscent of the Flk1 null mouse phenotype. Collectively, we provide compelling evidence that ER71 functions downstream of BMP, Notch, and Wnt signals and regulates FLK1⁺ mesoderm, blood, and vessel development.

S1-6: Molecular mechanism of familial Parkinson's disease

*Laboratory of Molecular Genomics
Nguyen Van Nhung*

Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by movement disorders and a loss of DA neurons. It mostly occurs sporadically by environmental toxins, but also genetically by mutations in a number of genes including *alpha-synuclein*, *LRRK2*, *parkin*, *PINK1*, *DJ-1* and *ATP13A2*. Recent PD studies have focused on studying the functions of these genes to investigate its central cause and suggested the following such as protein misfolding, abnormal protein accumulation, oxidative stress, and mitochondrial dysfunction. Among them, accumulating evidence has strongly suggested mitochondrial dysfunction as the primary cause for PD pathogenesis. More recently, we and others have demonstrated that mitochondrial dysfunction is a critical event toward PD pathology in *Drosophila*. Furthermore, PINK1 and Parkin are genetically linked in a linear pathway with Parkin acting downstream of PINK1. In parallel with the *Drosophila* results, mitochondrial dysfunction induced by PINK1 siRNA knockdown was markedly rescued by Parkin expression in the mammalian system, demonstrating the conservation of the PINK1-Parkin pathway between flies and mammals. This link between the two genetic factors has opened up a new avenue for the research of Parkinson's disease. However, major questions that should be addressed are the mechanism by which PINK1 regulates Parkin and of how they protect mitochondria. In our recent study, we therefore investigated the detailed relationship between PINK1 and Parkin and their function in the mitochondria. Surprisingly, PINK1 regulates the localization of Parkin to the mitochondria in its kinase activity-dependent manner. In detail, Parkin phosphorylation by PINK1 on its linker region promotes its mitochondrial translocation, and the RING1 domain of Parkin is critical for this occurrence. Finally, we demonstrate that mitochondria-translocated Parkin induces mitochondrial aggregation. These results demonstrate the biochemical relationship between PINK1, Parkin and the mitochondria, and thereby suggest the possible mechanism of PINK-Parkin-associated PD pathogenesis.

S1-7: Magnetic force driven enhanced siRNA-ss-PEG delivery using Pluronic/PEI nanocapsule encapsulating iron oxide nanoparticles

*Laboratory of NanoBiomaterial
Kyuri Lee*

Small interfering RNA (siRNA) has been attracted much attention for their effective gene silencing effect suppressing specific target protein in a post-transcriptional mRNA level. siRNA has substantial advantages for gene therapy of various diseases such as cancer and genetic disorders due to their excellent sequence specific powerful gene suppression capability with small amount. However, poor intracellular delivery, failure in endosomal escape and rapid enzymatic degradation in serum condition have restricted their various potential in vitro and in vivo applications. Previously, we demonstrated that PEGylated siRNA having cleavage disulfide bond between siRNA and PEG (siRNA-ss-PEG) greatly increased the serum stability when it was condensed into core-shell type PEC micelles with various cationic polymers. To further improve its efficacy, the multi-functional gene delivery vehicle introduced various functional agents is required.

Recently, inorganic nanoparticles, such as iron oxide nanoparticles, gold nanoparticles and Quantum dots were utilized as new tools for effective multi-functional drug delivery systems due to their unique magnetic, imaging properties. In particular, superparamagnetic iron oxide nanocrystals (e.g. γ -Fe₂O₃, Fe₃O₄) have been attracted for various bio-medical applications including hyperthermal therapy, magnetically driven drug delivery and magnetic resonance imaging (MRI). The magnetic properties of magnetic nanoparticles offer a feasible possibility to develop an enhanced drug or gene delivery system that can effectively deliver such agents to the cells by a magnetic force. In advance, by further adding various functional molecules such as anti-cancer drug, targeting moiety and imaging agent to magnetic nanoparticles, we can construct multi-functional nanoparticles that have sufficient magnetic properties for further application.

In this study, we developed Pluronic/PEI nanocapsules encapsulating oleic-acid coated iron oxide nanoparticles (PPMCs). The hydrophobic oleic-acid coated iron oxide nanoparticles were successfully dispersed in aqueous phase forming spherical core-shell structure with Pluronic nanocapsules shell crosslinked by PEI. The PEGylated siRNA containing disulfide bond (siRNA-ss-PEG) was electrostatically complexed with PPMCs, producing stable PPMCs/siRNA-ss-PEG complex while PEG would be placed outside of PPMCs. PPMCs and siRNA that complexed with PPMCs were pulled down to where the external magnetic force was exist. And in *in vitro* test, PPMCs exhibited improved cellular uptake efficiency and when it complexed with siRNA-ss-PEG, the cellular uptake of siRNA was also increased. In addition, enhanced GFP gene suppression effect of siRNA-ss-PEG was determined when PPMCs/siRNA-ss-PEG were transfected under external magnetic force, suggesting it can be effectively utilized as a novel siRNA delivery vehicle.

S2-1: Construction of a BAC-based chromosome physical map of CHO-DG44 cell

*Laboratory of Biochemical Engineering
Cao Yihua*

The cell lines derived from Chinese hamster are widely used in scientific research, especially Chinese hamster ovary (CHO) cells which are used in biopharmaceutical production. However the genomic information of CHO cell is not well analyzed while *Homo sapiens* genome has already been analyzed completely. It has been reported that Chinese hamster primary diploid cells have 22 chromosomes. The CHO-DG44 cell line has 20 chromosomes and chromosomal aberrations are frequently associated with the establishment of recombinant CHO cell lines.

In previous study, we constructed a CHO genomic bacterial artificial chromosome (BAC) library from a mouse dihydrofolate reductase (*Dhfr*) gene-amplified CHO DR1000L-4N cell line and later used it in combination with fluorescent *in situ* hybridization (BAC-FISH) for identifying the 20 individual chromosomes of CHO-DG44 cells. We found that chromosome A and B correspond to normal Chinese hamster chromosome 1, yet no other paired chromosomes could be identified because of remarkable chromosomal aberrations observed in the CHO-DG44 cells. After mapping more than 300 BAC clones to the chromosomes of CHO-DG44, a BAC-based physical map for each chromosome was constructed. According to this map, both chromosomes C and D were hybridized with same 16 BAC clones in same positions. The shorter chromosome D likely corresponded to long arm of chromosome C. The BAC-based chromosome map will be a powerful tool for research with CHO chromosome rearrangement during the establishment of recombinant CHO cell lines.

S2-2: Photoresponse analysis of phytochrome A in a non-photosynthetic parasitic plant, *Orobanche minor* Sm.

Laboratory of Cell Technology

Kazuteru TAKAGI

Light induces various morphological responses. Photoreceptors are involved in these responses. Among photoreceptors, phytochromes are red and far-red light photoreceptors. After light perception, phytochromes localize from cytoplasm to nucleus and control the expression of downstream genes. The entire network of light signaling has not yet been clear, because of its complexity. We focused on phytochromes in a non-photosynthetic parasitic plant, *Orobanche minor*. This plant cannot photosynthesize, and acquires energy from their host plants. Therefore, it is thought that *O. minor* has a light signaling system containing altered parts that are related to photosynthesis control, but retaining the function necessary to regulate morphogenesis. Amino acid sequence of phytochrome A (phyA) from *O. minor* (*OmphyA*) was compared to that of phytochrome A from *Arabidopsis thaliana* (*AtphyA*). The result revealed that *OmphyA* has 71% sequence identity and 26 amino acid substitutions which cannot be found in other photosynthetic plants. These substitutions are largely located in/around functional domains. For further analysis, we compared the light responses of *O. minor* with photosynthetic plants to find functional differences between *OmphyA* and other phyAs.

First we confirmed intracellular localization and gene expression pattern of *OmphyA* under various light conditions. As a result, it was revealed that at the molecular level *OmphyA* behave as other phyAs. Next we tested photoresponses of *O. minor* in germination, anthocyanin accumulation and stem elongation under several light conditions. Interestingly red light had no effect but far-red light inhibited the stem elongation indicating red light high-irradiance responses should be lacked during this stage in *O. minor*. Lastly we compared the gene expression involved in phyA signaling between *OmphyA* and *AtphyA* transfected *A. thaliana* protoplasts using qRT-PCR. It was revealed that the expression levels of genes involved in phyA signaling were different in *OmphyA* and *AtphyA* transfected protoplasts.

These results suggest that *OmphyA* function is different from that of *AtphyA*, and these differences may attribute to amino acid substitutions of *OmphyA*. Transcriptome and metabolome analyses are in progress using *OmPHYA* introduced *phyA* mutant of *A. thaliana*.

S2-3: Entomopathogenic Fungi as a Potential Bioresources of Bioactive Compounds

International Center for Biotechnology
Kei-ichi Ishidoh

Natural products obtained from microbes were widely used in pharmaceutical industry and in agroindustry. Although majority of the known bioactive compounds comes from prokaryotic species belonging to Actinomycetes, filamentous fungi have as well high potential as the producer of bioactive compounds, because whole genome information revealed that they have equivalent number of gene clusters of secondary metabolites.

Entomopathogenic fungi are ecologically classified as fungi that grow either inside of insect bodies or on the surface of their exoskeleton, which eventually causes the death of the host insect. One well-known species among these fungi is *Cordyceps sinensis*, and the insect complex of this species is designated as Tochu-Kaso which has been used as a traditional Chinese herbal medicine for more than 1500 years in both China and in Japan. However only very limited information is available with respect to the bioactive compounds produced by entomopathogenic fungi due to a difficulty of collecting such rare natural fungi.

A total of 49 strains, which were isolated from various areas in Japan and belonging to 9 genera of entomopathogenic fungi were used in this study aiming for finding novel bioactive compounds. Culture extracts of entomopathogenic fungi were analyzed by HPLC equipped with a photodiode array detector, and the basal or partial structures of detected compounds were predicted by the reference to the database which consists of UV spectra of secondary metabolites from microorganisms. Several compounds which have unpredictable structures were selected as candidates of novel compounds, purified and the structures of them were elucidated on the basis of spectroscopic analyses and chemical conversions.

As a result of this study, we found new derivatives of ATPase-inhibitory compound aurovertin from *Metarhizium anisopliae*. Biological assay using radish revealed that these compounds have herbicidal activities. Additionally, compounds obtained from culture broth of *Paecilomyces farinosus* and *Metarhizium flavoviride* were structurally elucidated based on their NMR spectra, and were identified to antimicrobial antibiotic aranorosinol and insecticidal, fungicidal compound N-(2-methyl-3-oxodecanoyl)-2-pyrroline together with its unsaturated derivative, respectively, while they were known compounds.

The result of this study gives new insight into the secondary metabolites produced by entomopathogenic fungi, and affirms the potential of them as good bioresources for the discovery of bioactive compounds.

Azumi, M. et al., Aurovertins F–H from the Entomopathogenic Fungus *Metarhizium anisopliae*. *J Nat Prod.* 2008 Feb;71(2):278-80

S2-4: Quantitative analysis of the bacteriophage Q β infection cycle

Laboratory of Evolution and Life System Science
Koji Tsukada

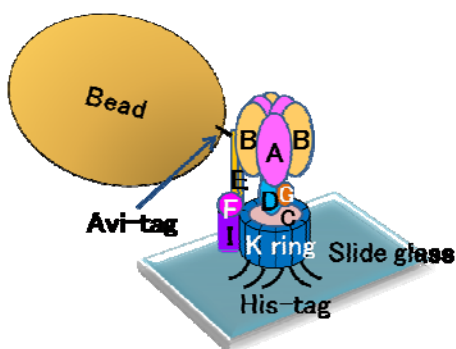
Viruses propagate themselves via an infection cycle. Since the basic properties of life are observed in this cycle, the study of viruses has long been an attractive field of research. In this study, the infection cycle of bacteriophage Q β was investigated. Adsorption of bacteriophage Q β to *Escherichia coli* is explained in terms of a collision reaction, the rate constant of which was estimated to be 4×10^{-10} ml/cells/min. In infected cells, approximately 130 molecules of β -subunit and 2×10^5 molecules of coat protein were translated in 15 min. Replication of Q β RNA proceeded in 2 steps—an exponential phase until 20 min and a non-exponential phase after 30 min. Prior to the burst of infected cells, phage RNAs and coat proteins accumulated in the cells at an average of up to 2300 molecules and 5×10^5 molecules, respectively. An average of 90 infectious phage particles per infected cell was released during a single infection cycle up to 105 min. The time distribution of progeny phage release was over 1 h. Therefore, the average number of released progeny phage was only 3–4% of the numbers of coat proteins or phage RNA that had accumulated in the infected cells by 45 min. It is interesting to assess whether the overproduction of phage proteins and RNA plays an important role in the survival and evolvability of the infectious Q β phage. Bacteriophage replication in particular has proved to be a good model system for understanding the principles of heredity, multiplication, and adaptive evolution, and research using these organisms has yielded a number of important findings. This quantitative information will make a significant contribution to our understanding of the mechanism of RNA bacteriophage proliferation, population of RNA phage quasispecies, and evolvability of RNA phage.

Tsukada et al. (2009), *Biochim. Biophys. Acta* 1790, 65-70.

S2-5: Attempt to see single molecule rotation of sodium transporting ATP synthase

Mizue Tanigawara

There are two types of ion transporting ATPases, F type ATPase and V type ATPase (vacuolar ATPase). F-ATPase which functions as an ATP synthase is found in mitochondria, chloroplast, and oxidative bacteria. V-ATPase functions mainly as a proton pump in acidic organelles, plasma membrane of eukaryotic cells. F-type and V-type ATPases resemble each other both structurally and functionally. V-ATPase consists of water soluble part of V1 and the membrane integrated sector Vo. Since isolated V1 from VoV1 complex rotates counter-clockwise hydrolyzing ATP, it is just called V-ATPase. ATP hydrolysis by V1 couples with the translocation of proton by Vo to establish the electro chemical proton potential across the membrane that serve as the proton motive force for many proton-coupled transporters. However, how these two molecular motor conjugate each other have yet to be seen; how much protons translocate by hydrolyzing one ATP? Simultaneous observation of a step of V1 which is generated by ATP binding and/or hydrolysis and the step of Vo which resulted in the binding and release of proton will provide an answer to this question. As the binding rate of proton to Vo is too fast to see its elementary step, it is necessary to decrease the rotation speed of Fo by changing the proton concentration in solution (this is consistent with pH). However, it is not a good idea to diverse pH because it results in denature of the protein. Here, a V-ATPase from *Enterococcus hirae* transports Na⁺ rather than H⁺ under physiological condition (called Na⁺-V_oV₁). Its ATPase activity strongly depends on the concentration of Na⁺ (Murata et al., 2001, JBC). To control the concentration of Na⁺ is much easier than that of H⁺. It is expected that the rotation speed of Vo will decreased enough to see Na⁺ binding step by decreasing the Na⁺ concentration in solution. The motivation of this study is to investigate the conjugation between two motors (ion/ATP) by observing the steps derived from ATP binding to V1 and Na⁺ binding to Vo simultaneously. We have constructed the system to see the rotation of Na⁺-V_oV₁. As a result, the rotation of Na⁺-VoV1 under the microscope was observed for the first time. This is the evidence that Na⁺-VoV1 is also the rotary molecular motor similar to FoF1 ATP synthase. However, the frequency to find the rotation was 1000 times lower than that of F1-ATPase. So, the reason why the rotating frequency was so low was investigated. As a result, the tag which binds the rotation probe proved to be not functional.



Experimental system of single molecule observation of Na⁺-VoV1. His-tag inserted into K subunit interacts Ni-NTA coated slide glass, and avi-tag which binds biotin was inserted in E subunit to attach on the streptavidin coated bead as a rotation probe.

S2-6: Transformation of rice with large DNA fragments using a bio-active beads method

*Laboratory of Dynamic Cell Biology
Naoki Wada*

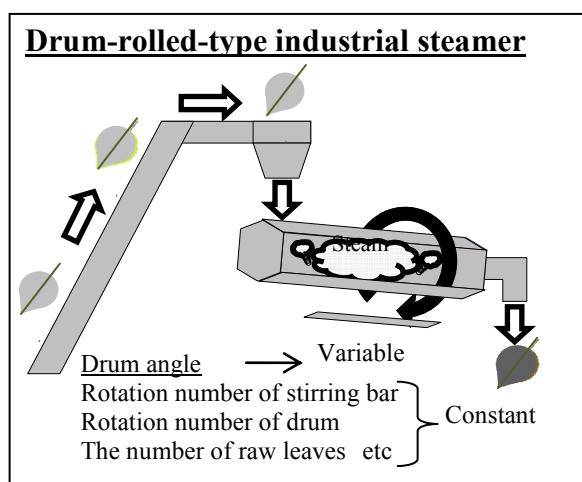
Transformation with large DNA molecules enables multiple genes to be introduced into plants simultaneously to produce useful transgenic plants with complex new phenotypes. The development of the transformation technology with large sized DNAs would also contribute to the application of plant artificial chromosomes which will offer multiple gene transfer at once and enable us to avoid position effect and rearrangement of transgenes in plant genome. These advantages will contribute to ease anxieties over genetically modified organisms by controlling the event more precisely during transformation processes. However, successful transformations of rice plants with large DNA molecules (>100 kb) have so far been limited.

In this study, BAC DNA (*ca.* 100 kb) containing a set of wheat hardness genes (*puroindoline a*, *puroindoline b* *GSP-1 gene*) was introduced into rice plants using a novel transformation method, i.e., bioactive bead method, which was developed previously by our group. As a result, nine transgenic rice plants were successfully obtained demonstrating that the method could transform plants using large DNA fragments. The presence of transgenes in the rice genome was confirmed by PCR, Southern blot, and FISH analyses. The results suggested that multiple transgenes were successfully integrated in all transgenic plants although rearrangement of transgenes occasionally happened. The presence of transgenes and their segregation were also investigated in the T₁ and T₂ generations. These results indicated that the transgenes were stably inherited in the successive generation. The expression of one of the transgenes, *puroindoline b*, was confirmed at the mRNA level and protein level in the T₂ generation. Our study thus clearly demonstrates that the bioactive beads method is capable of producing transgenic rice plants using the large sized DNA fragments.

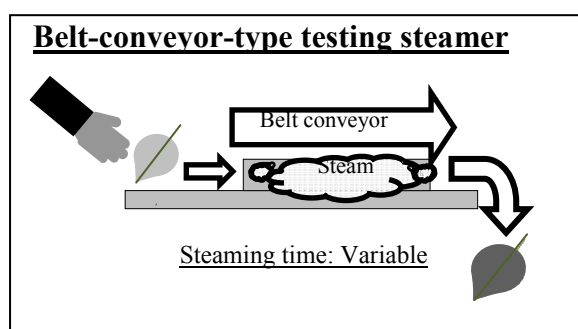
S2-7: Prediction of steaming index in green tea industrial process by means of FT-NIR-based Metabolic fingerprinting

Laboratory of Bioresource Engineering
Rui KAWAHARA

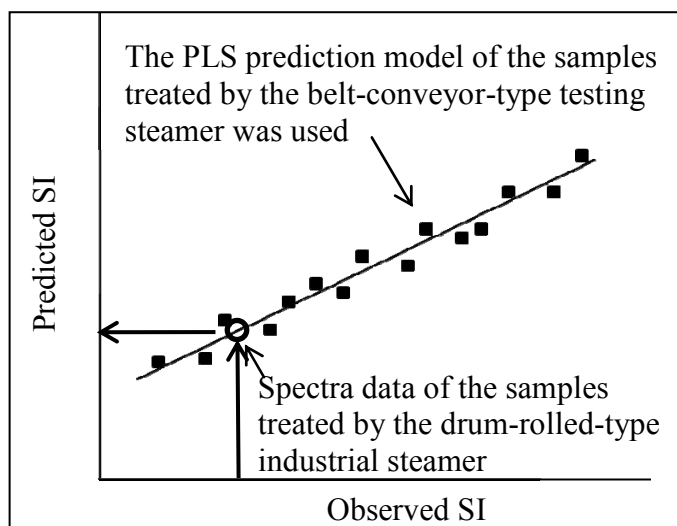
We successfully developed a technique in metabolic fingerprinting for determining the optimum operational parameters of an industrial green tea steamer; the use of this technique does not require any specialized skills. The steaming process has been thought to be the most significant step for determining the quality of green tea products. The quality of green tea can deteriorate by incorrect setting of the operational parameters of a factory machine used in the steaming process. Therefore, it is essential to develop a simple and robust system for setting the operational parameters for the steaming process. Metabolic fingerprinting based on pattern recognition method was employed to our research. We attempted to predict the steaming index (SI) of the leaf by developing a PLS regression model on the basis of the FT-NIR spectrum of the steamed leaf, which is used as a predictor. The PLS regression model was developed to predict the SI as a responsive variant from the FT-NIR spectra of tea leaf which is steamed by various parameters. This is the first study that proves the usefulness of the SI, which is a novel non-dimensional number, for optimizing the industrial condition required for the production of green tea.



Description of drum-rolled-type industrial steamer. This steamer is used in the real manufacturing process. A large number of tea leaves can be steamed simultaneously in this steamer.



Description of belt-conveyor-type testing steamer. This steamer has one operational parameter, namely, steaming time.



S3-1: CaV3.1 is a Tremor Rhythm Pacemaker in the Inferior Olive

*Neuragenetics laboratory
Young-Gyun Park*

Tremor is characterized by involuntary rhythmic muscle contractions that interfere with body control; however, the neural pacemaker underlying tremor rhythm remains obscure. Here, we report that mice lacking CaV3.1 T-type Ca²⁺ channels were resistant to a pharmacological model of essential tremor induced by harmaline. They also lacked the 4–10 Hz tremor-related oscillations that initiate in the inferior olive and propagate to the cerebellum in wild-type mice. In addition, CaV3.1-mutant inferior olive neurons had significantly lower subthreshold oscillations and failed to trigger synchronous rhythmic burst discharges in response to the membrane hyperpolarization induced by harmaline; thus, the CaV3.1 channels seem to be the molecular pacemaker that triggers the intrinsic oscillations of inferior olive neurons, leading to the rhythmic stimulation of motor circuits that occurs in essential tremor.

S3-2: Enhancement of Sialylation of Recombinant Erythropoietin in Chinese Hamster Ovary Cells.

*Laboratory of cellular Metabolic Engineering
Woo-Yong Shim*

Sialic acid, the terminal sugar in *N*-linked complex glycans, is usually found in glycoproteins and plays a major role in determining the circulatory lifespan of glycoproteins. In this study, we attempted to enhance the sialylation of recombinant erythropoietin (EPO) in Chinese hamster ovary (CHO) cells. To enhance EPO sialylation, we introduced human α 2,3-sialyltransferase (α 2,3-ST) and CMP-sialic acid synthase (CMP-SAS) into recombinant human EPO-producing CHO cells. The sialylation of EPO was increased by the expression of α 2,3-ST alone. Although the co-expression of α 2,3-ST and CMP-SAS did not increase sialylation, an increase in the intracellular pool of CMP-sialic acid was noted. Based on these observations, it was postulated that the transport capacity of CMP-sialic acid into the Golgi lumen was limited, thereby causing the reduced availability of CMP-sialic acid substrate for sialylation. Therefore, we co-expressed human α 2,3-ST and CMP-SAS, as well as also over-expressed Chinese hamster CMP-sialic acid transporter (CMP-SAT) in CHO cells, which produced recombinant human EPO. When α 2,3-ST, CMP-SAS, and CMP-SAT were over-expressed in CHO cells, there was a corresponding increase in sialylation compared to the co-expression of α 2,3-ST and CMP-SAS. The present study provides a useful strategy to enhance the sialylation of therapeutic glycoproteins produced in CHO cells.

S3-3: Intercellular trafficking of Vax1 homeotic transcription factor

*Laboratory of Neural Development
Nam Suk Kim*

Cells in the developing embryos keep in communication with their neighboring cells via direct interaction or indirect signaling processes. Especially, the reciprocal communication between neurons and glia is essential for their proper differentiation, survival, and migration into the specific target sites. The ventral anterior homeobox 1 (Vax1) transcription factor has been known to contribute to the development of nervous systems not only by facilitating glial fate acquisition through autonomous transcriptional regulation of cell fate determinants, but also by regulating the migration of neurons in a nonautonomous manner. We found that Vax1 protein can be secreted out from astrocytes to extracellular space, and then move into the neighboring neurons in mouse forebrain and optic nerve. Vax1 protein that is released into extracellular space was detected in exosome-rich fraction. These results indicate that Vax1 protein is secreted out in forms of vesicles instead of naked protein. Moreover, we found that the Vax1 secretion is facilitated by blocking either lysosomal degradation or trans-Golgi exocytosis pathway. Together, our finding intrigues the existence of non-autonomous regulation of neural development by Vax1 protein, which travels among glia and neurons

S3-4: Creating Dogs with Parkinson's diseases

*Laboratory of molecular physiology
Kang Ju Hyun*

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. It is characterized clinically by tremor, rigidity, bradykinesia (slowness of movement), and postural instability. The pathological hallmarks of the disease include the accumulation of cytoplasmic aggregates known as Lewy bodies in relatively specific brain regions, such as the substantia nigra and locus ceruleus, as well as the loss of dopaminergic neurons. The cause of PD is still unknown, but many factors have been shown to contribute to the development of disease which include aging, environment, and genes. Recent evidences indicate that at least 10 distinct genetic loci, *PARK1-PARK10*, are linked to the familial forms of PD. Among them, DJ-1, Parkin, and Pink1 are closely related to early onset Parkinson's disease. There were studies to find the cause of disease with knock out mouse model. However, the mouse did not show any significant symptom found in human. Our aim is to establish a canine disease model for Parkinson's, for dog shares many features of human disease. So far, we obtained canine clones containing genes causing the disease in human from the canine gene library and generated targeting clones, which will be used to transfect canine adult stem cells.

S3-5: Enhanced interferon- β production by CHO cells through elevated osmolality and reduced culture temperature

*Laboratory of Animal Cell Engineering
Young Kue*

For efficient production of native interferon- β (IFN- β) in recombinant CHO cell culture, the IFN- β molecular aggregation that occurs during culture needs to be minimized. To do so, we investigated the effect of hyperosmolality and hypothermia on IFN- β production and molecular aggregation in rCHO cell culture. Both hyperosmolality (470 mOsm/kg) and hypothermia (32°C) increased specific native INF- β productivity ($q_{\text{IFN-}\beta}$). Furthermore, they decreased the IFN- β molecular aggregation, although severe IFN- β molecular aggregation could not be avoided in the later phase of culture. To overcome growth suppression at hyperosmolality and hypothermia, cells were cultivated in a biphasic mode. Cells were first cultivated at 310 mOsm/kg and 37°C for 2 days to rapidly obtain a reasonably high cell concentration. The temperature and osmolality were then shifted to 32°C and 470 mOsm/kg, respectively, to achieve high $q_{\text{IFN-}\beta}$ and reduced IFN- β molecular aggregation. Due to the enhanced $q_{\text{IFN-}\beta}$ and delayed molecular aggregation, the highest native IFN- β concentration achieved on day 6 was 18.03 ± 0.61 mg/L, which was 5.30-fold higher than that in a control batch culture (310 mOsm/kg and 37°C). Taken together, a combination of hyperosmolality and hypothermia in a biphasic culture is a useful strategy for improved native IFN- β production from rCHO cells.

S3-6: *Caldibacter thermae* gen. nov., sp. nov., Thermophile of a previously uncultured lineage of the Phylum *Chloroflexi*, *Caldibacteria* classis nov.

Laboratory of Environmental & Molecular Microbiology
Hyung-Gwan Lee

A thermophilic, Gram-negative, non-spore-forming and rod-shaped bacterium, designated BM6-13 was isolated from a hot spring near the dormant volcano of Changbai mountain(N42°00'22", E128°03'24") in China. Growth occurs at 45-65°C, and the optimum temperature is 55-60°C. The optimum pH range for growth is 7.0-7.5. Phylogenetic analyses based on 16S rRNA gene sequence revealed that this strain belongs to an uncultured, previously recognized clone lineage of the phylum *Chloroflexi*. The bacterial phylum *Chloroflexi* has been recognized as a typical bacterial lineage that contains a number of uncultured environmental 16S rRNA gene clones with a few cultured representatives. Hitherto six major classes (subphyla) of the *Chloroflexi* have been resolved by comparative analysis of 16S rRNA genes. To date, only four of these classes have validated pure-culture representatives. We here propose a new genus and species, *Caldibacter thermae* gen. nov., sp. nov., with isolate BM6-13 as the type strain, and a new class for the subphylum to which it belongs, *Caldibacteria* classis nov. We also propose the subordinate taxa *Caldibacterales* ord. nov., *Caldibacteraceae* fam. nov.

S3-7: Efficient Gene Delivery by PEGylated Adenovirus using Specific Cell-targeting Ligand and Avidin-Biotin Interaction

*Laboratory of NanoBiomaterials
Jiwon Park*

Gene delivery systems using adenovirus vector exhibit high transduction efficiency and gene expression. However, the clinical application of adenovirus vector has some problems such as wide biodistribution of adenovirus and immune responses. In this study, the adenovirus (Ad) expressing the green fluorescent protein (GFP) was modified by polyethylene glycol (PEG) to reduce toxicity and immunogenicity. In addition, epidermal growth factor (EGF) was used as specific cell-targeting ligand to overcome problematic nonspecific transduction. Pegylated EGF was attached on the surface of adenovirus using avidin-biotin interaction. We confirmed that Ad-PEG-EGF complexes increased GFP expression level compared to Ad-PEG in EGF receptor positive cell line (A431 cell), but not in EGF receptor negative cell line (MCF-7 cell). Thus, the adenovirus immobilized with PEG-EGF based on biotin-avidin interaction both enhanced stability from nonspecific immune system and transfection efficiency on the specific cell line.

S4-1: Identification of new genes involved in the PHO pathway in *Saccharomyces cerevisiae*

Laboratory of Molecular Genetics
Ryosuke Kitakado

In *Saccharomyces cerevisiae*, the phosphate signal transduction pathway (PHO pathway) is involved in regulating several phosphate-responsive genes such as *PHO5*, encoding repressible acid phosphatase, and *PHO84*, encoding a major inorganic phosphate (Pi) transporter. In this pathway, a cyclin-dependent kinase inhibitor (Pho81) regulates the kinase activity of the cyclin (Pho80)-cyclin dependent kinase (Pho85) complex Pho80p-Pho85p, which phosphorylates the transcription activator Pho4p in response to intracellular phosphate levels. We reported that *PHO5* expression is strongly correlated with the intracellular phosphate levels and that the signaling defect in the *pho84* strain is likely to result from insufficient intracellular phosphate caused by a defect in phosphate uptake (1). Although the PHO pathway has been intensively investigated, how cells sense phosphate availability and transduce the phosphate signal to Pho81 still remains to be solved as one of the most challenging problems.

To elucidate the mechanism of signal transduction between Pho84 and Pho81 in the *PHO* pathway, we have systematically screened 4,828 deletion strains. We found that disruptants of *PLC1*, *ARG82*, and *KCS1*, which are involved in the synthesis of inositol polyphosphate, and *ADK1*, which encodes adenylate kinase, constitutively express *PHO5* in Pho81-dependent manner, suggesting that each of these genes functions upstream of Pho81 (Fig.1). This raises the possibility that diphosphoinositol tetrakisphosphate (PP-IP4) and/or bisdiphosphoinositol triphosphate ((PP)2-IP3) is important for phosphate regulation (2). On the other hand, Lee et al. (2008) recently reported that myo-D-inositol heptakisphosphate (PP-IP5) plays a role as a cellular component that stimulates Pho81-dependent inhibition of Pho80-Pho85 (3). To identify other components between Pho84 and Pho81 in the PHO pathway, we have isolated 67 *sef* mutants (the suppressors of *PHO* eighty four), which do not express *PHO5* in high-Pi media but still express *PHO5* in low-Pi media, like a wild-type strain, from *pho84 Δ* strains. All of the *sef* mutations are recessive and hypostatic to the *pho81Δ* mutation. We attempted to clone *SEF* genes but it was not successful. Now, we are trying to identify chromosome

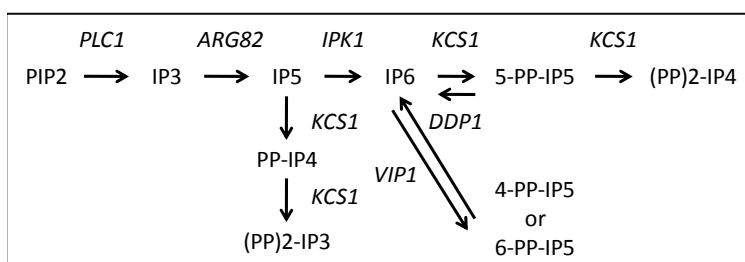


Fig. 1 Synthetic pathway of inositol polyphosphates

location of one of *sef* mutations, *sef6*, by chromosome arm loss mapping system. A preliminary result suggested that the *SEF6* gene may locate on the left arm of chromosome VIII, XI, XII, or XIV.

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S4-2: Subtilisin from the hyperthermophilic archaeon with modulated maturation process

Laboratory of Molecular Biotechnology
Shun-ichi Tanaka

Subtilisins have been widely used for industrial purposes as classical non-specific proteases. However, a common problem faced in industrial applications is ensuring the shelf life of the proteases. Active subtilisin is easily self-degraded due to its non specificity and high activity. Therefore, development of a method to keep subtilisin in an inactive state for long time and activate it at the time of use is desired.

Subtilisin is secreted in a pro-form with N-terminal propeptide, and matured upon autoprocessing and degradation of the propeptide. The propeptide inhibits the activity of a cognate subtilisin before and after autoprocessing. Therefore, inhibition of subtilisins by propeptides is a promising method to regulate subtilisin activity. Because propeptide is first autoprocessed from the mature domain and then degraded by it to produce active subtilisin, the best way to keep subtilisin in an inactive state is to keep it in a pro-form. However, subtilisin in a pro-form is usually unstable due to incomplete folding of the mature domain.

Tk-subtilisin from the hyperthermophilic archaeon *Thermococcus kodakaraensis* is a member of the subtilisin family. Our recent crystallographic studies show that the overall structure of Tk-subtilisin is similar to those of other subtilisins. However, the mature domain is completely folded to a highly stable structure before autoprocessing. This is due to the presence of a unique N-terminal insertion sequence of the mature domain. To take advantage of this unique folding mechanism of Tk-subtilisin, we attempted to construct the mutant protein of Tk-subtilisin, which is more resistant to autoprocessing than the wild-type protein.

According to the crystal structure of Tk-subtilisin, we constructed the mutant protein, in which the C-terminal residue of the propeptide in the S1 substrate pocket (Leu69) is replaced by Pro. It has been shown that subtilisins have the lowest preference for Pro in the P1 site. *In vitro* analysis showed that L69P mutant was not autoprocessed at the temperatures lower than 10°C. In contrast, this mutant was autoprocessed and efficiently matured at temperatures above 50°C. Based on these results, we propose that the combination of the introduction of a unique insertion sequence at the N-terminus of Tk-subtilisin and that of the proline residue at the C-terminus of the propeptide is an effective method to keep subtilisin in an inactive state and activate it at the time of use.

References

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S4-3: Quality assessment of commercial *Angelica acutiloba* roots through the application of metabolomics techniques

Laboratory of Bioresource Engineering
Sukanda Tianniam

Dried root of *Angelica acutiloba*, or toki in Japanese, is a folk medicinal plant that has become one of the important and popular pharmaceutical products possessing significant therapeutic traits- these include curing numerous women's gynecological diseases and potent immunomodulatory activities which is utilized in unison with conventional cancer therapy. Traditionally, experienced herbalists are employed to "judge" its quality with respect to appearance, flavor and olfactory characteristics which is inadequate for large-scale industrialized toki quality assessment. Metabolomics is a technique where it involves the complete evaluation of the biochemical fingerprints or profiles found in a biological sample in order to comprehend and discriminate the differences in status or origin through the application of statistical multivariate pattern recognition methods (chemometrics). This method was employed and investigated in order to establish an alternative efficient, reliable, automated-scientific-based QC procedure for the quality determination of *Angelica* roots in commercial markets. The utilization of high throughput analytical platforms, such as gas chromatography mass spectrometry (GC-MS), pyrolysis GC-MS and ultra-performance liquid chromatography mass spectrometry (UPLC-MS), were applied and results have shown distinct discrimination between the metabolic fingerprints of various *Angelica acutiloba* roots.

Fig. 1 GC-MS analysis of Toki

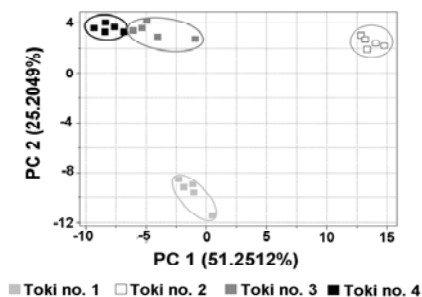


Fig. 2 Pyrolysis GC-MS analysis of Toki

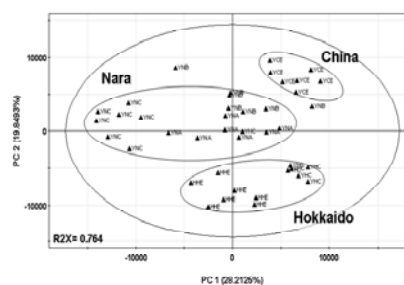
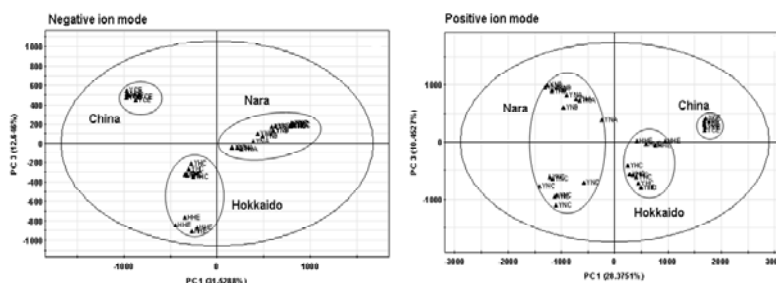


Fig. 3 UPLC-MS analysis of Toki



S4-4: Genome-wide identification of genes necessary for tolerance to lactic acid in yeast *Saccharomyces cerevisiae*

*Laboratory of Molecular Genetics
Suzuki Toshihiro*

Plant- and crop-based renewable plastic, including poly lactic acid, are being developed as low carbon resources. To achieve a sustainable society, these renewable plastics should be available at lower price compared with conventional petroleum-based plastics. *Saccharomyces cerevisiae* are intrinsically tolerant to low pH than the lactic acid bacteria mainly used for production of lactic acid as a monomer for poly lactic acid. This advantage offers the potential to reduce cost because the process of desalination of lactate is costly. A genetically-engineered *S. cerevisiae* strain was recently evaluated for efficient production of L-lactic acid and was found to produce high levels in the absence of pH control. However, it was still observed that the decrease in the yield of lactic acid due to a decrease of pH ($\text{pH} \leq 2.8$) resulted from the accumulation of lactic acid. To further improve production of lactic acid without a requirement for pH control, the acid tolerance of *S. cerevisiae* must be further enhanced. Purpose of this study is to elucidate the molecular mechanisms of tolerance against lactic acid and to create super lactic acid-tolerant yeast, using the following approaches: i) Identification of the genes whose disruption leads to lactic acid tolerance and ii) identification of the genes whose disruption causes lactic acid sensitivity. In this presentation, I will focus mainly on the second approach.

To identify genes which negatively control tolerance to lactic acid, we screened for lactic acid sensitive disruptants among the complete disruption collection for 4,828 non-essential genes of *S. cerevisiae* using 4.0% (w/v) L-lactic acid medium. We identified 107 genes that are essential for 4.0% lactic acid tolerance. Lactic acid sensitive disruptants were further examined for the sensitivity to other acids, i.e., acetic acid and hydrochloric acid. Only 7 of 107 sensitive disruptants displayed sensitivity specifically to lactic acid and four of them were disruptants for genes necessary for vacuolar function. Other three disruptants had mutations in genes which encode heat shock protein or two unknown proteins. This result indicates that vacuolar function is important for the tolerance to lactic acid. It is known that low environmental pH strongly affects the organization of cell wall. However, we noted that 19 so far tested among these sensitive disruptants did not show sensitivity to digestion by cell wall lytic enzyme, zymolyase, suggesting that they unlikely have the defect of cell wall integrity. Vacuolar acidification is important for some of cellular mechanisms. Therefore, we investigated vacuole acidification of sensitive disruptants with quinacrine. Quinacrine is known to be accumulated in the acidified organelle and generates fluorescence. Vacuolar morphology was also observed with FM4-64 which binds the membrane lipid and generates fluorescence. 37 disruptants showed abnormal acidification while 11 disruptants showed abnormal morphology. 10 disruptants showed both abnormal acidification and morphology. It was noted that 24 out of 107 disruptants have never been reported to be involved in vacuole function. These observations suggest that lactic acid sensitivity in these disruptants can be caused by abnormal acidification and morphology of the vacuole. From all of these observations, we suggest that a variety of genes are concerned with tolerance to lactate and enhancement of vacuole function would contribute to increased tolerance to lactic acid.

S4-5: Escherichia coli single-gene knockout mutants which are able to enhance P450 activity in whole-cell biocatalysis

Laboratory of Biochemical Engineering
Ying Zhou

A comprehensive single-gene knockout collection of *Escherichia coli* BW25113 (the Keio collection) was used to screen mutants which could enhance the deethylation of 7-ethoxycoumarin catalyzed by CYP154A1. Of the 3,978 mutants screened, four mutants were able to increase the CYP154A1-catalyzed bioconversion by approximately 1.4-1.7 times compared to that of the control strain. When the four deleted genes in *E. coli* BW25113 were replaced with kanamycin resistant (km^r) gene cassettes again using the same method as used in the construction of the Keio collection, three of them showed high levels of CYP154A1 activity. In-frame deletion mutants of these three mutants also exhibited high enzyme activity, indicating that the enhancement of the enzyme activity was attributed to the defects of these genes, but not to polar effects on their neighbor genes caused by the insertion of the km^r gene cassette. Single-copy recombinant plasmids, which carried these three corresponding genes, respectively, could restore the CYP154A1 activity to the level of the wild type. Another reconstructed mutant failed to enhance the CYP154A1 activity. This might be due to a spontaneous mutation in the chromosome. Further investigations are being carried out to clarify the underlying mechanisms. The present work demonstrated the potential to improve microbial hosts for P450-catalyzed bioconversions.

S4-6: Roles of calcium ions on folding and stability of Tk-subtilisin

Laboratory of Molecular Biotechnology
Yuki Takeuchi

Tk-subtilisin (a subtilisin homologue from *Thermococcus kodakaraensis*) is a highly thermostable subtilisin homologue from the hyperthermophilic archaeon *T. kodakaraensis*. Like bacterial subtilisins, Tk-subtilisin is matured from Pro-Tk-subtilisin upon autoprocessing and degradation of propeptide. However, the maturation process of Tk-subtilisin is different from that of bacterial subtilisins in the requirement of Ca^{2+} for folding of the mature domain. The crystal structure of an active site mutant of pro-Tk-subtilisin (pro-S324A) shows that its overall structure is similar to those of bacterial subtilisin-propeptide complexes, but the mature domain contains seven Ca^{2+} -binding sites (Ca1~Ca7). The Ca1 site is conserved in bacterial subtilisins, while the Ca2-Ca7 sites are unique for Tk-subtilisin. However, the role of each Ca^{2+} ion has not been understood yet. In this study, we constructed seven mutant proteins of Pro-S324A, which are designed to remove one of these Ca^{2+} -binding sites. The Pro-S324A derivative which lack Loop1 contain Ca1 site is termed ΔCa1 . The pro-S324A derivatives with the D226A, D225A, D224A, Q110A/E229A, D372A, and D121A/D314A/D315A mutations are designated a ΔCa2 , ΔCa3 , ΔCa4 , ΔCa5 , ΔCa6 , and ΔCa7 , respectively. The refolding rate constant of Pro-S324A, $\Delta\text{Ca2}/\text{pro-S324A}$, and $\Delta\text{Ca3}/\text{pro-S324A}$ is 1.3, 0.020, and 0.019 min^{-1} containing 10 mM CaCl_2 , respectively. On the other hand, the deletion of the Ca1, Ca4, Ca5, Ca6, and the Ca7 dose not seriously affect the foldability of Tk-subtilisin. From the result of DSC measurement, the denatured temperature of pro-S324A, ΔCa2 , ΔCa3 , ΔCa4 , ΔCa5 , and ΔCa7 is 110.9, 113.5, 113.2, 111.7, 107.2, and 106.9 °C, respectively, on the other hand, that of ΔCa1 and ΔCa6 is 84.3 and 99.2 °C. Additionally, the crystal structures of $\Delta\text{Ca2}/\text{pro-S324A}$, $\Delta\text{Ca3}/\text{pro-S324A}$, and $\text{Ca6}/\text{pro-S324A}$ are determined at 2.07, 2.40, and 2.09 Å resolutions. Based on these results, we discuss about the roles of calcium ions and the Loop2, unique Ca^{2+} -binding site for Tk-subtilisin.

S4-7: Development of a screening system for β -glucosidase-displaying yeasts using alginate beads

*Laboratory of Dynamic Cell Biology
Zhou Yan*

In recent years, there have been some reports about high speed screening systems for yeast cells reacting with fluorescence labeled molecules by using flow cytometry. However, flow cytometry could not be used for screening yeast cells displaying enzymes, since the fluorescent molecules disperse into a solution after degradation. On the other hand, in our laboratory alginate beads were used for immobilizing DNA, proteins and so on. By using this technology, to immobilize the yeast cells displaying enzymes into alginate beads, the dispersion of released fluorescent molecules would be prevented.

In this research, the yeast cells were immobilized in the alginate beads by the reverse micelle method using isooctane. The enzymatic reaction was performed by using TokyoGreen[®]- β Glu. The enzyme activity of the yeast cells displaying β -Glucosidase in alginate beads was confirmed. Alginate beads containing yeast cells displaying enzymes were successfully enriched from the mixed suspension with negative control which means beads containing yeast cells without enzyme by flow sorting. This system would become a useful method for high speed screening of yeast cells displaying various enzymes.

S5-1: Designing the substrate specificity of D-hydantoinase using a rational approach

Laboratory of Biomolecular Engineering

Sang-Chul Lee

Enzymes that exhibit superior catalytic activity, stability and substrate specificity are highly desirable for industrial applications. These goals prompted the designed substrate specificity of *Bacillus stearothermophilus* D-hydantoinase toward the target substrate hydroxyphenylhydantoin (HPH). Positions crucial to substrate specificity were selected using structural and mechanistic information on the structural loops at the active site. The size and hydrophobicity of the involved amino acids were rationally changed, and the substrate specificities of the designed *B. stearothermophilus* mutants were investigated. As a result, M63I/F159S exhibited about 200-fold higher specificity for HPH than the wild-type enzyme. Systematic mutational analysis and computational modeling also supported the rationale used in the design.

S5-2: Parent of origin specific gene expression changes in 39, XO mice suggest perturbations of common neural systems in Turner's syndrome, autism, and schizophrenia

Laboratory of Epigenetics

Ji Yun Song

Perseveration, leading to behavioural inflexibility, is a cognitive endophenotype common to many mental disorders, including autism and schizophrenia. Previous work has demonstrated deficits in behavioural flexibility in Turner's syndrome (TS, 45,XO), and a recent behavioural study of 39,XO mice has augmented this clinical finding. Subtly, in both these studies this cognitive endophenotype was only found when the single X was maternally derived (XmO). Using a *microarray and quantitative RT-PCR (qRT-PCR)* screen we examined whether genes implicated in mental disorders that exhibit perseverative behaviours show altered expression in brains of 39,XO mice, dependent on the parent of origin of the single X. We identified significant differences in expression between 39,XmO and 39XpO mice in a number of GABAA subunits, including a cluster of three genes on chromosome 11. Additionally, expression changes were detected in an uncharacterised gene thought be involved in RNA processing, and three genes that are important for neuronal proliferation and differentiation (Fath2, Fabp7 and Reln). Examination of expression in embryonic brain revealed a significant reduction of Reln in 39,XmO embryonic day 18.5 brain, suggesting possible neurodevelopmental abnormalities in these mice. And more supporting histone modifications on Reln promoter are found in brains of adult 39,XO mouse by *chromatin immunoprecipitation method*. Many of those genes that showed altered expression in 39,XmO mouse brain are implicated in the aetiology of schizophrenia, and to a lesser extent, autism (Gabbr2, Gabrg2, Gabra1 and Reln). Taken together, our data suggest perturbations of common neural systems in 45,XmO TS, autism and schizophrenia, possibly impacting on a shared cognitive endophenotype.

S5-3: cAMP signalling in mushroom bodies modulates temperature preference behaviour in *Drosophila*.

Laboratory for Neural Network of Cognition

Sung-Tae, Hong

Homoiotherms, for example mammals, regulate their body temperature with physiological responses such as a change of metabolic rate and sweating. In contrast, the body temperature of poikilotherms, for example *Drosophila*, is the result of heat exchange with the surrounding environment as a result of the large ratio of surface area to volume of their bodies. Accordingly, these animals must instinctively move to places with an environmental temperature as close as possible to their genetically determined desired temperature. The temperature that *Drosophila* instinctively prefers has a function equivalent to the 'set point' temperature in mammals. Although various temperature-gated TRP channels have been discovered, molecular and cellular components in *Drosophila* brain responsible for determining the desired temperature remain unknown. We identified these components by performing a large-scale genetic screen of temperature preference behaviour (TPB) in *Drosophila*. In parallel, we mapped areas of the *Drosophila* brain controlling TPB by targeted inactivation of neurons with tetanus toxin and a potassium channel (Kir2.1) driven with various brain-specific GAL4s. Here we show that mushroom bodies (MBs) and the cyclic AMP-cAMP-dependent protein kinase A (cAMP-PKA) pathway are essential for controlling TPB. Furthermore, targeted expression of cAMP-PKA pathway components in only the MB was sufficient to rescue abnormal TPB of the corresponding mutants. Preferred temperatures were affected by the level of cAMP and PKA activity in the MBs in various PKA pathway mutants.

S5-4: Magnetic bead based immunoassay with enzymes immobilized in mesocellular carbon foam.

Laboratory of Biomolecular Engineering

Yunxian, Piao

Aiming to create a sensitive and highly stable immunobiosensor we immobilized enzyme in a mesocellular carbon foam, MSU-F-C. The MSU-F-C, owing to its large surface area and pore volume, can be used as a host matrix for enzyme immobilization. Immobilization of enzymes in tailor-made nanoscale structures can significantly improve the performance of biocatalytic processes, since they can enhance the loading of bio-molecules and stability of the biocatalysts, as well as mass transport. In addition, by proper acid treatment, the MSU-F-C can be functionalized with carboxylate groups so that the carbon surface can be labeled with various bio-molecules. Here we introduce an application of the enzyme immobilized in nanoporous carbon to immunoassay for the first time. Briefly, MSU-F-C was loaded with horseradish peroxidase (HRP), followed by cross-linking of the enzyme using glutaraldehyde (GA) and modification of the surface with anti-human IgG by EDC/sulfo-NHS chemistry. The resulting MSU-F-C/HRP/anti-human IgG stably retained immobilized enzymes and antibodies, and showed much higher thermal stability compared to the HRP/anti-human IgG conjugate. In magnetic bead-based immunoassays, signal amplification using MSU-F-C/HRP/anti-human IgG has led to the successful colorimetric detection of a model analyte, human IgG, in picomolar quantity. Optimal conditions for construction of high performance immunoassay were established, and employed for immunoassay for the target antigens.

**S5-5: *Vadicola ignavus* gen. nov., sp. nov., the member of *Actinomycetaceae* family,
first isolated from environment**

Laboratory of Environmental & Molecular Microbiology

Liang Wang

A Gram-positive and cocci shaped bacterium, KC011^T, was isolated from Gapcheon stream nearby Daejeon city in South Korea. Growth occurs at 25-40°C, the optimum temperature is 30-37°C. The pH range for growth is 6.5-8.5. Phylogenetic analyses based on 16S rRNA gene sequence revealed that this strain belong to the family *Actinomycetaceae*, in which the most closely related genera were *Actinobaculum* and *Arcanobacterium*. Hitherto, none of the validated members in the family *Actinomycetaceae* have had environmental originus. Here, we propose a new genus and species, *Vadicola ignavus* gen. nov., sp. nov., with isolate KC011^T as the type strain, which belong to the family *Actinomycetaceae*.

S5-6: Modulation of MEK/ERK and BMP4 signaling pathways induces efficiently differentiation of human embryonic stem cells toward hemangioblast-like cells

Laboratory of Development and Differentiation

Sang-Wook Park

Human embryonic stem cells (hESCs) have potential of self-renewal and pluripotency. So far, various specialized cell types have been derived from hESCs with inefficiency. Here, we demonstrate that hESCs could efficiently differentiate into bipotent hemangioblast-like cells by regulating two signaling pathways. Inhibition MEK/ERK signaling and activation of BMP4 signaling enhanced synergistically expression of mesoderm markers in hESCs. The induced hESCs could efficiently differentiate into CD34⁺ hemangioblast-like cells with high efficiency, ranging from ~10% to 20%. hESC-derived CD34⁺ cells could further develop to vascular lineage cells such as endothelial cells and smooth muscle cells. The hESC-derived endothelial cells took up acetylated-low density lipoprotein (LDL) and formed vascular-like structures on the Matrigel *in vitro*. The CD34⁺ cells could also differentiate into hematopoietic cells such as erythroid and granulomacrophage. Moreover, hESC-derived CD34⁺ cells improved blood perfusion and limb salvage in ischemic model mice by involving neovascuogenesis. Thus, functional hemangioblast-like cells could be generated from hESCs with high frequency by combined regulation of MEK/ERK and BMP4 signaling pathways. Our findings suggest that modulation of signaling pathways may be efficient to induce hESCs into a specialized lineage.

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