Symposium on Interdisciplinary Researches in Okayama

Date: 20th-21st, November, 2012
Venue: The 50th Anniversary Hall, Okayama University
Welcome Message

Professor Masahiko Sisido  
Director, Research Core for Interdisciplinary Sciences (RCIS)  
Okayama University

On behalf of the organizing committee of the "Symposium on Interdisciplinary Researches in Okayama", I cordially express my gratitude to the speakers and other active participants for attending the symposium. This symposium is organized by the faculty members of RCIS, Okayama University.

The RCIS was established in July, 2008 for promoting interdisciplinary sciences. Eleven talented young tenure-track faculty members joined us and started their independent research about 4 years ago.

The tenure-track system, although it is common in the United States and in some European countries, is still in immature stage in Japanese universities. In the past and even at present, most of faculty members in Japan have been enjoying academic stability, after they were recruited at the starting position, often as collaborators supervised by full professors. This recruit system assures and supports long-term research that may not provide answers of the current and urgent problems. It has been an advantageous point of the Japanese academy. But because scientific interest changes more and more rapidly in recent years, especially in the interdisciplinary fields, we built a new management system of faculty members that is flexible enough to deal with rapid progress of science. The tenure-track system of RCIS, Okayama University assures independent research for the members and provides with enough research funds, but requires rigid evaluation after about 4 years. So far, the members are doing very well in research and in getting external research funds. Three of the first eleven members left the RCIS getting tenure positions in Okayama and other universities, and other three got tenure qualification already. We welcomed two additional tenure-track members in the last 2 years and currently there are 10 members belonging to RCIS.

It is my best pleasure to introduce here the members of RCIS who beautifully organized this international symposium. Also, I would like to ask all the participants to enjoy the fresh fruits of interdisciplinary sciences.
Organizing Committee

This symposium is organized by Research Core for Interdisciplinary Sciences, Okayama University
http://rcis.vbl.okayama-u.ac.jp/RCIS/

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10:00~11:30  Session 1 (Biology)
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13:00~14:30  Session 2 (Biology)
15:00~17:00  Poster Session I (Biology)
18:00~20:00  Dinner Party (Peach Union 4F)

Day 2 ◇ November 21(Wed), 2012

10:00~11:30  Session 3 (Chemistry, Physics, Mechanics)
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15:30~16:30  Session 4 (Chemistry, Physics, Mechanics)
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*1 Opening Address
   Dr. Shinichi Yamamoto, Executive Director/Vice President of Okayama University

*2 Closing Address
   Dr. Masahiko Sisido, Director of Research Core for Interdisciplinary Sciences
Venue

Okayama University (Tsushima Campus)
Address: 1-1-1 Tsushima-Naka, Kita-ku, Okayama-shi, 700-8530, Japan

Sessions: 50th Anniversary Hall
Opening, Closing, Plenary Talks: Main hall (1-2F)
Poster presentations: Poster room (2F)

Dinner party: Peach Union 4F
Participation fee is ¥5000.
Advance entry: You can apply the dinner party by sending an e-mail (satoha@cc.okayama-u.ac.jp) with your name and affiliation by 10th Nov.
On-site entry: You will be able to enter for the dinner party until reaching the fixed number.
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Plenary Talks
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Biogenesis, function, and defects of the Golgi in diseases

Yanzhuang Wang
Associate Professor, University of Michigan, USA

The Golgi apparatus is a membrane-bound organelle that serves as a central conduit for protein and lipid modification, processing, trafficking and secretion. Proteins encoded by over one-third of the genes in the human genome, such as hormones (e.g. insulin), neurotransmitters, growth factors, digestive enzymes, antibodies and extracellular matrix proteins, all go through the Golgi, where they are processed, concentrated, sorted, and transported to the correct location. A unique feature of this membrane organelle is the stack of the flattened cisternae, but the molecular mechanism and biological significance of stack formation remain elusive. Dr. Wang’s research aims to understand how this unique stacked structure is formed, why this structure is important for its function, and how a new Golgi is generated when the cell divides. In particular, Dr. Wang is interested in studying the proteins that help “glue” the cisternae together to form a stack and the role of ubiquitin in regulation of Golgi membrane dynamics during the cell cycle. In the last few years, he and his colleagues have developed a unique multidisciplinary approach employing biochemistry, cell biology, electron microscopy, and more recently proteomics, combined with a novel in vitro reconstitution assay, to provide a mechanistic explanation for Golgi structure formation and function. He found that stack formation directly involves the Golgi stacking proteins GRASP65 and GRASP55, which play complementary and essential roles by forming mitotically regulated trans-oligomers that hold the Golgi membranes into stacks. Using GRASP65/55 as tools to manipulate Golgi stack formation, he demonstrated that Golgi stacking functions as a flux regulator to ensure accurate protein modifications. He also discovered that monoubiquitination of Golgi membrane proteins during mitosis is essential for subsequent membrane fusion and identified HACE1 and VCIP135 as the ubiquitination and deubiquitinating enzymes whose activity is required for post-mitotic Golgi reassembly. Most recently, he has gathered intriguing preliminary data concerning the structural and functional defects of the Golgi in cell culture and mouse models for cancer as well as cardiovascular and Alzheimer’s diseases.
Previously we demonstrated that BMP signaling is re-activated during endogenous regeneration, and BMP2 and 7 can induce regeneration response in the regeneration-incompetent digit. Both endogenous and BMP-induced regeneration are associated with blastema formation. However it is poorly understood how BMP involves in the blastema formation of mammals. In this study, we focus on the identification of BMP target cells and downstream mechanism of BMP signaling during neonatal digit regeneration. We found blastema cells were attracted by BMP source, suggesting migration of blastema cell is mediated by SDF-1α/CXCR4 signaling. We showed SDF-1α was upregulated by BMP2 in the endothelial cells and exogenously treated BMP2 increased in the numbers of SDF-1α+ and CXCR4+ cells, but these cells are not overlapped. Immunostaining study revealed SDF-1α is expressed in CD31+ endothelial cells. Furthermore, using BRE-LacZ transgenic mice, we demonstrated that endothelial cells directly respond to BMP. BMP-responding endothelial cells express SDF-1α to recruit blastema cells such as osteogenic progenitor cell. Taken together, in this study we defined the BMP signaling involves in regeneration via SDF-1α/CXCR4 signaling to recruit regenerating cells in mammals.
Molecular errors, cryptic genetic variation, and evolvability

Joanna Masel
Associate Professor, University of Arizona, USA

Making genes into gene products is subject to predictable errors, each with a phenotypic effect that depends on a normally cryptic sequence. The distribution of fitness effects of these cryptic sequences, like that of new mutations, is bimodal. For example, a cryptic sequence might be strongly deleterious if it causes protein misfolding, or it might have only a minor effect if it preserves the protein fold and tweaks function. Few sequences have effect sizes that fall in between.

Strongly deleterious sequences can be subject to some selection even while they are cryptic, and expressed only at low levels that depend on a molecular error. Strongly deleterious effects can be avoided globally by avoiding making errors (e.g., via proofreading machinery) or locally by ensuring that each error has a relatively benign effect. The local solution requires powerful selection acting on every cryptic site, and so evolves only in large populations. Small populations with less effective selection evolve global proofreading solutions. However, we also find that for a large range of realistic intermediate population sizes, the evolutionary dynamics are bistable and either solution may result. The local solution, which does not occur in very small populations, facilitates the co-option of cryptic sequences and therefore substantially increases evolvability. By describing this increased evolvability with respect to a genotype network, we find that surprisingly, increased evolvability can occur even in genetically uniform populations.

Purging selection due to translational errors can likely explain how noncoding sequences can be converted to coding during evolution. Examples will be given both for C-terminal protein sequences that evolved from 3'UTRs, and of a new 28 amino acid polypeptide in Saccharomyces cerevisiae. The latter was detected from ribosomal profiling data, which also shows that many “noncoding” transcripts show extensive physical association with ribosomes. This association allows for purging selection to operate on translational errors. Evolutionary capacitor can even switch “on” previously cryptic stocks of variation. Molecular switches that do this, such as the yeast prion [PSI+], can be favored by natural selection. In other words, evolvability can evolve.
A ‘genes-to-ecosystems’ approach to ecology merges the tools and theories of population genetics and evolutionary biology with those of community and ecosystem ecology. Like diversity among species, genetic diversity within species is increasingly recognized as having an important influence on how communities are assembled and how ecosystems function. In genotypic diversity experiments, we found positive responses of arthropod species diversity, including herbivore and predator diversity, to increasing numbers of genotypes in experimental plots. Additionally, these relationships were non-additive, or many more arthropod species than predicted by the sum effects of individual plant genotypes. Intraspecific diversity can also affect the structure of the communities within the same trophic level. We found the success of colonizing plant species is mediated by genetic variation in functional traits within native dominant plants. In colonization experiments, there were considerable differences in understory plant communities depending on the competitive abilities of overstory genotypes. Taken together, this approach can further our understanding of the importance of biodiversity across levels of organization from individuals to entire ecosystems.
Without understanding its evolutionary history and drivers we can not claim a complete understanding of a biological system, predict its diversity among organisms, or hope to be able to reliable manipulate it (in the context of medicine and engineering). In this talk, I will demonstrate mathematical and computational approaches towards deciphering the evolutionary processes that can lead to specific systems properties at the cellular level. In turn, these properties can channel further evolution creating an interesting interplay between systems level properties and evolutionary dynamics. Describing two recent projects in detail, I will highlight fluctuating environments and nonlinear dynamics as an example of this interplay and its effect of system robustness and evolvability. The talk will conclude with general remarks on the relevance of the emerging field of evolutionary systems biology in our quest to better understand (and manipulate) cellular systems.

Orkun S Soyer is a senior lecturer of systems biology at the University of Exeter. He received a PhD from University of Michigan under the supervision of Richard Goldstein in 2004. His postdoctoral work focused on the evolution of signalling networks, and was done under the mentorship of Sebastian Bonhoeffer at the ETH, Zuerich. Orkun's lab is interested in deciphering the evolutionary and ecological principles that can explain the structure and dynamics of biological systems at molecular level. A connected aim is to use the resulting insights towards manipulating existing biological systems or designing novel ones.
Plants defend themselves against microbial invaders and developed unique immune systems, called plant innate immunity. The timely recognition of invading microbes and the rapid induction of defense responses are essential for plant disease resistance. At least two recognition systems are used by plants. Plant defenses are often initiated by pathogen-associated molecular patterns (PAMPs), such as chitin and flagellin. This basal defense is dependent on a much less specific recognition system. Both animals and plants can recognize invariant PAMPs that are characteristic of pathogenic microorganisms.

Perception of the peptide fragment of flagellin in Arabidopsis thaliana depends on the leucine-rich repeat type receptor kinase flagellin sensing 2 (FLS2). The fls2 mutant Arabidopsis is more susceptible to the bacterial pathogen than wild-type plants, suggesting that recognition of PAMPs by plant cells potentiates defense responses. Pathogens produce avirulence (Avr) proteins (effectors) to facilitate the infection by suppression of basal defense. In turn, plants obtained robust defense system, a gene-for-gene interaction between a dominant plant resistance (R) gene and an Avr gene, which provides race-specific resistance that is easily overcome by mutation of the Avr gene.

Understanding these plant signaling systems creates an opportunity to manipulate these systems to enhance resistance in crops. FLS2- and R-gene products share similarities with components of the animal innate immune system, suggesting that some downstream signaling components are common between plants and animals.

I will introduce and discuss downstream signaling components of plant immune systems, such as activation of mitogen-activated protein kinase (MAPK) cascades, production of reactive oxygen species (ROS) and nitric oxide (NO).
Minimally-invasive cell surgery and its applications

Yoko Yamanishi
Associate Professor, Nagoya University, Japan

We have successfully operated enucleation of oocyte by using microelectric knife without any thermal collateral damage. Minimally-invasive cellular-scale ablation was achieved by the monodispersed microbubbles which were generated by a pulse discharge of microelectrode. The discharged output power and conductive area of microelectrode were controlled by glass shell insulation around the copper micro-wire. Fig.1 shows the concept of the micro-electric bubble knife. A small space which is called "bubble reservoir" between the Cu wire and glass tip contribute to stabilize the electric discharge and directional bubble generation. Fig. 2 shows high-speed camera photos of the phenomenon of a line of mono-dispersed microbubbles, and which can ablate the cell surface successfully (Fig.3). The confocal microscope image of the fluorescent cytoplasm and zona pellucida dyed by rhodamine B confirmed that the micro-electric bubble knife can process the cell membrane and control the depth of ablation with limited damage with a resolution of approximately a few \( \mu \text{m} \). Fig.4 shows the comparison of the ablation region after the enucleation and it was confirmed that the ablation area by microelectric bubble knife is about a half of that by manual operation with glass capillary. This low cost microelectric bubble knife has possibilities to extend to fabricate any objective material under various environments and contribute to a new top-down fabrication method in the micro-nano bioengineering field.

Fundamentals and Applications of Electro-conjugate Fluid

Kenjiro Takemura
Associate Professor, Keio University, Japan

Electro-conjugate fluid (ECF) is a kind of functional fluid, which produces an active flow when subjected to high DC voltage. Since the ECF only requires a tiny electrode pair in several hundreds micrometers to generate flow, it could be an attractive micro fluid power source, i.e., bulky pumps are no longer required. Fig. 1 shows a needle-ring electrode pair used for generating the ECF flow. Although electrodes are in the submillimeter order, it could generate pressure of about 30 kPa and flow rate of 50 ml/min with 6 kV applied. In addition, the pressure and flow rate could easily be increased by placing several electrode pairs in series and parallel, respectively. If you integrate 10 electrode pairs in series, you may get 0.3 MPa of pressure, which is almost equivalent to that used in pneumatic systems.

Fig. 2 shows an ECF flexible micro hand with 70 mm long, 40 mm wide and 40g weight. It consists of flexible fingers composed of fiber reinforced rubber tubes, a palm, and needle-ring electrode pairs located at the base of fingers. The palm, electrode pair and fingers respectively work as tank, pump and actuator. Although small, lightweight and dexterous, the hand has all essential components inside. Other than the ECF hand, there are several applications of ECF flow such as ECF micromotors, ECF liquid rate gyroscopes, ECF artificial muscle actuators, etc.

The ECF is an attractive fluid power source as mentioned, however, the flow mechanism is still under investigation. The author proposed a flow model of ECF, in which an electric force induced to dielectric fluids under an electric field is introduced to an equation of motion of Newtonian fluid. Note that the electric force could be calculated with Korteweg-Helmholtz equation. Fig. 3 compares flow velocity distributions obtained with numerical simulation and visualization experiment of the flow. They are obviously corresponding and could prove our flow model of ECF is reasonable.

As mentioned, the ECF is an attractive subject of fundamental research, and at the same time, it may have a potential applications. I expect many researchers to get interested in this attractive fluid and hope the ECF to become a breakthrough of new actuation system.

Fig. 1 Electrode pair Fig. 2 ECF hand Fig. 3 Flow distribution
Realizing Atom-Economical Tandem Chemical Processes for the Efficient Assembly of Bioactive Complex Molecules

Philip Wai Hong Chan
Assistant Professor, Nanyang Technological University, Singapore

Research in our group is focused on the development of new atom-economical metal-catalyzed methods that can construct skeletally diverse complex molecules of current biological interest from simple and readily accessible substrates under mild reaction conditions.[1][5] In this presentation, we will disclose a selection of our recent contributions to this increasing important aspect of synthetic chemistry in the preparation of a wide variety of potentially bioactive nitrogen heterocycles.

Selected Recent References

Proton Exchange Membrane Fuel Cells (PEMFCs) for Near Term Power Applications: Materials Challenges and Opportunities

Rajesh Bashyam, Ping He and Shanna Knights
Senior Research Scientist, Ballard Power Systems Inc., Canada

Due to concerted efforts in recent years between fuel cell developers, universities and government laboratories, fuel cell products are presently gaining momentum in a number of specific market applications. However, certain technological challenges remain to increase market acceptance over a broad range of applications. Combined with reduced cost, durability is one of the most critical requirements for successful commercialization of PEM fuel cell technology for both stationary and automotive applications. Considerable material improvements in the membrane electrode assembly (MEA) are required to meet durability goals. Opportunities for MEA durability improvements include: (i) catalyst surface area stability against agglomeration, dissolution and poisoning; (ii) catalyst support resistance to corrosion; (iii) reduction in catalyst migration through the polymer electrolyte membrane; (iv) chemical stability of the membrane material to peroxide attack; (v) stability of the catalyst layer against cell reversal; and (vi) stability of the gas diffusion layer against carbon oxidation.

Extensive research has been done to develop cathode catalysts for the oxygen reduction reaction (ORR) in PEM fuel cells. Though significant performance gains have been obtained through some of the newly developed catalysts like PtCo/C, PtCoNi/C, and PtCoMn/C in comparison to conventional Pt/C, tolerance to a range of operating conditions is still a major concern. In addition, wide adoption of fuel cell technology in the near term market will be facilitated by increased fuel flexibility, including efficient operation on reformed fuels. A PEM fuel cell stack operated on reformed fuel generally needs to tolerate at least a small amount of carbon monoxide (CO) in H₂, but even a small amount of CO (0.2 - 2 ppm CO) can poison the anode Pt/C catalyst, which results in significant reduction in performance. However, significant improvements in CO tolerance have been observed with the development of PtRu/C anode catalysts. In addition to H₂ oxidation reaction (HOR) and ORR, the O₂ evolution reaction (OER) may also occur in fuel cells in the event of cell reversal due to fuel starvation; for this case, ruthenium in the form of RuO₂ has been used to promote the oxygen evolution reaction at the anode. Unfortunately Ru is not stable at potential >0.4 V vs RHE. In particular, under conditions of air/air start-up/shutdown, and fuel starvation leading to cell reversal, oxidation of Ru followed by dissolution occurs at potential (>0.9 V vs RHE). The dissolved ruthenium crosses over to the cathode through the polymer electrolyte membrane and deposits on the cathode Pt catalyst. The Ru contaminated cathode reduces the activity of the Pt cathode catalyst for the ORR and hence a significant overpotential occurs at the cathode, which ultimately reduces the overall performance of the fuel cell and could hamper even some of the newly developed cathode catalysts mentioned above. Ru crossover related failure modes have been documented for DMFC but not addressed well in literature for PEMFC operated with CO contaminated H₂ or PEM operated with cell reversal tolerant anode. Ballard recently addressed this issue and demonstrated mitigation strategies. An overview of some of the challenges and opportunities, with an emphasis on the anode catalyst will be presented.
Solution-Crystallized Organic Field-Effect Transistors with High Charge Carrier Mobility

Takafumi Uemura
Assistant Professor, Osaka University, Japan

Organic thin-film transistors are regarded as an important technology for broad range of applications such as flexible displays and other large-area imaging devices because of their superior mechanical flexibility and cost advantage in printable technologies. Recently, a number of high-performance organic semiconductor materials are developed, so that their applicability to display technology is highly expected.

This presentation covers recent progress in our studies of solution-crystallized organic transistors. In order to promote development of solution-processed organic field-effect transistors, we have investigated techniques of crystallization during the fabrication of organic semiconductor thin films from solution. Regulating direction of the crystal growth in the process, we successfully formed crystalline semiconductor films with the mobility as high as 12 cm²/Vs from newly developed organic semiconductor compounds of 2,9-dialkyl-dinaphtho[2,3-b:2',3'-f]thieno[3,2-b]thiophene (C₁₀-DNTT) [1,2]. Figure 1 shows the typical transistor characteristics of solution-crystallized C₁₀-DNTT transistor. In our comprehensive measurements of Hall-effect and temperature dependent mobility tell us that their fundamental charge transport mechanism is band transport. In addition, recently we have demonstrated solution-processed C₁₀-DNTT active-matrix (AM) backplanes to drive liquid crystalline displays (LCD). Figure 2 shows a photograph of the C₁₀-DNTT AM-LCD. The size of the display is 2.3 inch in diagonal. The number of pixels is 30 × 23 and the pixel size is 1.5 mm × 1.5 mm. The averaged carrier mobility was over 3 cm²/Vs, which is the highest mobility reported for solution-processed organic AM backplanes. The performance is satisfactory to drive the LCD panel within applied voltages of 20 V, so that high contrast switching is realized.

![Typical transistor characteristics of solution-crystallized C₁₀-DNTT TFT](Fig. 1)

![Active-matrix LCDs with high-mobility solution-crystallized C₁₀-DNTT TFTs](Fig. 2)

References
Poster Sessions
(Poster room, 2F)
1. The role of phosphorylation of a COPII coat protein, Sec31, in molecular export from the endoplasmic reticulum

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Molecular export from the ER is thought to be mediated by COPII vesicles. It has been shown that an essential COPII coat component, Sec31 in yeast, is phosphorylated and its phosphorylation has been implicated in COPII vesicle budding. However, the molecular mechanisms, including the phosphorylation sites and the kinases for this phosphorylation, are unknown. Hypothesizing that the phosphorylation-dephosphorylation cycle of the coat components of COPII vesicles may regulate molecular export from the ER, we recently discovered the phosphorylation of mammalian Sec31. Here we show that membrane recruitment of Sec31 is dependent on its phosphorylation. This suggests that the molecular export from the ER may be related to the phosphorylation of Sec31 in mammalian cells.

2. Muscle reconstruction in axolotl limb regeneration

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Urodele amphibians have a great capability to regenerate various organs, including limbs. They can regenerate lost limbs, when their limbs are amputated. Previous studies have suggested that dermal fibroblasts carry limb positional information. After amputation, dermal fibroblasts are transformed into blastema cells, which are undifferentiated multipotent cells. Blastema cells derived from dermal fibroblasts can redifferentiate into varied cell types, such as dermal cells and cartilaginous cells. However, they are not able to redifferentiate into muscle cells. Myogenic cells in a blastema have been implied to come from a stump muscle tissue. It is suggested that blastemal dermal fibroblasts have limb positional information, but it is not clear that blastemal myogenic cells have the information as a limb muscle since myogenic cells do not come from dermal fibroblasts. We examined a presence of the memory of a muscle in a regenerating limb by using a novel study system. We have succeeded in an induction of an ectopic limb in a flank. A limb induced on a flank possessed splendid muscles. To investigate their origin, we tested whether non-limb muscles could participate into limb regeneration. As a result, we revealed tail muscles can participate into limb regeneration. Moreover, a HGF/c-met signaling regulates muscle cell migration into a blastema in an axolotl similarly as in higher vertebrates. In association with those findings, we would like to discuss limb muscle formation in limb regeneration, development and evolution.
Dorsal root ganglia (DRGs) are clusters of sensory neurons that convey somatosensory information, such as touch, temperature, and pain to the central nervous system. DRGs arise from a subset of migrating neural crest cells. We previously found that DRG neurons do not develop in zebrafish mutant for either *erbb2* or *erbb3*, genes that encode receptor tyrosine kinases that function as ErbB2/ErbB3 heterodimers. By following neural crest migration in live transgenic embryos, we hypothesize that ErbB2/ErbB3 signaling regulates target recognition of DRG progenitors, so that they stop migrating in a location where they receive DRG-inducing signals.

We found that Erk, a downstream regulator of ErbB signaling is phosphorylated only in a very few population of migrating neural crest. This is consistent with the fact that only a very few neural crest cells receive ErbB2/ErbB3 signaling and becomes DRG neurons. However the inhibition of MAPK did not affect for DRG neuron formation. DRG neurons also appear to be normal after inhibition of PI3K, another downstream regulator of ErbB signaling. We are currently investigating how some neural crest cells receive ErbB signaling and which pathway ErbB signaling is through within neural crest cells.

4. Early regulation of axolotl limb regeneration

Akira Satoh
Research Core for Interdisciplinary Sciences, Okayama University, JST PRESTO

Amphibian limb regeneration has been studied for a long time. In amphibian limb regeneration, an undifferentiated blastema is formed around the region damaged by amputation. The induction process of blastema formation has remained largely unknown because it is difficult to study the induction of limb regeneration. The recently developed accessory limb model (ALM) allows the investigation of limb induction and reveals early events of amphibian limb regeneration. The interaction between nerves and wound epidermis/epithelium is an important aspect of limb regeneration. During early limb regeneration, neurotrophic factors act on wound epithelium, leading to development of a functional epidermis/epithelium called the apical epithelial cap (AEC). AEC and nerves create a specific environment that inhibits wound healing and induces regeneration through blastema formation. It is suggested that FGF-signaling and MMP activities participate in creating a regenerative environment. To understand why urodele amphibians can create such a regenerative environment and humans cannot, it is necessary to identify the similarities and differences between regenerative and non-regenerative animals. Here, I would like to summarize our recent works.
5. Sexual interaction influences daily activity pattern in *Drosophila melanogaster*.

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Social interaction is one of the time resetting signals for the *Drosophila* circadian clock, but it is not well understood which interaction can influences the clock. Sexual behavior is a prominent social interaction and has been reported to be under control of the clock in *Drosophila*. Here we investigated whether the sexual interaction synchronizes the clock as a time resetting cue so-called “Zeitgeber”. To examine this, we have recorded circadian locomotor activity rhythms when male and female are paired. Wild-type flies exhibit bimodal activity showing the peaks just at lights-on and before lights-off in light-dark cycle when they are separately recorded. Those behavioral patterns are influenced by pairing clock mutants and wild-type flies, suggesting that the social interaction is a potent Zeitgeber. We further investigated whether the sexual interaction affects the brain neurons that control the activity rhythms. Our immunohistchemical experiment showed that a subset of the clock neurons in the brain was influenced by pairing male and female, thus indicating that the sexual behavior can change the circadian rhythm through the brain clock neurons.

6. Artificial selection on developmental period affected the circadian rhythm in *Drosophila melanogaster*

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Generally, faster circadian clocks are expected to speed up the development time, suggesting a potential genetic correlation between developmental period and circadian rhythm. Such genetic correlation between developmental period and circadian rhythm has been demonstrated in two fly species, *Drosophila melanogaster* and *Bactrocera cucurbitae*. *Period* mutants in *D. melanogaster* and disruptive selection lines in *B. cucurbitae* show correlated effects on developmental period and circadian rhythm. However, whether natural genetic variation on developmental period and circadian rhythm is correlated with each other in *D. melanogaster* is still unknown. To examine this genetic correlation in *D. melanogaster*, we performed an artificial disruptive selection on developmental period, and evaluated the circadian rhythm. As a result, we found a significant response to the selection for longer developmental period, and it correlated effect to prolong the free-running period.
7. HSP90 as a capacitor of behavioral evolution

Masahiro Tsujino\(^1\), Taishi Yoshii\(^2\) and Kazuo Takahashi\(^1\)
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Because rapid adaptation to varying environmental condition is ensured by genetic variation, maintenance of genetic diversity is essential for organisms. Recently, the agent called evolutionary capacitor plays a main role in the maintenance of genetic variation, and has attracted a lot of attention. HSP90, one of the molecular chaperons, has been known to buffer morphological variation and may have similar effect on behavioral traits. In fact, inhibition of HSP90 activity was shown to increase behavioral variation in a previous study, suggesting a possibility that HSP90 acts as an evolutionary capacitor of behavioral traits. To investigate this hypothesis, we examined the effect of HSP90 inhibition on the locomotor activity in \emph{Drosophila melanogaster}. HSP90 inhibition was done by pharmacological inhibitor, Geldanamycin, and RNA interference. Here, 20 isogenic deficiency strains from DrosDel project were used to examine the effect on genetic variation, Locomoter activity was evaluated using indices cited from previous studies, e.g. circadian rhythm, activity, sleep, and evening anticipation, under constant dark and at a constant temperature.

8. Multiple capacitors for natural genetic variation in wing shape of \emph{Drosophila melanogaster}

Kazuo Takahashi
Research Core for Interdisciplinary Sciences, Okayama University

Genetic diversity is essential for every organism to adapt to changing environment. Theoretical and empirical studies have shown that the more a population is genetically diverse, the faster it adapts to new environments. The mechanism of how genetic diversity is maintained in natural populations has been studied for a long time, and recently, the role of evolutionary capacitor has attracted a lot of attention. An evolutionary capacitor buffers genetic and environmental perturbations and keeps genetic variation cryptic, accumulating mutations in a population. In my current project, I used wings of \emph{D. melanogaster} as a model system and searched for genomic regions that regulated the amount of natural genetic variation in wing morphology using isogenic deficiency strains. As a result, multiple genomic regions were shown to affect the amount of quantitative genetic variation and significantly increased broad-sense heritability of wing shape. In addition, several genomic deletions revealed qualititative cryptic genetic variation in wing morphology such as extra wing compartment, lack of wing vein etc. These results indicate the existence of multiple capacitors for natural genetic variation, and the genomic regions with effect on natural genetic variation may encompass candidate genes for evolutionary capacitors.
The cell cycle is a series of events that lead to cellular duplication. An error in the cell cycle can cause severe cellular dysfunctions such as cancer. It is considered that the robustness of the cell cycle is guaranteed by multiple feedback regulations within the control system. However, the complete picture of the feedback regulations is still unclear. We previously developed a method named genetic Tug-Of-War, by which we can assess the robustness of the cellular system against the increase in the copy number of a certain gene.

In this study, we tried to identify novel feedback regulations in the fission yeast cell cycle, by measuring the increase of the protein expressed from the gene of which the copy number is increased by the genetic Tug-Of-War method. If the ratio \[ \frac{\text{increase in protein amount}}{\text{increase in gene copy number}} \] is >1, a positive feedback regulation is predicted in the system. If the ratio is <1, a negative feedback regulation is predicted. We have assessed 20 cell cycle regulatory genes, and found potential positive feedbacks in the controls of \textit{cdc10} and \textit{chk1} regulations, and negative feedbacks in the controls of \textit{cdc16} and \textit{mik1} regulations.

We also found that the negative feedback in the \textit{mik1} regulation was performed through the degradation of Mik1 protein activated by its own protein kinase activity. We are now analyzing the molecular detail of the \textit{mik1} regulation, and mechanisms of feedbacks regulating other genes.

10. Robustness analysis of cellular systems

Hisao Moriya
Research Core for Interdisciplinary Sciences, Okayama University

Robustness is a property that allows a system to maintain its function despite perturbations. Robustness is considered to be one of the general design principles of biological systems, because they are complex evolvable systems. Robustness of biological systems is observed as tolerance against perturbations such as environmental change, biochemical noise and mutation, all of which can be referred as “intracellular parameters”. Robustness of biological systems has been studied mainly with theoretical analyses using computer simulations, yet the robustness of real biological systems is still unclear because there had been no experimental method to effectively assess the robustness. We have developed an experimental method named genetic tug-of-war (gTOW) to measure robustness of cellular systems. Using gTOW, we can measure the limit of gene overexpression, and thus assess the robustness of cellular system against the change in a gene expression parameter. In my laboratory, we have applied this method to measure the robustness of cellular systems in yeasts. In this poster presentation, I would like to summarize our current effort to understand the cellular robustness.
11. Quantifying cytotoxicity caused by protein overproduction

Koji Makanae and Hisao Moriya
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In most cases, gene overexpression is harmful for the cell and affects the growth rate. However, the mechanisms that exert the cytotoxicity on cells are still not clear. Here, we constructed various Green-Fluorescent Proteins (GFPs) with the following manipulations, and measured the copy number limits of overexpression using a method called 'genetic tug-of-war (gTOW)' to elucidate causes for the cytotoxicity as follows; (1) Addition of signal sequences to examine where the overexpressed GFPs exert the cytotoxicity. (2) Introducing mutations into GFPs to cause misfolding to examine the involvement of the sequestration of chaperones.

As a result, whereas the copy number limit of a GFP gene with a nuclear localization signal (NLS) was not different from the one of normal GFP gene, the limit of a GFP gene with a nuclear export signal (NES) was significantly low, indicating the strong cytotoxicity of NES-GFP. Misfolded GFP also showed significant cytotoxicity, addition of NLS to misfolded GFP slightly rescued the cytotoxicity. We thus developed tools to evaluate the cytotoxicity of overexpressed proteins, and we are now analyzing the cellular responses upon the overexpression of the GFPs.

12. A novel strategy to explore lower limit of gene expression:
reverse genetic tug-of-war

Masataka Sasabe, Sayumi Shintani, Koji Makanae and Hisao Moriya
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Biological systems are robustly maintained against internal and/or external perturbations and thus organisms can survive even if parameters such as gene expression levels are fluctuated. To understand the acceptable range of parameters to maintain proper cell functions, our group has previously developed a method called 'genetic tug-of-war (gTOW)' to determine the copy number limit of gene overexpression. Conversely, the lower limit is largely unknown due to the lack of methodology, though it is inevitably important to argue the cellular robustness and fragility. In this study, we designed a modified system termed reverse-gTOW (r-gTOW), to determine the lower limit of gene expression by applying Tobacco Etch Virus (TEV) protease induced protein inactivation (TIPI). The lower limit of gene expression can be measured by genetically appending degron sequence to a target, as well as modulating and monitoring the copy number (i.e. expression level) of TEV protease gene. We confirmed the feasibility of the r-gTOW and expanded it to explore the lower limits of expression of cell division cycle regulators.
13. Genetic interaction between mitochondrial dosage sensitive gene and a protein phosphatase gene PPH3

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We previously reported a method designated genetic tug-of-war (gTOW), by which we can measure the limit of gene overexpression in S. cerevisiae. In gTOW, a target gene with its native regulatory regions is cloned into a particular plasmid, and the copy number limit of overexpression of the target gene is evaluated. Genome wide gTOW screening identified 115 dosage sensitive genes (DSGs) that have very low limits of ≤10 copies.

Using a genome-wide suppressor screening, we identified a protein phosphatase gene PPH3 as a suppressor of a DSG encoding a mitochondrial pyruvate carrier (MPC2). We further tested if PPH3 could suppress the dosage sensitivities of other DSGs, and found that PPH3 specifically suppressed the dosage sensitivities of mitochondrial DSGs. Pph3 is known to function in the recovery process from the DNA damage checkpoint. We thus think that the overexpression of mitochondrial DSGs may cause DNA damage as a result of the reactive oxygen species production.

14. The involvement of NF-κB in isothiocyanate-regulated cell proliferation

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Isothiocyanates (ITCs) are electrophilic compounds that abundantly occur in cruciferous vegetables. Benzyl isothiocyanate (BITC), an ITC compound isolated from papaya, has been explored as a promising chemopreventive agent, since it induces cell cycle arrest and apoptosis in cancer cells. Nuclear factor-κB (NF-κB) is a transcription factor that regulates cell proliferation, apoptosis, and inflammation. However, the relation between BITC-reduced cell proliferation and NF-κB remains unclear. In this study, we clarified the regulating role of NF-κB in BITC-reduced cell proliferation in a human colon cancer cell line, HT-29.

We found that lower concentrations of BITC (<10 μM) increased the phosphorylation of IκB kinase α/β (IKK α/β) and the nuclear translocation of NF-κB. These effects were mitigated by inhibitors for MAPKs. BITC-increased nuclear translocation of NF-κB was not observed in the other cancer types. BITC (5 μM), however, decreased the mRNA level of cyclin D1 which is a regulator of cell cycle progression. This concentration of BITC also enhanced the interaction of NF-κB with β-catenin, transcriptional activator for cyclin D1, and reduced binding of β-catenin on the cyclin D1 promoter. Taken together, our data suggested that BITC regulates cell proliferation via MAPK-induced NF-κB nuclear translocation in HT-29 cells.
15. ArfA-mediated ribosome rescue in *Escherichia coli*

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Nishioka, Kazuya Nishigaki, Yuma Oka, Junki Morikawa and Tatsuhiko Abo
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Translation terminates when translating ribosome encounters an in-frame stop codon. When mRNA without the in-frame stop codon (non-stop mRNA) is translated, ribosomes stall at its 3’ end and form non-productive translation complex (NTC) containing peptidyl-tRNA. In eubacteria, such stalled ribosomes are mainly rescued by SsrA-mediated *trans*-translation. Recently we and other groups reported that *Escherichia coli* has at least two alternative ribosome-rescue factors: ArfA (YhdL) and ArfB (YaeJ). ssrA and arfA mutations are synthetically lethal, suggesting the importance of ribosome rescue. Interestingly, ArfA expression is negatively regulated by *trans*-translation, suggesting that ArfA has a fail-safe function in rescuing ribosome. ArfB has the GGQ-motif, the essential motif for peptidyl-tRNA hydrolysis in ribosome, and resolves NTC by itself, whereas ArfA requires cellular factors for ribosome rescue. Biochemical analyses revealed that RF2 is a factor that cooperates with ArfA to hydrolyze peptidyl-tRNAs located in the P-site of the stalled ribosome. Molecular mechanism of ArfA-mediated ribosome rescue will be discussed.

16. Genetic diversity of *cytochrome P450 sterol demethylases* on rice genome

Yuko Aono1, Kousuke Ikeda1, Graham Etherington2, Katrin Geisler3, Kauhiro Toyoda1, Mikihiro Yamamoto1, Tomonori Shiraishi1, Yuki Ichinose1, Jo Dicks2, Anne Osbourn3 and Yoshi-Shige Inagaki1

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Triterpenoids, including triterpene glycosides (saponins) have various functions, anti-cancer, anti-oxidant, and anti-microbial. Compared to dicots, monocots including cereals and grasses are generally deficient in this secondary metabolite except for oats (*Avena*). The biosyntheses of steroids and triterpenoids involve the initial cyclization of 2,3-oxidosqualene to sterols and triterpenes, followed a series of oxidations by cytochrome P450s. It is reported that both oats and *Arabidopsis thaliana* produce triterpenoid compounds from contiguous co-regulated gene clusters. Here, we probe the rice genome sequence to characterize the diversity of a key gene family *Cytochrome P450 sterol demethylase* (CYP51Gs & OsCYP51Hs). Same as OsOSCs, 12 CYP51 genes and pseudogenes were also discovered and characterized. It is likely that one is for rice steroids biosynthesis pathway, and another is for rice triterpenoids biosynthesis pathway. However, we found no evidence for a triterpenoid gene cluster in rice. These findings provide new insights into the evolution of triterpenoid synthesis in monocots.
17. Genetic diversity of OxidoSqualene Cyclases on rice genome

Tatsu-ichiro Nishira1, Kousuke Ikeda1, Yuko Aono1, Graham Etherington2, Katrin Geisler3, Kazuhiro Toyoda1, Mikihiro Yamamoto1, Tomonori Shiraishi1, Yuki Ichinose1, Jo Dicks2, Anne Osbourn3 and Yoshi-Shige Inagaki1

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Triterpenes are one of the largest classes of plant secondary metabolite and important for use as drugs, immunostimulants and other commercial applications. Triterpenes are formed from the isoprenoid pathway by cyclization of 2,3-oxidosqualene, and so share a common biosynthetic origin with sterols. The cyclization of 2,3-oxidosqualene to cycloartenol is first committed step in the synthesis of essential sterols and is catalyzed by the oxidosqualene cyclase (OSC) enzyme cycloartenol synthase. In higher plant, 2,3-oxidosqualene is also a precursor for nonsteroidal triterpene such as α-amyrin, β-amyrin, lupeol, and so on. Triterpenes are common in dicots, but seldom found in monocots except for oats (Avena). Here we probe the rice genome sequence, to characterise the diversity of a key gene family which is OSC gene involved in triterpene synthesis and to examine the possibility of a triterpene gene cluster in rice. We discover and characterise 12 OSC genes and pseudogenes. It is likely that no one out of twelve was involved in the triterpenoid biosynthesis pathway. We also uncover a key event for tandem duplication in monocot triterpene diversification. We conclude considerable diversity of OSC gene family in rice. These findings provide new insights into the evolution of triterpene synthesis in monocots.

18. The causal pathogen of black spot on strawberry cultivar HS-138 was the strawberry pathotype of A. alternata

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The Alternaria black spot of strawberry is caused by the strawberry pathotype of Alternaria alternata, which produces host-specific toxins, called AF-toxins, and affects only one Japanese strawberry cultivar, Morioka-16. In 2009, the occurrence of black spot on the recently bred strawberry cultivar HS-138 and the causal pathogen were reported as Alternaria sp. by Misawa et al. In this research, the pathogenic isolate E11 from HS-138 was confirmed to have pathogenicity only on the host plants of A. alternata strawberry pathotype. Isolate NAF8 of the strawberry pathotype A. alternata showed pathogenicity to HS-138. Production of AF-toxins by the isolate E11 was found by chromatographic analysis and bioassay on the leaves of Morioka-16 and Nijisseiki. On the other hand, HS-138 plants were affected by isolate NAF8 by spore inoculation and by AF-toxin I to the same degree as Morioka-16. These results suggest that HS-138 plants are susceptible to the strawberry pathotype because of their sensitivity to host-specific AF-toxin. As the above results, the causal pathogen of black spot on strawberry cultivar HS-138 was the strawberry pathotype of A. alternata.
19. HopH1, a putative Zn-dependent protease, of *Pseudomonas syringae* induces hypersensitive response in non-host *Solanum torvum* Sw. cv. Torubamubiga

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The gram-negative phytopathogenic bacterium *Pseudomonas syringae* delivers the hypersensitive response (HR) and pathogenicity (Hrp) effectors via type III secretion system to elicit HR in non-host plants and cause disease in host plant. Recently, our group found that Rip36 (a putative Zn-dependent protease) of *Ralstonia solanacearum* Rs1000 is an avirulent factor to induce HR in *Solanum torvum* (St) Sw. cv. Torubamubiga. The ortholog of rip36 was also found in *P. syringae* pv. *tomato* (Pto) DC3000 (HopH1) and *P. syringae* pv. *syringae* (Psyr) B728a (hopH1) but not in *Pseudomonas syringae* pv *phaseolicola* (Pph) 1448A. To examine whether HopH1 induces HR in St Torubamubiga, a hopH1 mutant (denoted ΔhopH1) was constructed in Pto DC3000. The wild types (WT) of Pto DC3000 and Psyr B728a and ΔhopH1 mutant induced HR in St Torubamubiga, whereas WT of Pph 1448A did not. However, when we introduced hopH1 of Pto DC3000 and Psyr B728a into Pph 1448A, the resultant transgenicants acquired HR inducing activity. These results indicate that HopH1 of *P. syringae* is an avirulent factor to induce HR and HR-induction ability of ΔhopH1 mutant of Pto DC3000 was due to presence of other elicitor.

20. Chemical biology of xylem differentiation in *Arabidopsis thaliana*

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Xylem is the channels of vascular plants that conduct water and mineral and is essential for the evolution of land plants. Xylem differentiation is regulated by the interaction of multiple signaling molecules but the interaction mechanism remains unknown. Recently, our chemical biology approach has shown that the counteraction of thermospermine to auxin regulates the timing and spatial pattern of xylem differentiation. Thermospermine is a structural isomer of spermine and is produced through the action of thermospermine synthase, ACAULIS5 (ACL5) in *Arabidopsis thaliana*. Thermospermine suppresses xylem differentiation and its deficiency in the acl5 mutant results in a severe dwarfism associated with excessive xylem differentiation. To elucidate the molecular basis of the function of thermospermine, we screened chemical libraries for compounds that can affect xylem differentiation in the acl5 mutant. We found that the auxin analogs remarkably enhanced xylem vessel differentiation in acl5 seedlings but not in the wild type. The effectiveness of auxin analogs was dependent on their metabolic stability and cellular accumulation. The xylem-inducing effect of auxin analogs was suppressed by thermospermine and inhibitors of auxin perception. These results suggest that the auxin signaling that promotes xylem differentiation is normally limited by thermospermine. In addition, our chemical screening also identified novel suppressors of xylem differentiation that will provide valuable insights into xylem differentiation.
21. A chemical biology approach to understand root morphology during mechanical stimulation in *Arabidopsis thaliana*

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We have previously reported that a new and simple growing system to apply mechanical stimulation on arabidopsis root tip: growing horizontally on dialysis membrane-covered agar. (Okamoto et al, 2008) When Arabidopsis seedlings allowed to grow horizontally on the system, the roots showed characteristic ethylene phenotypes: 2-fold reduction in root growth, increase in root diameter, decrease in cell elongation, and ectopic root hair formation. Genetic and physiological studies showed that the defects in root gravitropism (*aux1-7, iaa14/slr-1, arf7arf19*) and ethylene response (*ein2-1*) nullified the root morphology. In order to obtain components involved in the signal transduction during mechanical stimulation in arabidopsis roots, we employed chemical biology approach and screened about 500 pharmacologically active compounds to enhance or nullify the morphology of roots. Possible mechanism behind the morphology of roots will be discussed based on physiological roles of candidate compounds and genetic studies.

Reference

22. Use of radiocarbon to estimate diet ages of earthworms across different climate regions

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Natural abundance of radiocarbon (\(^{14}C\)) has been applied to estimate the turnover time of soil carbon (C) across different climate regions. However, despite the important functional role played by soil animals in decomposition processes, little is known about variation in their radiocarbon concentrations across different climate regions. In this study, we tested whether the diet ages (defined as time elapsed since C in the diet of earthworms was fixed from atmospheric CO\(_2\) by photosynthesis) differed according to feeding habits and across study sites in various climate regions, ranging from cool temperate forests to tropical savanna. Multiple regression analysis showed that the diet ages of earthworms were significantly affected by both feeding habits and study sites. The diet ages of endogeic (soil-feeding) earthworms (8.3 years) were significantly older than those of epigeic (litter-feeding) earthworms (2.6 years), with anecic (litter-/soil-feeding) earthworms (5.7 years) having intermediate diet ages. Mean diet age differed significantly across the sites, but it did not necessarily become younger in warmer climate regions. These results suggest that the degree of decomposition of soil organic matter used by earthworms differs among the study sites, or that the difference in the turnover time of soil organic C used by earthworms across the sites is relatively small and variable due to factors other than temperature, such as vegetation and soil texture.
23. Arboreal and ground ant fauna in a temperate forest in Japan

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Arboreal and ground ant fauna was studied in seven vegetations of different stand ages (time since last cut event; 1 yr, 6 yr, 11 yr, 24 yr, 50 yr, 59 yr, 105 yr) in a temperate forest in Japan. We set up a 30 × 30 m plot in each of the vegetation. In each plot, we collected arboreal ants from the selected five to seven canopy trees through direct observation. We also collected ground ants through direct observation and using tuna and honey baits. We compared abundance and species composition of arboreal ants, and that of ground ants, among the vegetations. A total of 9 ant species were collected from the ground and a total of 7 ant species from the canopy trees. Total abundance of ground ants was higher in 1~6 yrs than in the other vegetations. The abundance of each ground ant species varied among the vegetations. *Ponerinae* ants was only collected from the ground in 105 yr. Total abundance of arboreal ants was higher in 1~6 yr and 105 yr than in 11~59 yrs. Ants nesting in the crown of canopy trees was only one ant species, *Crematogaster teranishii*, in 105 yr. In 24~59yr, there was no ants foraging on canopy trees.

24. ESTABLISHING A NEW METHODOLOGY FOR GENOME MINING AND BIOSYNTHESIS OF NATURAL PRODUCTS THROUGH FUNGAL MOLECULAR GENOMICS

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Polyketides (PKs) and nonribosomal peptides (NRPs) have been isolated from *Streptomyces* and many other source organisms. In recent years, gene clusters encoding PK synthases (PKSs) and NRP synthetases (NRPSs) have been discovered through fungal genome sequencing. While on average 50 gene clusters are identified in a single fungal genome, fewer fungal PK and NRP products can be isolated from a fungal culture grown under a typical growth condition. Thus, simple artificial reactivation of the cryptic gene cluster may be insufficient for an efficient natural product biosynthesis. To circumvent these obstacles, we examined upregulation of 60 gene clusters encoded in chromosomal DNA of four fungal species, *Aspergillus fumigatus*, *A. flavus*, *A. oryzae* and *Chaetomium globosum*, using the aforementioned fungal molecular genetics. Thus far, we have isolated seven new PK and NRP compounds successfully. Subsequently, we used our recombination cloning-based yeast expression system to reconstitute these biosynthetic gene clusters quickly and efficiently. Our preliminary results clearly demonstrate successful expression of seven *C. globosum* PKS gene clusters in *Saccharomyces cerevisiae*, three of which led to the production of new natural products whose identities have been characterized spectroscopically. Our methodology will facilitate the efforts in isolating novel natural products and rationally engineering in the biosynthetic pathways for production of analogs possessing comparable if not more potent bioactivity.
Session II (Chemistry, Physics, Mechanics)
November 21 (Wed) 13 : 30～15 : 30

25. Sulfonamides identified as plant immune-priming compounds in a high-throughput chemical screening increase disease resistance in Arabidopsis thaliana

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Plant activators are agrochemicals that activate plant immune system contributing to crop protection from diseases. Since plant activators work on plants but not on pathogens, no new drug-resistant strains have come out so far. This durability is the most prominent feature of the plant activators compared with pesticides. To isolate lead compounds for practical plant activators, we screened two different chemical libraries composed of various bioactive substances by using a screening procedure that can selectively identify immune-priming compounds. Among various candidate molecules we isolated, here we report characterization of a group of sulfonamide compounds, sulfameter, sulfamethoxypyridazine, sulfabenzamide and sulfachloropyridazine. These sulfonamide compounds potentiated cell death of Arabidopsis suspension culture induced by avirulent Pseudomonas bacteria, and increased disease resistance in Arabidopsis plants against the virulent strain. The possible action mechanisms of the sulfonamides are discussed based on their bioactivity and molecular structures.

26. ImprimatinA and B, novel plant immune-priming compounds identified via a newly-established high-throughput chemical screening target salicylic acid glucosyltransferases in Arabidopsis thaliana

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Plant activators are compounds that protect plants from pathogens by activating their immune system. Here we report the establishment of a high-throughput chemical screening procedure to identify plant immune-priming compounds which potentiate but do not directly induce cell death in Arabidopsis cell suspension cultures induced by Pseudomonas syringae pv. tomato DC3000 avrRpm1. From screening of a library of 10,000 structurally diversified small organic molecules and derivative analysis of the isolated candidates, we identified five compounds designated Imprimatins for “immune priming chemicals”. These compounds were classified into two groups, ImprimatinA and –B, with structural similarity. These Imprimatins enhanced disease resistance against Pseudomonas bacteria in Arabidopsis plants. Pretreatments increased the accumulation of endogenous salicylic acid (SA), but reduced its metabolite, SA-O-ß-D-glucoside. We found that Imprimatins inhibited both a known and a previously unknown SA glucosyltransferase (SAGT) in vitro. Each single and their double knockout Arabidopsis plants for these SAGTs phenocopied the Imprimatin-induced phenotypes. Our results indicate that Imprimatins can provide a novel mode of action to prime plant immunity, and that SA glucosylation is a target for developing novel crop protectants.
27. ImprimatinC1, a novel plant immune-priming compound, functions as a partial agonist of salicylic acid

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Plant activators are agrochemicals that protect crops from pathogens. They confer durable resistance to a broad range of diseases by activating intrinsic immune mechanisms in plants. To obtain leads regarding useful compounds, we have screened a chemical library using an established method that allows selective identification of immune-priming compounds. Here, we report the characterisation of one of the isolated chemicals, imprimatinC1, and its structural derivative imprimatinC2. ImprimatinC1 functions as a weak analogue of salicylic acid (SA) and activates the expression of defence-related genes. However, it lacks antagonistic activity toward jasmonic acid. Structure-activity relationship analysis suggests that imprimatinC1 and C2 can be metabolised to 4-chlorobenzoic acid and 3,4-chlorobenzoic acid, respectively, to function in \textit{Arabidopsis}. We also found that imprimatinC1 and C2 and their potential functional metabolites acted as partial agonists of SA. Thus, imprimatinC compounds could be useful tools for dissecting SA-dependent signal transduction pathways.

28. Diuretics prime plant immunity in \textit{Arabidopsis thaliana}

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Plant activators are agrochemicals that activate the plant immune system, thereby enhancing disease resistance. Due to their prophylactic and long-lasting effects on a wide spectrum of diseases, plant activators can provide synergistic crop protection when used in combination with traditional pest controls. Although plant activators have achieved great success in wet-rice farming practices in Asia, their use is still limited. To isolate novel plant activators applicable to other crops, we screened a chemical library using a method that can selectively identify immune-priming compounds. Here, we report the isolation and characterization of three diuretics, bumetanide, bendroflumethiazide and clopamide, as immune-priming compounds. These drugs upregulate the immunity-related cell death of \textit{Arabidopsis} suspension-cultured cells induced with an avirulent strain of \textit{Pseudomonas syringae pv. tomato} in a concentration-dependent manner. The application of these compounds to \textit{Arabidopsis} plants confers disease resistance to not only the avirulent but also a virulent strain of the pathogen. Unlike salicylic acid, an endogenous phytohormone that governs disease resistance in response to biotrophic pathogens, the three diuretic compounds analyzed here do not induce \textit{PR1} or inhibit plant growth, showing potential as lead compounds in a practical application.
29. A microfluidic device to reduce treatment time of intracytoplasmic sperm injection

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Sperm collection in the conventional intracytoplasmic sperm injection (ICSI) method is a time-consuming process for samples with low sperm concentrations. To reduce the ICSI treatment time for the severe conditions, we propose a sperm collection method using a microfluidic chip device, which can facilitate easy sperm observation without use of centrifugation. We compared the ICSI treatment time required for intracytoplasmic injection of porcine sperm using the method employing the microfluidic device and the conventional microdroplet method before conducting clinical assisted reproductive technology (ART) study. The average ICSI treatment time using the method employing the microfluidic device with poor quality semen (sperm concentration, $2.0 \times 10^4$ cells/ml) was shorter than that using the conventional microdroplet method [265 ± 15 s (n = 43) vs 347 ± 19 s (n = 50); P < 0.05]. Using the microfluidic device, the ICSI treatment time for low sperm concentrations can be reduced. The results suggest that this device may be clinically useful for ICSI in human assisted reproductive technology laboratory supports.

30. Use of a mechanical vibration system to observe the effects of mechanical stimuli on in vitro mouse embryo development

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Mammalian embryos are transported to the uterine cavity through the fallopian tubes during development. Embryo development can be regulated not only by hormones but also by applying mechanical stimuli (MS), such as shear stress (SS), compression, and/or friction force, in the fallopian tubes before implantation. To mimic the mechanical environment in in vitro culture, mechanical vibration systems were developed; these systems enhanced porcine and human blastocyst development. In this study, we examined embryo motion, SS induced by vibration, and blastocyst development to understand the effects of mechanical vibration on embryo development. Frozen two-cell stage embryos from ICR mice were defrosted and cultured in 50 μl of mW culture medium for 3 days. In the mechanical vibration group, the embryos were cultured at a frequency of 74 Hz for 5 seconds at 15-min intervals. The rate of embryo development to the blastocyst stage was 48% (N = 235) and 51% (N = 235; P > 0.05) in the vibration and control culture groups, respectively. There was no significant difference in either blastocyst development rate or average cell number between the two groups. Using this culture system, we can apply SS and mechanical vibration to the rotating embryos in microdroplets to facilitate diffusion not by medium motion but by mechanical vibration of embryo.
31. Electrode fabrication using conductive nano-ink and microfluidic technology for bio-applications

Ikuyo Sugimoto, Koji Matsuura, Mieko Kodama and Masayuki Kanehara
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Printed electronics technology used to economically prepare printing conductive patterns onto flexible materials is both fundamental and crucial for the successful integration of electronics with textiles or fluidics. Conductive nanoink (NI) is a suspension of hydrophilic organic–inorganic hybrid nanoparticles (NPs) in aqueous solution. The NI composition can impart electrical conductivity to a surface-protecting ligand layer of conductive inorganic NPs, and do not require any post-treatment such as the removal of ligands. The use of conductive inorganic NPs dispersed in water enables the simplification of the electrode preparation process at room temperature. In this study, we developed a transparent breadboard and NP microelectrodes in the microfluidic channel for measurement of the number of particles. The electrode fabrication in the microfluidic channel can be acquired using conventional laboratory equipment without the need for costly ultra-vacuum deposition instruments. We are in the process of preparing the microfluidic channel which is equipped with the NP microelectrodes, and which is integrated with electrical measurement systems to count cells and/or microparticles. The microfluidic fabrication of electrodes and electrical measurement technologies may potentially be applied to the technology for micro–nano fluidics and bio-applications such as cell counting or ion sensing.

32. Observation of cell focal adhesions using surface plasmon resonance microscopy without labeling

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Observation of cell adhesion site without chemical labeling contributes to the understanding of intracellular adhesion dynamics of biomolecules including integrin and other adhesion proteins. To satisfy the demands to investigate cell adhesion site without labeling, we employed a scanning localized surface plasmon microscopy (SLSPM) and observed the adhesion sites of the cell on a substrate with spatial resolution of 200 nm. By combining a non-scanning surface plasmon microscopy (SPRM) and SLSPM, we observed a cell adhesion site and identified the regions of interest. The main advantage of our system is that it has the highest spatial resolution among other SPRM imaging systems without fluorescent labeling. Our optical system is useful for recognizing and detecting the adhesion dynamics because of its high spatial resolution. In addition, we discuss the objectives to use the SLSPM and SPRM systems reported by other groups for cell observation.
33. Simultaneous investigation of intracellular calcium concentration and mechanical stimuli to mouse blastocysts

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Mammalian embryos are transported to the uterine cavity through the fallopian tube during cell cleavage and blastocyst development. Based on ciliary movement, similarly-sized tubal lumens of the ampulla and isthmus, and the diameter of the embryo, fertilized oocytes may be subjected to mechanical stimuli (MS). To investigate the responses of the mammalian embryos to MS, we developed a dynamic air actuation system to apply MS in a microfluidic channel by deforming a 0.1-mm membrane, and investigated changes of intracellular calcium ion concentration ([Ca²⁺]) in mouse blastocyst applied MS. [Ca²⁺], was measured in a stained mouse embryo with Fluo-4 AM using confocal fluorescence microscopy. When blastocysts were compressed, fluorescence intensity (FI) in the blastocyst also increased in response to the applied MS. The sum of FIs increased by a factor of 1.1-1.2 times compared with those observed before MS. The increase in the sum of FI indicated that enhancement of [Ca²⁺] would be induced by these MS. Molecular mechanosensing systems such as mechanosensitive ion channels (MSIs) would play an important role in responses to these MS. By investigating the relationship between the kinetics of FI changes and MS using selective inhibitors for the MSIs, we could determine the embryonic sensor proteins.

34. Natural Products in Organic Synthesis

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For the construction of a sustainable society, it is important to find new unused resources that can be alternatives. However, the development of unused resources has the possibility to conflict with the achievement of a sustainable society. Most of these unused resources contain a lot of impurities of complex shapes; therefore, purification of the resources consumes a large amount of energy, produces a lot of waste and harmfully influences the environment.

In the present study, these resources are used as both catalysts and starting materials for organic reactions. There remain a number of unsolved problems in organic synthesis, problems that are difficult to tackle with a purely synthetic chemistry-oriented viewpoint, for example, the transformation of hydrocarbon and biomass. Current achievements using these materials will be disclosed.
The catalytic enantioselective construction of all-carbon quaternary stereocenters remains a difficult problem in synthetic chemistry. A reliable approach toward this challenge has been the asymmetric conjugate addition of carbon-based nucleophiles to suitable α,β-unsaturated carbonyl acceptors. The majority of asymmetric conjugate additions for the synthesis of quaternary centers involve the use of highly reactive organometallic reagents to a variety of unsaturated electrophiles with copper catalysts. These reactions uniformly involve air and moisture sensitive organometallic reagents that require rigorously anhydrous reaction conditions. In the industrial chemistry, however, the reaction conditions displaying a tolerance to water and air are highly desirable.

In the present study, the first palladium-catalyzed asymmetric conjugate addition of arylboronic acids to β-substituted cyclic enones would be demonstrated employing an easily accessible chiral pyridinooxazoline (PyOX) ligand. These reactions generate a wide array of benzylic all-carbon quaternary stereocenters under an atmosphere of air and without the need for purification or distillation of any commercially obtained materials.

36. Nonprecious metal catalysts based TiO$_2$ and their structural properties

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Transition metal (TiO$_2$) catalysts coexist with nitrogen and carbon as a function of various concentrations has been prepared through sol-gel route. In a typical process imidazole and Vulcan XC 72 was used as nitrogen and carbon source respectively. Also, the addition of Mn impurity into the TiO$_2$ host lattice was performed and is served as to improve the overall conductivity, which is more beneficial for catalytic performance. XRD results revealed the formation of anatase phase tetragonal system of TiO$_2$. Presence of carbon peak at about 25° has overlapped with the anatase phase peak of TiO$_2$. Using the Scherrer’s relation, the particle sizes were estimated and are ~14 nm in diameter. Stretching Vibrations of C=C, C-N, O-Ti-O showed the corresponding peaks at about 1554, 1270 and 790 cm$^{-1}$ respectively in the FTIR spectra. The prepared catalysts were well oriented and ordered in a regular spherical shape, identified through SEM micrograph. TEM observation also clears the catalysts are at nanoscale and is well correlate with the estimation of particle size from Scherrer’s relation.
37. Electron conductive π-junction Au nanoparticles

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Gold (Au) nanoparticles play important roles in different branches of science, such as in nanoelectronics, nonlinear optics, biological labeling, oxidation catalysis, etc. For the application of nanoparticles in nanoelectronic devices, exploiting the organo-electronic π-orbital interactions, which are generally used in electron-conductive polymers and organic transistors etc., are quite important in light of the reduction of the interparticle resistance of the surrounding ligands. Here we report the preparation, structural analysis and unique optical and electronic properties of π-junction Au nanoparticles. Multidentate macrocyclic phthalocyanine derivatives were synthesized and used to protect Au nanoparticles, where the phthalocyanine ligands densely protect the Au nanoparticles in a face-coordination fashion to make π-junction Au nanoparticles. The Q-band of the phthalocyanine rings on Au nanoparticles was disappeared, indicating the direct electronic interaction between the phthalocyanine π-electrons and the Au orbital. The phthalocyanine π-junction Au nanoparticles can be used as direct printable electron conductive material under ambient condition.

38. Piezoelectric polymer flexible displacement sensor for soft actuators

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This research aims to realize a displacement detection sensor that can be attached on flexible soft bellows actuators. These types of actuator can be applied to micro hands. The sensor material is a piezoelectric polymer: poly(vinylidene fluoride-trifluoroethylene) [P(VDF/TrFE)]. The P(VDF/TrFE) has the advantage of the piezoelectric constant g31. This constant shows the performance as the piezoelectric sensor. The fabrication process for the sensor is simple. Accordingly P(VDF/TrFE) is suitable for the sensor element. By adjusting thickness, temperature and applied voltage in the polarization process, fabrication conditions were optimized. As a result, the piezoelectricity has been improved.

In addition, the P(VDF/TrFE) sensor was mounted on the actuator having a bellows structure. The sensor film was formed from piezoelectric polymer P(VDF/TrFE) pellet. A gold electrode was deposited on the sensor film with the sputtering. Next, the film was polarized by applying the direct current 250V at 140°C. A conductive paste was used as wiring in order to keep flexibility.

The actuator is curved by applying a negative pressure. As a result, we have succeeded in the defecting of actuator displacement by the sensor when the negative was applied pressure up to 40kPa.
39. Rubber tube actuator for assisting colonoscope insertion

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Insertion of a colonoscope requires very technical procedure and in some cases it is very
difficult even for experienced doctors. We have developed a new mechanism which can add
self propellant ability to the colonoscope. In this research, we have realized a soft rubber
actuator which is tube shape and can support colonoscope insertion. The soft rubber actuator
generates ellipsoidal motion on the surface by applying pneumatic pressure. Therefore by
using multiple actuators and driving them with phase difference, traveling waves are
generated. So the colonoscope with actuators can have self-propelling ability. This ability
depends on the cross-sectional shape of the rubber tube. We have optimized the shape by
nonlinear FEM (Finite Element method) and fabricated the actuator. Fundamental
experiments were carried out for confirming the self propelling ability using a dummy
endoscope. As a result, optimized actuator realized 2.2 times higher velocity compared to the
previous one. Additionally effect of decreasing load to colon wall of a phantom could be
recognized experimentally.

40. Miniature of McKibben Actuator

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McKibben actuator that is known as artificial muscle, is made of rubber tube and knitted
fiber and can generate contraction force. This actuator has several advantages as follows,
flexibility, simple structure, light weight and high power/volume ratio. Therefore many types of
artificial muscles have been studied. However almost of these artificial muscles are several
millimeters in diameter. On the other hand, muscle of actual creatures is a bundle of many
fiber muscles which are micro size. Therefore if some muscle fibers in a bundle were broken,
the bundle can compensate its actuation by the other muscle fibers. In this study, we have
fabricated a McKibben actuator that is about 20 [mm] in length of the actual contraction part
and 1.0 [mm] in outer diameter. This size is the smallest diameter in the world at present. The
fiber angle is 43.8 [deg]. This actuator has been fabricated by molding a silicone rubber, and
winding fibers on the surface of the rubber. Through some experiments, the actuator can
generate 234 [mN] and contract about 10.5 [%] by applying pneumatic pressure of 600
[kPaG].
41. Variable Stiffness Colonoscope driven by air pressure

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Recently, the need of colonoscopy is increasing because of the rise of colonic diseases including cancer of the colon. However, current colonoscopy depends on doctor’s skill strongly. Therefore, a large intestine endoscope that can be inserted without any special techniques of doctors with high safety is required.

We have developed the variable stiffness endoscope that consists of multi pneumatic rubber devises. The device is cylindrical rubber structure with reinforcement fibers. By applying air pressure, stiffness of the device can be changed without its deformation. Therefore the endoscope can changed own stiffness partially by controlling air pressure and easy insertion will be achieved by switching the stiffness. An evaluation phantom which has sensors to measure load to the intestinal wall has been developed, and we have investigated the relation between stiffness change pattern and load to intestinal wall experimentally. From the experiments optimum stiffness change pattern to reduce the load in colonoscopy has been derived.

42. Novel occlusal check device detecting teeth sound using soft suction cups

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For healthy chewing, normal occlusion without local overload is necessary. However, the current occlusal examination depends on techniques, skills, and sense of doctors.

In this research, we are focusing on that occlusal condition is related to sound from teeth, and have developed a novel occlusal checking system detecting the sound.

The system is configured with multiple sound listening devices. Each device consists of a soft suction cup and a miniature microphone. The suction cup can be adhered on a tooth by applying negative pressure through a tube, and the microphone is inserted into the tube. Therefore when a patient bites, generated sound from the target tooth can be detected by the microphone.

we have found out the relationship between adhesion force and applied negative pressure to the suction cup, and decided optimum value of negative pressure. Then, by using two devices, we have conducted experiments of detecting occlusal sound using a jaw model for confirming the potential of this system. Furthermore, we carried out the experiments in the oral cavity, and succeeded in detecting sounds from tooth by using fabricated devices.
43. Electronic Structure of low-dimensional molecular aggregate

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Most work in the field of organic electronics has focused on $\pi$-conjugated molecules with a closed-shell configuration, which accommodate their $\pi$-electrons in only bonding orbitals and consequently are quite stable. The ground state of such molecules can be well described by a single electron configuration, in which two electrons occupy the highest occupied molecular orbital (HOMO). In general, closed-shell molecules have wide energy gaps between the HOMO and the lowest unoccupied molecular orbital and the intermolecular interactions between such molecules are much weaker than intramolecular interactions such as covalent bonds. On the other hand, the molecules called organic biradicals possesses much strong intermolecular interaction compared with closed-shell molecules. The biradical molecule carries tow-unpaired electrons and the interaction between the two unpaired electrons leads to extremely strong intermolecular interactions, leading to intermolecular covalency. Organic biradicals have a high degree of potential for a new building block for a highly ordered organic semiconducting film in organic electronics. This work has studied the crystalline film growth and the electronic structure of low-dimensional molecular aggregates of several organic biradical molecules. In the presentation their anomalous electronic structures are discussed.
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