CHULALONGKORN UNIVERSITY-NAIST JOINT SYMPOSIUM ON BIOLOGICAL SCIENCES

Aim: This joint symposium is planned to establish substantial exchanging activities in research and education between Department of Biochemistry, Faculty of Science, Chulalongkorn University and Graduate School of Biological Sciences, NAIST. Presentations by four faculty members from each university will help understanding each other and lead active discussion to share future possibilities of collaboration and joint research.

Date: **March 26, 2015 Thursday**

Venue: Large Lecture Room in Biological Sciences building

Program:

10:00	**Opening remark** (Program host: Associate Professor ***Taro Kawai***) **Welcome speech** (Professor ***Toshio Hakoshima***, Dean of Graduate School of Biological Sciences)
10:10-11:00	**Session I: Biomedical Science** (Chairman: Associate Professor ***Taro Kawai***) 10:10 Professor, Dr. ***Anchalee Tassanakajon***, Head of Department of Biochemistry Shrimp immune proteins and their function in defense against pathogen infection 10:40 Associate Professor ***Yasumasa Ishida*** Gene discovery in haploid ES cells
11:00-11:40	**Session II: Plant Biology** (Chairman: Professor ***Takashi Hashimoto***) 11:00 Associate Professor ***Teerapong Buaboocha*** Identification of genes responsible for salt tolerance in local Thai rice by omics approaches 11:20 Professor ***Seiji Takayama*** Self/non-self discrimination in plant self-incompatibility
11:40-12:20	**Session III: Microbiology** (Chairman: Professor ***Hisaji Maki***) 11:40 Assistant Professor ***Saowarath Jantaro*** Enhanced production of polyhydroxybutyrate in engineered *Synechocystis* sp. PCC 6803 12:00 Professor ***Kazuhiro Shiozaki*** Fission yeast as a genetic model to unravel signaling pathways in diseases
12:20-13:30	**Lunch break**
13:30-14:00	**Session IV: Structural Biology** (Chairman: Professor ***Toshio Hakoshima***) 13:30 Assistant Professor ***Kuakarun Krusong*** Amylomaltase: From Structure to Function 13:50 Assistant Professor ***Yoshiki Tanaka*** Crystal structure of multi-drug transporter MATE
14:00-14:10	**Closing remark** (Professor ***Kazuhiro Shiozaki***, Chair of International Education Committee)

Shrimp immune proteins and their function in defense against pathogen infection

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In common with other invertebrates, shrimp lack a true adaptive immune system and rely on effective innate immune responses to combat invading pathogens. So far, several immune effector molecules that participate in the innate immunity in shrimp have been identified and characterized. Pattern recognition receptors (PRRs) that bind to microbial cell wall components and subsequently activate a variety of immune responses have been reported in shrimp including lectins, toll-like receptors, Down Syndrome Cell Adhesion Molecule (DSCAM) receptors and lipopolysaccharide- and beta glucan binding proteins (LGBPs). *Pm*LGBP recognizes and binds to LPS and b-1,3-glucan and subsequently activate the melanization cascade which is an important immune response of shrimp against bacterial infection. In addition, we recently found a serine proteinase, *Pm*clipSP2 and serine proteinase homologs (*Pm*SPH1 and 2) that act as pattern recognition molecules and involved in the activation of the melanization cascade in *Penaeus monodon*. Antimicrobial peptides are important components of the shrimp defense system. They exhibit broad spectrum of antimicrobial activity against various shrimp pathogens and their production is possibly regulated by the Toll and IMD pathways like those found in insects. Hemocyte homeostasis-associated protein (*Pm*HHAP), a highly responsive protein to WSSV infection, has been shown to play an important role in controlling the hemocyte homeostasis by regulating the hemocyte apoptosis. Gene silencing of *Pm*HHAP resulted in an increase in the number of apoptotic cells and dramatically induced a high level of caspase 3/7 activity in hemocyte and also resulted in the characteristic of apoptotic DNA ladder. The knowledge on function of these immune components leads to a better understanding of shrimp immunity which could facilitate the new strategies to effectively control disease in shrimp.

Gene discovery in haploid ES cells

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We previously reported generation of a bank of bi-allelically mutated ES cells derived from a diploid ES-cell line (Horie, K. et al. *Nat. Methods*, 8: 1071-1077, 2011). However, its generation required complicated procedures including transient Bloom-gene (*blm*) inactivation for elevation of crossing-over after introduction of a mono-allelic mutation. To separate bi-allelic from mono-allelic mutants, we developed a conditionally convertible selection cassette (cNP cassette) containing the neomycin (neo)- and puromycin (puro)-resistance genes in addition to the splice acceptor/hygromycin-resistance gene for disruption of gene function. The neo and puro genes were arranged in a tail-to-tail configuration and flanked by inversely oriented *lox*2272 sequences. In the default condition, the phosphoglycerate kinase-1 (*Pgk*) promoter induces the expression of only neo, not puro, resulting in G418-resistant and puromycin-sensitive ES cells. Cre-mediated recombination induces inversion of the cNP cassette, resulting in G418-sensitive and puromycin-resistant ES cells. Since the bi-allelically mutated ES cells have two copies of the cNP cassette, G418-plus-puromycin double selection after transient Cre expression permits their isolation if one of the disrupted alleles is flipped to show puromycin-resistance while the other stays in the G418-resistant configuration.

We have adopted a similar strategy for isolation of gene-trapped haploid ES cells. Difficulty of the isolation is high rate of spontaneous conversion of haploid to diploid ES cells. Haploid ES cells bearing insertion of a *Tol2* transposon-based cNP cassette undergo spontaneous diploidization, resulting in generation of ES cells containing two copies of the cNP cassette. However, newly generated diploid ES cells basically do not convert further into tetraploid ES cells. Thus, the G418-plus-puromycin double selection results in isolation of diploid ES cells derived from gene-trapped haploid ES cells. We should be able to exploit these 'bi-allelically gene-disrupted' ES-cell clones in a variety of genetic screens.

Identification of genes responsible for salt tolerance in local Thai rice by omics approaches

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Plants have evolutionarily developed several mechanisms to tolerate salt stress. To develop more salt-tolerant plants, it is crucial to identify the key components of the salt-response network. Here, two omics approaches have been employed to identify genes responsible for salt tolerance in rice (*Oryza sativa* L.). In the first approach, we used genome-wide association mapping based on SNPs to identify causative loci in the indica rice genome. For phenotyping, photosynthetic parameters and yield components were collected from one hundred local Thai rice varieties grown under normal or salt stress (9 dS m^{-1}) condition. For genotyping, exome sequencing of their genomes by exome capture was performed. On average, 15.6 M reads were obtained from each variety resulting in more than 300,000 SNPs and several candidate genes identified, which will be further characterized. In the second approach, a more directed transcriptome profiling between the wildtype and the transgenic rice overexpressing OsCaM1-1, a calmodulin sensor protein, grown under salt stress condition was carried out. The *OsCam1-1* overexpression was previously shown to confer a better salt-tolerant ability to the transgenic rice. The transcriptome profiles revealed differentially expressed genes in the sucrose/starch metabolism, TCA, and glyoxylate cycles and some of the stress-related transcription factors including AP2/EREBP and WRKY. From these results, we hypothesize that the salt tolerant ability of the transgenic rice overexpressing *OsCam1-1* may result from the enhanced central energy metabolism of the cell.

Self/non-self discrimination in plant self-incompatibility

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Flowering plants have developed self-incompatibility (SI) as a genetic system to prevent inbreeding and thus promote outcrossing to generate genetic diversity. SI is based on the self/non-self discrimination between male and female. In many plants, SI is controlled by a single locus, designated *S*, with multiple haplotypes. Each *S*-haplotype encodes both male-specificity and female-specificity determinants (*S*-determinants), and the self/non-self discrimination is accomplished by the *S*-haplotype-specific interaction between these *S*-determinants. Recent studies have revealed that plants utilize diverse self/non-self discrimination systems, which can be classified into two fundamentally different systems, self-recognition and non-self-recognition systems. The self-recognition system, adopted by Brassicaceae and Papaveraceae, depends on a specific interaction between male and female *S*-determinants derived from the same *S*-haplotype, and their interaction triggers incompatible responses to prevent the fertilization. The non-self-recognition system, found in Solanaceae, depends on non-self (different *S*-haplotype)-specific interaction between male and female *S*-determinants, and their interaction positively supports the fertilization process. The number of male *S*-determinant genes in Solanaceae has been increased through gene duplication and gene conversion events to collaboratively recognize diverse non-self female *S*-determinants. Furthermore, recent studies have revealed that these families with SI also contain high proportion of selfing (self-compatible) species with disrupted SI systems. Selfing is disadvantageous when selfed offspring store recessive traits, but it may nevertheless be needed for reproductive assurance under environments where pollinators or mates are scarce. Considering these together, flowering plants must have repeatedly lost and re-acquired SI systems in the course of their evolution.

Enhanced production of polyhydroxybutyrate in engineered *Synechocystis* sp. PCC 6803

Saowarath Jantaro

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Currently bioplastic plays a well-known role more than in the previous days as an alternative source of plastic based on their biodegradability. It is environmentally friendly and easily decomposed by microorganisms in nature. The wider use of bioplastic is then a promising strategy that potentially relieve the increasing effect of non-degradable plastic waste. The alternative resources of bioplastic have been focused by the scientists to various microorganisms such as, bacteria and especially, photoautotrophic cyanobacteria. The PHB biosynthetic pathway in cyanobacterium *Synechocystis* sp. PCC 6803 is mainly started from acetyl-CoA which is catalyzed by several enzymes including beta-ketothiolase (encoded by *phaA*), acetoacetyl-CoA reductase (encoded by *phaB*) and PHB synthase (encoded by *phaC* and *phaE*). *Synechocystis* PCC 6803 strains with overexpressing *Pha* genes were constructed and characterized for PHB production. These *Pha* genes-overexpressing strains had slightly reduced growth rates compared to wild type. Under N-limited condition, the strains overexpressing *phaAB*, *phaEC* and *phaABEC* significantly accumulated higher PHB contents when compared with wild type. The maximum PHB content, a 2.7 fold increase was obtained from the strain overexpressing *phaAB* grown for a short term of 7 days in N-limited medium. The larger size of PHB granules in *phaAB* overexpressing strain were clearly visualized by TEM imaging. A further increase of PHB production was obtained after adding 0.4% (w/v) acetate into N-limited medium, especially in *phaAB* overexpressing strain with produced up to 35.4 % PHB (w/w) after 9 days of treatment. In conclusion, our finding indicated that the increase of PHB content in *Synechocystis* PCC 6803 can be achieved by overexpression of *phaA* and *phaB* whose transcripts were up-regulated when grown under N-limited condition with acetate addition.

Fission yeast as a genetic model to unravel signaling pathways in diseases

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The long-term objective of our research is to understand at a molecular level how cells perceive and respond to diverse stress by elucidating the structure and function of intracellular stress-signaling pathways. Our studies are being carried out with the fission yeast *Schizosaccharomyces pombe*. This organism has served as an outstanding model system for studying basic cellular functions conserved among eukaryotes.

In diverse eukaryotic species, mitogen-activated protein kinase (MAPK) cascades are utilized in sensing and integrating various stress signals to induce protective responses. We have demonstrated that the Spc1 MAPK pathway in *S. pombe* is remarkably homologous to the mammalian SAPK (stress-activated protein kinase) pathways, which determine cancer cells' response to chemotherapy and radiotherapy as well as inflammatory responses in diseases such as asthma, arthritis and bacterial infection.

In both mammals and fission yeast, the stress MAPK pathways have important roles in linking growth controls with environmental signals. Our current research interests are (1) to understand how cells sense and transmit diverse stress stimuli, including osmostress, heat shock and oxidative stress to activate Spc1 MAPK; (2) to uncover how the stress MAPK pathway interacts with signaling pathways involving the TOR (Target Of Rapamycin) protein kinase. The TOR kinase is highly conserved from yeast to human and regulates cell proliferation/metabolism in response to environmental cues. We are particularly interested in the regulation of TOR complex 2 (TORC2), a mediator of insulin signaling in higher eukaryotes. We expect that these studies in *S. pombe* will lead to a better understanding of the human signaling pathways in cancers, inflammatory diseases and diabetes.

Amylomaltase: From Structure to Function

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Amylomaltase (4-α-glucanotransferase; EC 2.4.1.25) catalyses inter- and intra- molecular transglycosylation of α-1,4 glucans, yielding linear or cyclic α-1,4 glucan products. Unlike cyclic α-1,4 glucans produced by cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19), cycloamyloses produced by amylomaltase has a large degree of polymerisation (DP), ranging from DP of 16 or higher. Large-ring cyclodextrins (LR-CDs) are highly soluble in water and can form inclusion complexes with various guest molecules. This helps improve properties of guest molecules such as their solubility, stability, reactivity, volatility and bioavailability. Therefore, LR-CDs can be widely used in pharmaceutical, cosmetics and food industries. Amylomaltase gene from a mesophilic bacteria *Corynebacterium glutamicum* was cloned and the recombinant enzyme was characterized. Amylomaltase from *C. glutamicum* (*Cg*AM) produced LR-CDs with a DP of 19 or higher. X-ray crystal structure of *Cg*AM showed a $(\beta, \alpha)_8$ barrel as a core structure and has a similar arrangement of the catalytic side-chains (two Asp residues and one Glu residue) as shown in members of the α-amylase superfamily. The acarbose- and maltotriose-bound *Cg*AM structures revealed amino acids residues essential for the enzymatic activity.

Crystal structure of multi-drug transporter MATE

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Toxic compounds essentially have to be exported from the cell to maintain life. MATE (multidrug and toxic compound extrusion) transporters function in the efflux of endogenous cationic and lipophilic substances using an electrochemical gradient of H^+ or Na^+ across the membrane, and are ubiquitously distributed in archae, bacteria, and eukaryotes. Bacterial MATE transporters confer multidrug resistance (MDR) to pathogens.

Here we present the crystal structures of the H^+-driven MATE transporter from *Pyrococcus furiosus* in two distinct apo-form conformations, and in complexes with a derivative of the antibacterial drug norfloxacin and in vitro selected thioether-macrocyclic peptides. The structure of PfMATE consists of 12 transmembrane-helices (TMs) forming two pseudo-symmetrical lobes, an N-lobe (TM1-TM6) and a C-lobe (TM7-TM12), which are related by a pseudo two-fold symmetry axis. The PfMATE structure adopts a V-shaped conformation, with the central cleft open toward the extracellular side, representing an outward-open state. In the outward-open state we found that it adopts two distinct conformations, the “bent” and “straight” conformations, in terms of the structure of the TM1 helix in the N-lobe. In the bent conformation, it is unstrained and kinked at Pro26 and Gly30, and bent toward the TM2 side. As a result, the N-lobe cavity is collapsed in the bent conformation. The structures, combined with functional analyses, revealed that the protonation of Asp41 on the N-terminal lobe induces the bending of TM1, which in turn collapses the N-lobe cavity, thereby extruding the substrate drug to the extracellular space. The protonation of Asp41 induces the bending of TM1 at Pro26, which collapses the N-lobe cavity and extrudes the bound substrate into the extracellular space. Moreover, two of the macrocyclic peptides bind the central cleft in distinct manners, which correlate with their inhibitory activities.