RIKEN International Symposium

Frontiers in Integrated Symbiology

June 4th, 2018 (Monday)

Small Hall, Tower Hall Funabori, Tokyo

主催：iSYM RIKEN

後援：日本共生生物学会

RIKEN International Symposium “Frontiers in Integrated Symbiology”
June 4th, 2018 (Monday)
Small Hall, Tower Hall Funabori, Tokyo

Program

10:00-10:10 Opening remarks

10:10-10:50 **Ifor WILLIAMS**
(Pathology & Laboratory Medicine, Emory University, USA)
“M Cells Contribute to Intestinal Symbiosis by Promoting Generation of Microbiota-Reactive Secretory IgA”

10:50-11:30 **Neil MABBOTT**
(The Roslin Institute & Royal (Dick) School of Veterinary Sciences, University of Edinburgh, UK)
“The role of crypt-associated macrophages in the maintenance of the intestinal stem-cell niche”

11:30-12:00 **Hiroshi OHNO**
(Laboratory for Intestinal Ecosystem, IMS, RIKEN)
“Small intestinal microbes act in concert to exacerbate autoimmune inflammation in the central nervous system”

12:00-13:00 Lunch

13:00-13:30 **Wataru SUDA/Masahira HATTORI**
(Laboratory for Microbiome Sciences, IMS, RIKEN)
“Long-read metagenomic analysis of human gut microbiomes”

13:30-14:00 **Moriya OHKUMA**
(Microbe Division, JCR, BRC, RIKEN)
“Next generation technology for integrated symbiology and microbial resources of symbionts”

14:00-14:40 **Tomoyoshi NOZAKI**

(Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo)

"Divergent evolution of mitochondria under anaerobic conditions"

14:40-15:10 **Jun KIKUCHI**

(Environmental Metabolic Analysis Research Team, CSRS, RIKEN)

"Ecoinformatics approach for fish・microbiota and ecosystems"

15:10-15:30 Coffee Break

15:30-16:10 **Kiwamu MINAMISAWA**

(Microbial Symbiosis, Graduate School of Life Sciences, Tohoku University)

"Environmental dynamics of plant-associated bacteria: From rhizobia towards plant microbiomes"

16:10-16:40 **Ken SHIRASU**

(Plant Immunity Research Group, CSRS, RIKEN)

"Rhizosphere interactions regulate antibiotics production of root-associated microbes"

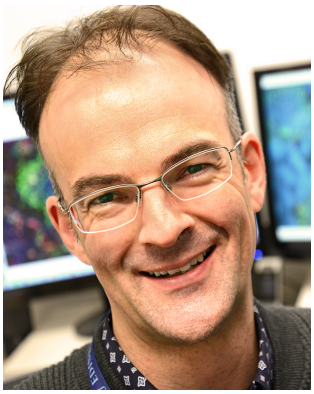
16:40-17:00 Closing remarks

Abstracts

Ifor Willimas
(Pathology & Laboratory Medicine, Emory University, USA)

M Cells Contribute to Intestinal Symbiosis by Promoting Generation of Microbiota-Reactive Secretory IgA

The mammalian intestine includes an associated mucosal immune system that promotes a stable symbiotic relationship with the resident gut microbiota while simultaneously supporting a vigilant surveillance program aimed at rapid containment of any potentially pathogenic microbes encountered at the mucosal interface. Secretory IgA (SIgA) directed against gut resident bacteria assists the mammalian mucosal immune system in establishing homeostasis with the commensal gut microbiota after weaning. Germinal centers (GCs) in Peyer's patches (PP) are critical inductive sites where naive B cells specific for bacterial antigens encounter their cognate antigens and receive T-cell help fostering their differentiation into IgA-producing plasma cells. We investigated the role of antigen sampling by intestinal M cells in initiating the SIgA response to gut bacteria by developing mice in which receptor activator of nuclear factor-κB ligand (RANKL)-dependent M-cell differentiation was abrogated by conditional deletion of the RANKL receptor RANK (encoded by *Tnfrsf11a*) in the intestinal epithelium. These conditional knockout mice lacked intestinal M cells and displayed profound delays in PP GC maturation and the establishment of a full complement of lamina propria IgA plasma cells, resulting in persistently diminished levels of fecal SIgA. The phenotype of mice specifically lacking intestinal cells indicates that M-cell-mediated sampling of commensal bacteria is a critical initial step for the efficient induction of both microbiota-reactive SIgA and intestinal homeostasis.



Neil A. Mabbott
(The Roslin Institute & Royal (Dick) School of Veterinary Sciences, University of Edinburgh, UK)

The role of crypt-associated macrophages in the maintenance of the intestinal stem-cell niche

The cytokine colony stimulating factor 1 (CSF1) controls the growth and differentiation of macrophages, acting through the CSF1 receptor (CSF1R). Studies have also suggested that Paneth cells express CSF1R and have implicated CSF1-CSF1R signalling in the maintenance of the intestinal stem cell niche. However, in contrast to previous reports our data show that in the intestinal epithelium CSF1R mRNA expression is undetectable in Paneth cells and any other epithelial cell lineage. Instead, in the lamina propria CSF1R mRNA expression is restricted to macrophages that are intimately associated with the crypt epithelium. Our data also show that these crypt-associated CSF1R-dependent macrophages influence intestinal epithelial differentiation and homeostasis. Macrophage ablation following prolonged CSF1R-blockade affects Paneth cell differentiation and leads to a reduction of *Lgr5*$^{+}$ intestinal stem cells. The disturbances to the crypt caused by macrophage depletion adversely affect the subsequent differentiation of certain intestinal epithelial cell lineages, notably resulting in a change in the balance between goblet cell and M cell differentiation. These data suggest that modification of the phenotype or abundance of macrophages in the gut wall, for example after pathogen infection or following pharmacological CSF1R-blockade, could adversely affect the development of the intestinal epithelium and the ability to sample particulate antigen from the gut lumen.

Hiroshi OHNO
(Laboratory for Intestinal Ecosystem, IMS, RIKEN)

Small intestinal microbes act in concert to exacerbate autoimmune inflammation in the central nervous system

Accumulating evidence indicates that gut microbes play a role in pathogenesis of autoimmune diseases including multiple sclerosis (MS). Here, we show that two distinct gut microbial signals coordinately activate myelin oligodendrocyte glycoprotein (MOG)-specific autoreactive T cells in the small intestine (SI). After induction of experimental autoimmune encephalomyelitis (EAE), an animal model for MS, MOG-specific CD4 T cells can be observed in the SI. Germ-free (GF) mice monocolonized with SI microbes demonstrated that a newly isolated Erysipelotrichaceae strain adheres to SI epithelial cells and acted like adjuvant to antigen-nonspecifically enhance Th17 responses, via inducing serum amyloid A and IL-23, that were associated with an increased susceptibility to EAE. Shotgun sequencing of SI contents revealed that a *Lactobacillus* strain possesses potential mimicry peptides to MOG. While monocolonization of GF mice with the *Lactobacillus* strain did not enhance EAE development or severity, co-colonized mice with Erysipelotrichaceae and *Lactobacillus* strains resulted in more severe EAE than Erysipelotrichaceae-monocolonization. These data suggest that the several SI microbes with a different role in the pathogenesis of MS might be therapeutic targets or provide preventive strategies for the disease, and we need to consider the synergistic effects on the pathogenesis by these microbes.

Wataru SUDA/Masahira HATTORI
(Laboratory for Microbiome Sciences, IMS, RIKEN)

Long-read metagenomic analysis of human gut microbiomes

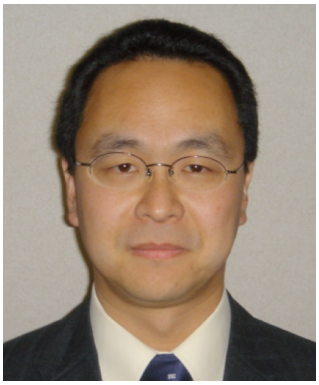
The number of commensal bacteria constituting human microbiomes is estimated to be hundreds of trillions, which are profoundly associated with host physiological states including disease. Thus, it is obviously important to comprehensively and precisely evaluate the whole pictures of microbiome structure. To this end, our laboratory applies NGS-based metagenomics to elucidate human microbiomes including gut, oral, and skin in Japanese individuals.

In most of studies on human microbiome, short reads of ~300 bp produced by Illumina sequencers have been used. However, short-read sequencing has several intrinsic limitations for completeness of the analysis largely due to existence of high-similar repetitive sequences such as ribosomal RNA genes in microbial genomes, sometimes resulting in ambiguous and insufficient outcomes. Here, we show results for metagenomics of human gut microbiomes by a long-read PacBio sequencer, generally producing reads of ~10 kb in genomes sequencing. The results showed that reads of 9 kb on the average were successfully obtained from 12 human fecal DNA samples, and assembly of them followed by binning generated ~100 high-quality chromosomal bins including seven complete circular contigs. In addition, we also found ~90 small circular contigs, many of which were assigned to hitherto unknown plasmids and phages.

Moriya OHKUMA
(Microbe Division, JCR, BRC, RIKEN)

Next generation technology for integrated symbiology and microbial resources of symbionts

Many of microbial symbionts are formidably difficult to culture and usually comprise a complex community. In order to understand the nature of a symbiotic system, the structure and function of a whole community, roles of the individual constituents, and interactions with each other and with their host should be clarified. It goes without saying that isolation and cultivation of microbial symbionts are crucial. My group has been working as a world-leading microbial resource center for cultured, taxonomically identified microbial diversity, and collecting, preserving, and distributing microbial symbionts isolated by ourselves and others. Such microbial resources, though still limited, are promising to enhance researches in symbioses. Metagenomic and metablomic approaches are advantageous for investigating the whole symbiotic systems, and in this project sophisticated technologies have been developed. In addition, single-cell technologies are anticipated for filling the gaps between the advances in studies on whole communities and the limit of microbial cultures. My group has also been investigating the termite-gut microbial community responsible for efficient utilization of recalcitrant lignocellulose. Through analyses of single-cell genomes of the symbionts, symbiotic relationships of various species-specific, coevolving associations between cellulolytic protists (single-cell eukaryotes) and bacteria in this community have been unveiled.



Tomoyoshi NOZAKI
(Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo)

Divergent evolution of mitochondria under anaerobic conditions

Hydrogenosomes and mitosomes are mitochondrion-related organelles (MROs) in anaerobic/microaerophilic eukaryotes with highly reduced and divergent functions. *Entamoeba,* the causative agent of human amebiasis, possesses a highly reduced and divergent MRO known as the mitosome. We previously demonstrated by proteomics that sulfate activation is a major function of *E. histolytica* mitosomes. We identified cholesteryl sulfate (CS) and other sulfated lipids as final products. We further demonstrated that CS is engaged in stage differentiation from the motile disease causing trophozoites to the dormant transmissible cysts. Taken together, these results indicate that CS and mitosome functions are uniquely involved in fundamental biological process necessary for transmission of this parasite between hosts. Interestingly, *Mastigamoeba balamuthi*, an anaerobic, free-living amoebozoan species, also has the sulfate activation pathway in MROs, but does not possess the capacity for CS production. Hence, we proposed that a unique function of MROs in *Entamoeba* contributes to adaptation of its parasitic life cycle.

Understanding of metabolite trafficking across the two mitosomal membranes is important to understand metabolic functions of mitosomes. We recently discovered a novel mitosomal β-barrel outer membrane protein of 30 kDa (MBOMP30) and several novel membrane-spanning proteins from a list of the mitosome proteome. We experimentally confirmed their localization and integration to mitosome membranes by Percoll-gradient fractionation, carbonate fractionation, immunofluorescence assay, and immunoelectron microscopy. These new class of mitosomal membrane proteins including MBOMP30 likely play unique and indispensable roles in *Entamoeba* mitosomes.

We have recently discovered that two dynamin-related proteins, DrpA and DrpB, are involved

in mitosome fission, while no indication of misotome fusion has been gained. Expression of a mutant form or gene silencing of these Drps caused abnormal morphology of mitoses and growth defect, suggesting that mitosome fission is mediated by these Drps.

Jun KIKUCHI
(Environmental Metabolic Analysis Research Team, CSRS, RIKEN)

Ecoinformatics approach for fish・microbiota and ecosystems

Ecosystem services are important for human life as well as sustainability of biological diversity. Analysis of environmental impact on fishery, both aquaculture production and catch fishes, are important to approve Marine Stewardship Council and Aquaculture Stewardship Council. Environmental homeostasis can be evaluated by data mining of analytical parameters from natural samples[1,2]. Here, I introduce ecoinformatics approach for integrated analysis of natural fishes, sediments and water[3]. Moreover, I will demonstrate that the deep learning computation can be used to discriminate geographic origin[4] and size-dependent metabolic profiles of analytical big-date from natural fishes[5].

References: 1) Kikuchi & Yamada (2017) *Analyst* 142, 4161-4172; 2) Oita et al., (revised) *Sci. Total Environ.*; 3) Wei et al. (2018) *Sci. Rep.*8, 3478; 4) Date, Y. & Kikuchi, J. (2018) *Anal. Chem.* 90, 1805-1810; 5) Asakura et al. (in press) *Anal. Chim. Acta.*

Kiwamu MINAMISAWA
(Microbial Symbiosis, Graduate School of Life Science, Tohoku University)

Environmental dynamics of plant-associated bacteria: From rhizobia towards plant microbiomes

Plant environments provide a diversity of ecological niches for microorganisms including rhizobia, plant growth-promoting microbes, and pathogens. Among them, rhizobia have been extensively studied for their dynamically-changing genome structures, polyphasic interactions with host plants, and biogeochemical functions as representative plant-associated microbes. Thus, I would like to introduce our two topics of rhizobia including nitrogen transformation in legume rhizosphere and strain-specific nodulation restrictions (incompatibility between host legume and rhizobia and subsequent genome dynamics in rhizobia), which indicate the dynamic evolution of rhizobial genomes with host plants and environments. Plant microbiomes is recently paid the most attention to address the community-level functions. Our microbiome studies on paddy rice root revealed CH_4 oxidation by methanotrophs is a driving force in shaping bacterial communities in rice roots grown in CH_4-rich environments. A hypothesis was proposed for the interplay between rice plant genes, root microbiomes, and their biogeochemical functions (Minamisawa *et al.* 2016). New experimental approaches have been recently developed for plant microbiome research: synthetic engineering approach to plant microbial communities in gnotobiotic systems (Bai *et al.* 2015) and informatics approach to identification of "core microbes" (Toju *et al.* 2018). Integration of these new approaches with conventional techniques and knowledge will open the new door of plant microbiomes and their application to agriculture.

Ken SHIRASU
(Plant Immunity Research Group, CSRS, RIKEN)

Rhizosphere interactions regulate antibiotics production of root-associated microbes

Many plant roots-associated actinomycetes are known antagonists toward plant pathogens, often by producing various types of antibiotics. To investigate whether plants influence the root-associated actinomycetes for antibiotic production, we assayed the antibiotic activities of the strains isolated from *Arabidopsis*, the model plant. We show that the isolates altered their antibiotic activities in the presence of the *Arabidopsis* root extracts. In particular, one strain of *Streptosporangium* sp., produced the red pigment when cultured with the root extracts but not with shoot extracts. Scanning electron microscopy (SEM) revealed that the hyphae extensively colonized the root surface, especially around the root hairs. To identify the secondary metabolite gene cluster(s) responsible for the red pigment production and its regulation, we sequenced its genome using PacBio Single Molecule Real-Time (SMRT) sequencing. Based on antiSMASH analysis and phylogenetic analysis of the chain length factor of type II polyketide synthases (PKSs), we identified at least one type-II PKS that is predicted to synthesize the red pigment. The Arabidopsis-*Streptosporangium* pair and the genomic information of the bacterium provide a novel model system for studying of plant-actinomycete interactions. Using bacterial culture collections, their anti-Ralstonia activities were also altered by root extract, suggesting that plants control their associating microbes to produce anti-pathogenic compounds for self-protection.