Published by>
Office for Research Promotion
Research Institute for Microbial Diseases
Osaka University

3-1 Yamadaoka, Suita, Osaka 565-0871, Japan
Tel +81-6-6879-8357
e-mail biken-pr@biken.osaka-u.ac.jp

**http://www.biken.osaka-u.ac.jp/en/**

Osaka University RIMD Research Institute for Microbial Diseases 2019-2020

# RIMD

Research Institute for
Microbial Diseases

2019-2020

# RIMD

Research Institute for
Microbial Diseases

大阪大学微生物病研究所

Research Institute for Microbial Diseases (RIMD), Osaka University is a world's foremost institute for basic biological researches including microbiology, immunology and oncology.

We dedicate to stimulate fundamental biological researches as a MEXT Joint usage/research center.

## Contents


- **Organization** ..... 4
- **Division of Infectious Disease** ..... 6
  - Department of Molecular Bacteriology
  - Department of Viral Infections
  - Department of Molecular Virology
  - Department of Immunoparasitology
  - Department of Infection Microbiology
  - Institute for Advanced Co-Creation Studies
- **Division of Host Defense** ..... 16
  - Department of Molecular Immunology
  - Dept of Immunochemistry
- **Division of Cellular and Molecular Biology** ..... 20
  - Department of Molecular Microbiology
  - Department of Oncogene Research
  - Department of Signal Transduction
  - Department of Cellular Regulation
  - Department of Homeostatic regulation
- **Genome Information Research Center** ..... 30
  - Department of Experimental Genome Research
  - Department of Genome Informatics
  - Department of Infection Metagenomics
  - Next-Generation Sequencing (NGS) Core Facility
- **Research Center for Infectious Disease Control** ..... 38
  - Department of Bacterial Infections
  - Department of Virology
- **International Research Center for Infectious Diseases** ..... 41
  - Laboratory of Clinical Research on Infectious Diseases
  - Laboratory of Emerging Viral Diseases
  - Pathogenic Microbes Repository Unit
- **Research Center for Mechanism and Regulation of Aging** ..... 44
- **Research Center for Infectious Disease Control** ..... 46
  - Yabumoto Department of Intractable Disease Research
  - Department of Molecular Protozoology
- **Thailand-Japan Research Collaboration Center** ..... 50
  - Section of Bacterial Infections
  - Section of Viral Infections
  - Section of Bacterial Drug Resistance Research
  - Section of Antiviral Research
  - Mahidol-Osaka Center for Infectious Diseases
- **BIKEN Innovative Vaccine Research Alliance Laboratories** ..... 56
  - Vaccine Creation Project
  - Mucosal Vaccine Project
  - Vaccine Dynamics Project
- **Animal Resource Center for Infectious Diseases** ..... 60
- **Office for Research Promotion** ..... 61
- **Common Research Facilities** ..... 62
- **About RIMD** ..... 64
- **For Students and Researchers who wants to study in RIMD** ..... 72
- **Grad Students Studying in RIMD** ..... 74




# Message from the Director

In 1934, the Research Institute for Microbial Diseases (RIMD), the first institute attached to Osaka University, was established for the study of microbial diseases. For more than 80 years since its foundation, the RIMD has concentrated on basic researches in infectious diseases, immunology, and oncology and made significant contributions to the control of infectious diseases through the identification of new pathogens, the elucidation of pathogenesis of microbes, and the development of vaccines and diagnostics based on these basic research findings. In addition, the RIMD has achieved an outstanding contribution in the progress of life sciences through the discovery of oncogenes and cell fusion phenomena and the elucidation of innate immune system.

The RIMD is certified as the Joint Usage/ Research Center by the Ministry of Education, Culture, Sports, Science and Technology Japan (MEXT). In addition to the collaborative research conducting in our facilities, the RIMD is also responsible to support for international scientific community through the provision of the bacteria stored at our Pathogenic Microbes Repository Unit. Faculties at the RIMD are also affiliated to the Graduate Schools of Medicine, Frontier Biosciences, Science, and Pharmaceutical Sciences at Osaka University and accepted many graduate students worldwide for contribution to the development of human resource in the next generations.

The RIMD produces world-leading research on infectious diseases from basic research to clinical applications through tight collaborations with BIKEN, public interest incorporated foundation engaged in development, production and supply of a wide variety of vaccines based on the research findings in the RIMD, and the Immunology Frontier Research Center (IFReC), established by the great efforts of 4 researchers in the RIMD to develop new research projects designed to clarify immune responses in a spatio-temporal manner in the body.

The RIMD will continue to dedicate our efforts in the progress of the basic research of infectious diseases, immunology, oncology, developmental biology, and cell biology based on the past outstanding achievements, and to focus on the development of young researchers either in Japan or abroad who are highly motivated to discover and establish new scientific paradigms by themselves.

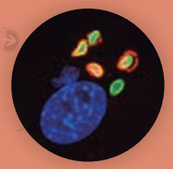
Y. Matsuura

Yoshiharu Matsuura D.V.M., Ph.D.
Director
Research Institute for Microbial Diseases
Osaka University

# Organization

Research Institute for Microbial Diseases (RIMD) was established as a research center for microbiology, immunology and oncology in 1934. We have performed outstanding researches in these fields and we also contribute extensively to growth in the basic sciences in Japan thorough advanced research and the development of human resources. Now, we are also developing new research fields such as gene engineering, genome research and always exploring breakthrough in biological science.





RIMD
Research Institute for Microbial Diseases
大阪大学微生物病研究所

## Research Divisions

To explore the pathogenesis of microbes

### Division of Infectious Disease

| | |
|---|---|
| Dept. of Molecular Bacteriology | Horiguchi Lab |
| Dept. of Viral Infections | Shioda Lab |
| Dept. of Molecular Virology | Matsuura Lab |
| Dept. of Infection Microbiology | Mimuro Lab |
| Dept. of Immunoparasitology | Yamamoto Lab |
| Institute for Advanced Co-Creation Studies | Okamoto Lab |

To explore the mechanisms that protect against microbes

### Division of Host Defense

| | |
|---|---|
| Department of Molecular Immunology | Yamasaki Lab |
| Dept of Immunochemistry | Arase Lab |

To explore regulatory mechanisms in cancer cells

### Division of Cellular and Molecular Biology

| | |
|---|---|
| Department of Molecular Microbiology | Hara Lab |
| Department of Oncogene Research | Okada Lab |
| Department of Signal Transduction | Takakura Lab |
| Department of Cellular Regulation | Miki Lab |
| Department of Homeostatic regulation | Ishitani Lab |

## Common Research Facilities

- Central Laboratory for Biological Hazardous Microbes
- Central Instrumentation Laboratory
- Radioisotope Laboratory

## Administration

- General Affairs Section
- Accounting Section
- Research Cooperation Section

## Office for Research Promotion

## Special Research Facilities

To overcome infectious diseases

### Research Center for Infectious Disease Control

| | |
|---|---|
| Department of Bacterial Infections | Iida Lab |
| Department of Virology | Kobayashi Lab |

To understand our body system from genetic information

### Genome Information Research Center

| | |
|---|---|
| Department of Experimental Genome Research | Ikawa Lab |
| Department of Genome Informatics | Daron Lab |
| Department of Infection Metagenomics | Iida Lab |
| Laboratory of Genome Research | Miwa Lab |
| Network Administration Office | |

To develop new therapeutic approaches to infectious diseases

### International Research Center for Infectious Diseases

| | |
|---|---|
| Laboratory of Clinical Research on Infectious Diseases | Kamitani Lab |
| Laboratory of Emerging Viral Diseases | Iwasaki Lab |
| Laboratory of Pathogen Detection and Identification | Nakamura Lab |
| Pathogenic Microbes Repository Unit | |

### Animal Resource Center for Infectious Diseases

### Research Center for Mechanism and Regulation of Aging

- Division of Aging Model Organism
- Division of Cellular Senescence

### Thailand-Japan Research Collaboration Center

- Section of Bacterial Infections
- Section of Bacterial Drug Resistance Research
- Section of Viral Infections
- Section of Antiviral Research
- Mahidol-Osaka Center for Infectious Diseases

### Endowed Chair

| | |
|---|---|
| Yabumoto Department of Intractable Disease Research | Kinoshita Lab |
| Department of Molecular Protozoology | Horii Lab |

To develop novel vaccines with high safety and efficacy

### BIKEN Innovative Vaccine Research Alliance Laboratories

| | |
|---|---|
| Vaccine Creation Project | Yoshioka Lab |
| Mucosal Vaccine Project | Sato Lab |
| Vaccine Dynamics Project | Aoshi Lab |

Division of Infectious Disease

# Department of Molecular Bacteriology

Some pathogenic bacteria cause specific disease symptoms including flaccid/spastic paralyses, paroxysmal coughing, skin exfoliation, and osteogenesis imperfecta, besides general symptoms such as fever and inflammation. Our major questions are as to how these specific symptoms appear in response to bacterial infections or what kinds of bacterial virulence factors are involved in them. We hope that we will understand the nature of bacterial infections by answering these questions.

## Staff

Asst. Prof. : Yukihiro Hiramatsu ／
Postdoc. : Takashi Nishida ／
Grad. Student 3

## Yasuhiko Horiguchi

**Professor**

Dr. Horiguchi received his Ph.D. from Osaka Prefecture University in 1987. After working at the Kitasato Institute for 3 years, he has worked for RIMD since 1990. He took his positon as Postdoc in 1990, a Research Associate in 1992, an Associate Professor in 1998. He was appointed current position in 2001.

## Publication


(1)The Eukaryotic Host Factor 14-3-3 Inactivates Adenylate Cyclase Toxins of Bordetella bronchiseptica and B. parapertussis, but not B. pertussis. Fukui-Miyazaki A., et al ***mBio*** (2018) 9:49–15.

(2)Ectopic Expression of O Antigen in Bordetella pertussis by a Novel Genomic Integration System. Ishigaki K., e tal. ***mSphere*** (2018) 3:e00417–17–11.

(3)Protective effects of in vivo-expressed autotransporters against Bordetella pertussis infection. Suzuki K., et al. ***Microbiology and Immunology*** (2017) 61:371–379.

(4)The bvg-repressed gene brtA, encoding biofilm-associated surface adhesin, is expressed during host infection by Bordetella bronchiseptica. Nishikawa S., et al. ***Microbiology and Immunology*** (2016) 60:93–105.

(5)Detection of genes expressed in Bordetella bronchiseptica colonizing rat trachea by in vivo expressed-tag immunoprecipitation method. Abe H., et al. ***Microbiology and Immunology*** (2015) 59:249–261.

(6)Polymorphisms influencing expression of dermonecrotic toxin in Bordetella bronchiseptica. Okada K., et al. ***PLoS ONE*** (2015) 10:e0116604.



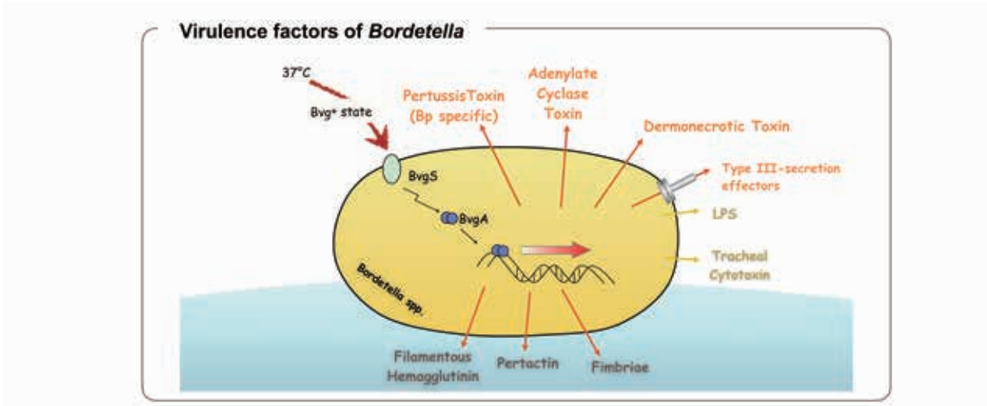

## ●To understand the mechanism of infection

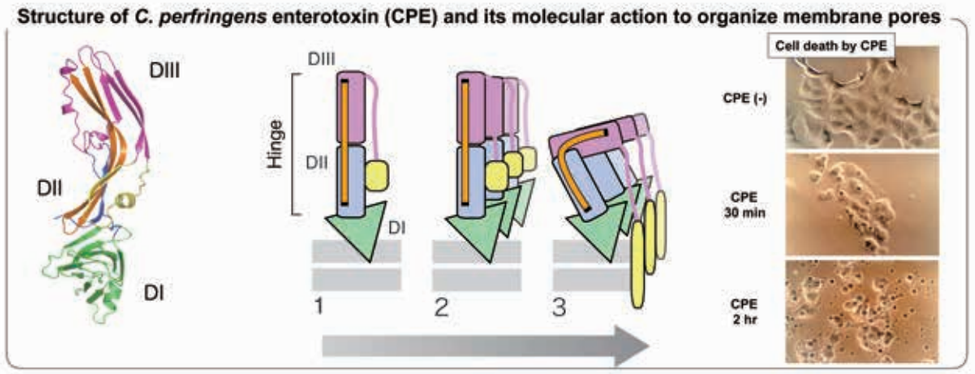
*Bordetella pertussis*, which is one of our research subjects is a representative pathogenic bacteria of *Bordetella* and causes whooping cough. In addition to *B. pertussis*, *B. parapertussis* and *B. bronchiseptica* are categorized in the genus *Bordetella*. Although these pathogenic organisms share homologous virulence factors and commonly cause respiratory infections with characteristic coughing, their host specificities and the course of disease manifestation are quite different: *B. pertussis* is a strict human pathogen causing the acute disease whereas *B. bronchiseptica* infects a wide range of mammals and causes chronic infections. We are trying to understand what determines host specificities and distinct disease manifestations in *Bordetella* infections. Understanding the molecular mechanism by which the bacteria cause coughing in hosts is our another goal of the *Bordetella* research.

## ●Analyzing the structure-function relationship of bacterial protein toxins.

Bacterial protein toxins cause a variety of specific symptoms manifested in bacterial infections. Many bacterial protein toxins are essentially multifunctional biomolecules, which travel in a host body, bind to target molecules or cells, and modify target molecules with high specificity. Some bacterial toxins are known as the most poisonous substances on the earth. We are analyzing the structure-function relationship of these bacterial protein toxins to understand how they exert such powerful toxicities on target cells and intoxicated animals. We believe that these results should give an insight into the mechanism causing specific symptoms observed in bacterial infections.

To achieve the above-mentioned goals, we are conducting the research work by using every experimental technique based on bacteriology, molecular and cellular biology, biochemistry, medical and veterinary science.



Division of Infectious Disease

# Department of Viral Infections

Viruses are simple organisms composed of proteins, nucleic acids, and, in some cases, lipids. Nevertheless, they interact with several host factors and ultimately cause disease in humans. Our laboratory focuses on the molecular mechanisms underlying viral diseases, including human immunodeficiency virus (HIV).

## Staff

Assoc. Prof. : Emi E. Nakayama ／
Undergrad. Student 1 ／
Grad. Student 1

## Tatsuo Shioda

**Professor**

Dr. Shioda obtaind his B. Sc. from the University of Tokyo in 1982 and his Ph.D. form the same institution in 1990. After working at the Institute of Medical Science in the University of Tokyo and University of California San Francisco, he was appointed as Associate Professor in 1997. He took his current position as a Professor of RIMD in 2000.

## Publication


(1) Naturally Occurring Mutations in HIV-1 CRF01_AE Capsid Affect Viral Sensitivity to Restriction Factors. Nakayama E.E., et al., ***AIDS Res Hum Retroviruses.*** (2018) doi:10.1089/AID.2017.0212.

(2) SL1 revisited: functional analysis of the structure and conformation of HIV-1 genome RNA. Sakuragi S., ***Retrovirology***. 2016 Nov 11;13(1):79.

(3) Genome-wide association study of HIV-related lipoatrophy in Thai patients: Association of a DLGAP1 polymorphism with fat loss. Uttayamakul S., et al. ***AIDS Res Hum Retroviruses.*** (2015) Aug;31(8):792-6.

(4) Impact of TRIM5α in vivo. Nakayama E.E., et al. ***AIDS.*** (2015) Sep 10;29 (14):1733-43.

(5) A Single-Nucleotide Polymorphism in ABCC4 Is Associated with Tenofovir-Related Beta2-Microglobulinuria in Thai Patients with HIV-1 Infection. Likanonsakul S., et al. ***PLoS One*** (2016) Jan 25;11(1):e0147724.

(6)Novel mutant human immunodeficiency virus type 1 strains with high degree of resistance to cynomolgus macaque TRIMCyp generated by random mutagenesis. Sultana T., et al. ***J Gen Virol***. (2016) Apr;97(4):963-76. a




## ●Antiviral host factors and their application to a cure for HIV infection

HIV does not establish a productive infection in any monkey other than the chimpanzee. Also, the sensitivity of HIV infection and rate of disease progression vary from individual to individual. To date, several anti-HIV host restriction factors, including TRIM5α and TRIMCyp, have been identified as responsible for these phenomena. Currently, we are trying to elucidate the molecular mechanism(s) underlying the anti-HIV activity of these factors. We also aim to cure HIV infection by establishing novel reproductive medicine-based strategies, including iPS cells from HIV patients.

## ●Analysis of HIV-1 genome RNA dimerization

The genome of retroviruses such as HIV-1 always exists as a dimer; genome dimerization plays an important role at various stages of the viral life cycle, including genome packaging and reverse transcription as well as the genome recombination processes involved in viral diversification. Therefore, genome dimerization is a novel target for anti-HIV therapies. We are currently analyzing computer-assisted structural models of HIV-1 Dimer Initiation Sequences, which are the most important factors involved in genome dimerization, to get a more complete picture of HIV genome dimerization. We are also analyzing genome recombination in HIV-1 by constructing a novel system to measure recombination efficiency.

## ●Human genome analysis of HIV-associated neurocognitive disorders

Despite successful antiretroviral therapy, nearly a quarter of HIV patients develop mild-to-severe neurocognitive disorders (HAND). We aim to undertake genome analysis of HAND patients to elucidate the underlying molecular mechanisms, thereby developing therapeutic strategies to treat/prevent this disease.

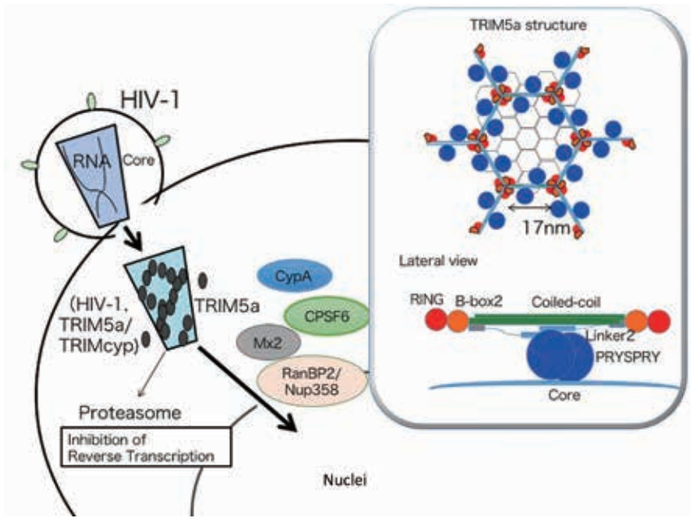



Fig. 1.Host factors involoving in early replication steps of HIV

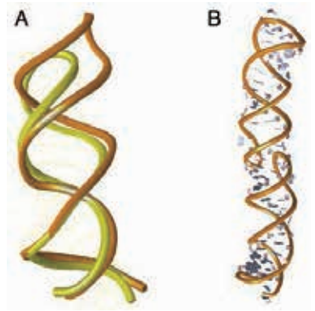



Fig. 2.Newly identified structure of DIS. A. Comparison of the current model (brown) with the previously proposed model (yellow). B. Structure in a dimeric form. The newly identified model shows more extended DIS structure possibly allowing stronger molecular interaction.

Division of Infectious Disease

# Department of Molecular Virology

Viruses “know” cells better than human beings and have evolved to replicate in living cells. We are working to understand the molecular mechanisms underlying the interplay between viruses and host cells through research on hepatitis viruses, flaviviruses, and insect viruses.

## Staff

Assoc. Prof. : Yusuke Maeda ／
Assoc.Prof. : Takasuke Fukuhara ／
Asst. Prof. : Chikako Ono ／
Undergrad. Student 3 ／ Grad. Student 6

## Yoshiharu Matsuura

**Professor**

Dr. Matsuura received his PhD from Hokkaido University in 1986 and worked at Research Institute of Daiichi Seiyaku Co. Ltd for . He was appointed Professor in RIMD in 2000 after working at NERC Institute of Virology in Oxford University as a postdoctoral fellow and at the National Institute of Infectious Diseases as a head of Laboratory of Hepatitis Viruses in Department of Virology II. He serves as Director of RIMD from 2015.

## Publication


(1) Infection with flaviviruses requires BCLXL for cell survival. Suzuki T., et al., ***PLoS Pathog***. (2018) 14(9):e1007299.

(2) Characterization of SPP inhibitors suppressing propagation of HCV and protozoa. Hirano J etal. ***Proc Natl Acad Sci U S A.*** 2017 Dec 12;114 (50):E10782-E10791.

(3) Host-derived apolipoproteins play comparable roles with viral secretory proteins Erns and NS1 in the infectious particle formation of Flaviviridae. Fukuhara T et al., ***PLoS Pathog***. 2017 Jun 23;13(6):e1006475.

(4) Characterization of miR-122-independent propagation of HCV. Ono C, et al. ***PLoS Pathog.*** 2017 May 11;13(5):e1006374.

(5) TRC8-dependent degradation of hepatitis C virus immature core protein regulates viral propagation and pathogenesis. Aizawa S. & Okamoto T., et al. ***Nat. Commun.*** (2016), doi: 10.1038/ncomms11379.

(6) Lipoprotein receptors redundantly participate in entry of hepatitis C virus Yamamoto S. & Fukuhara T.,et al. ***PLoS Pathog.*** (2016), doi: 10.1371/journal.ppat.1005610.




## ●Molecular biology of hepatitis viruses

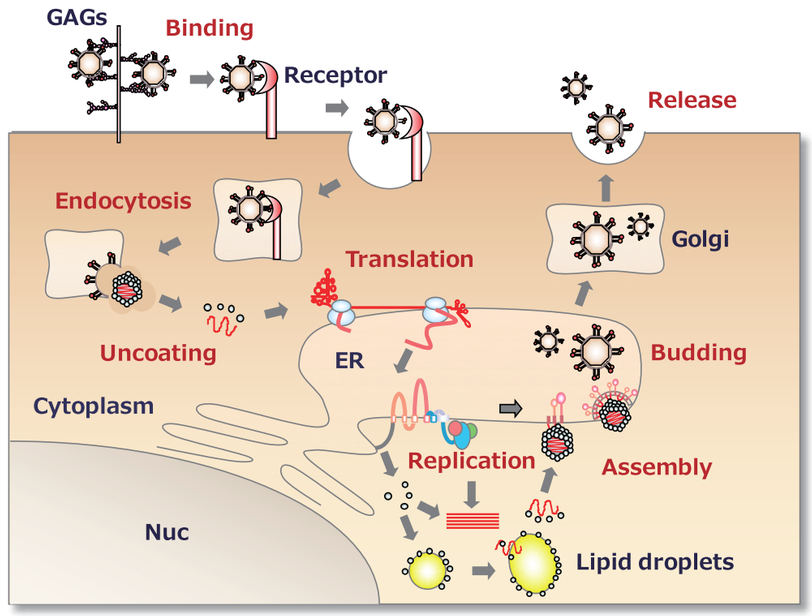
Hepatitis C virus (HCV) infects over 170 million individuals worldwide and is one of the most common etiologic agents of chronic liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC). Although novel innovative anti-HCV drugs that act directly on viral proteins have achieved a sustained virological response in hepatitis C patients, drug-resistant viruses emerge easily. Therefore, host factors necessary for HCV replication are ideal targets for the development of new therapeutics for chronic hepatitis C; such drugs will lessen the possibility of drug-resistant breakthrough viruses emerging because the frequency of mutation is much lower than that in the viral genome.

Upon infection with HCV, viral RNA is directly translated into viral proteins. Viral RNA replicates in the cytoplasm using various host factors and organelles. Viruses replicate in living cells, and some of them, including HCV, are pathogenic to the host. We are focusing on trying to understand the molecular mechanisms underlying the interaction between the virus and host by identifying the host factors involved in the propagation and pathogenicity of HCV. We have shown that the HCV core protein participates not only in the assembly of viral particles but also in the development of liver steatosis and HCC. We have also shown that host proteins, including molecular chaperones and apolipoproteins, participate in viral replication and in the formation of infectious particles. Novel therapeutic agents targeting the host factors crucial for propagation and pathogenesis of HCV could be available if we can elucidate the molecular mechanisms underlying infection and replication of HCV. We are also working on hepatitis B virus and Japanese encephalitis virus, also members of the *Flaviviridae*.

## ●Development of baculoviral vectors

Development of viral vectors capable of safely transducing foreign genes into target cells is essential for future gene therapy. We are working on developing the insect baculovirus, *Autographa californica* nucleopolyhedro virus, as a versatile viral vector for gene delivery. Baculovirus is capable of entering a variety of mammalian cells and facilitates expression of foreign genes under the control of mammalian promoters; however, the viral genome does not replicate. We are working on developing viral vectors that have the advantages and characteristics of baculovirus.

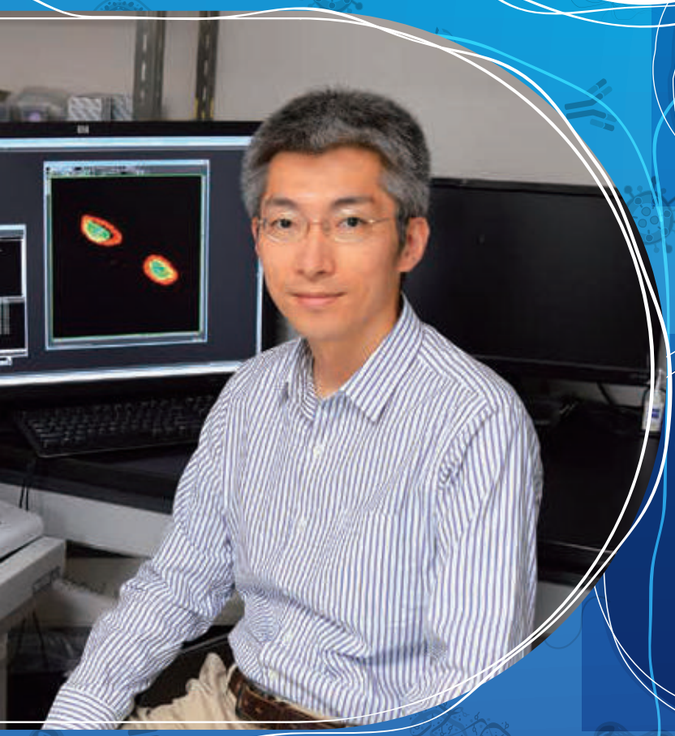
## HCV life cycle



Division of Infectious Disease

# Dept. of Immunoparasitology

In our immunoparasitology laboratory, we use the apicomplexan protozoan parasite *Toxoplasma gondii* as a model for exploring host defense systems and pathogenesis. Our research goal is to elucidate the molecular mechanisms underlying the interface between the host and pathogen.



## Staff

Assoc. Prof. : Miwa Sasai ／
Asst. Prof. : MA JISU ／
Postdoc. : Masaaki Okamoto ／
Grad. Student 4

## Masahiro Yamamoto

**Professor**

Received B. Sc. from the University of Tokyo (2001) and Ph.D. from Osaka University (2006). Promoted to an assistant professor (2006), an associate professor (2010) at the graduate school of medicine in Osaka University, an independent associate professor (2012) at RIMD in Osaka University. Appointed current position as a Professor of RIMD in 2013.

## Publication


(1) Inducible Nitric Oxide Synthase Is a Key Host Factor for Toxoplasma GRA15-Dependent Disruption of the Gamma Interferon-Induced Antiparasitic Human Response. Bando H., et al. ***MBio***. (2018) 9(5).

(2) Essential role for GABARAP autophagy proteins in interferon-inducible GTPase-mediated host defense. Sasai M., et al., ***Nat Immunol.*** (2017) 18 (8):899-910.

(3) p62 plays a specific role in interferon-γ-induced presentation of a *Toxoplasma* vacuolar antigen. Lee Y., et al. ***Cell Rep.*** (2015) 13:223-33.

(4) RabGDIα is a negative regulator of interferon-γ-inducible GTPase-dependent cell-autonomous immunity to *Toxoplasma gondii*. Ohshima J., et al. ***Proc Natl Acad Sci USA.*** (2015) 112:E4581-90.

(5) Selective and strain-specific NFAT4 activation by the *Toxoplasma gondii* polymorphic dense granule protein GRA6. Ma J.S., et al. ***J Exp Med.*** (2014) 211:2013-32.

(6) A cluster of interferon-γ-inducible p65 GTPases plays a critical role in host defense against *Toxoplasma gondii*. Yamamoto M., et al. ***Immunity*** (2012) 37:302-13.




*Toxoplasma gondii* is an obligatory intracellular protozoan pathogen that causes lethal toxoplasmosis in humans and animals. One third of the global population is thought to be infected with this pathogen, making it the "most successful parasite." *T. gondii* infects virtually all nucleated cells in warm-blooded animals. The parasite forms a special membranous structure called a "parasitophorous vacuole (PV)." The host-parasite interaction takes place through the PV. In response to *T. gondii*, the host immune system produces inflammatory cytokines such as interleukins, chemokines, and interferons. Interferon-γ (IFN-γ) is the most important host factor for inducing anti-*T. gondii* responses, which suppress and kill the parasites. One of the main projects in our laboratory is to identify the IFN-γ-induced anti-*T. gondii* host defense mechanisms involved in innate and adaptive immunity. Recently, we found that IFN-γ-inducible GTPases called GBPs are important for *T. gondii* PV disruption, and that their function in anti-*T. gondii* responses requires autophagy proteins; this suggests an unexpected link between IFN-γ-induced immunity and autophagic pathways.

On the other hand, virulent *T. gondii* suppress IFN-γ-induced host immunity and even manipulate host immune cells to maximize the virulence of the parasite. Another main project in our laboratory is to identify novel virulence mechanisms used by *T. gondii*. For example, we recently showed that a *T. gondii*-secreting virulence factor, GRA6, directly activates the host transcription factor NFAT4 to induce chemokines and recruit neutrophils to eradicate the parasite. Thus, our laboratory is focusing on host-parasite interactions via immunoparasitological mechanisms.

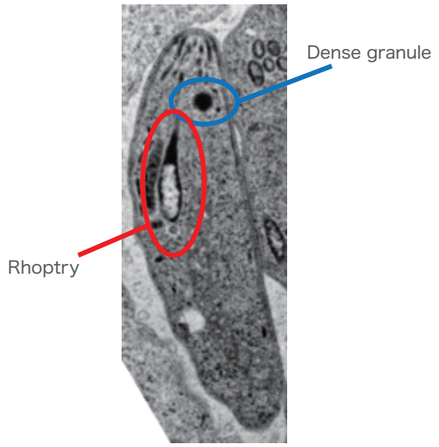



Fig. 1. *Toxoplasma gondii*
Pathogenic proteins are secreted from Dense granules and Rhoptry.

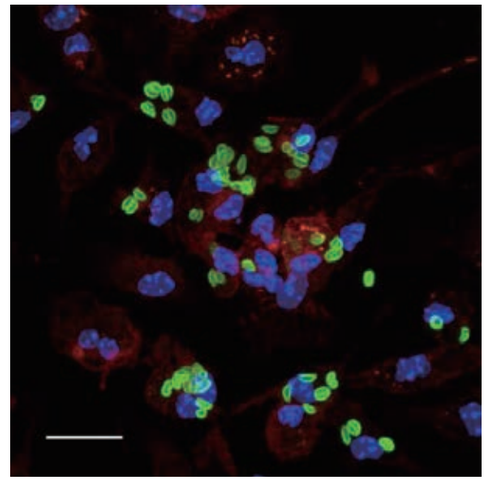


Fig. 2. *Toxoplasma gondii* (green) proliferating inside macrophages (red).

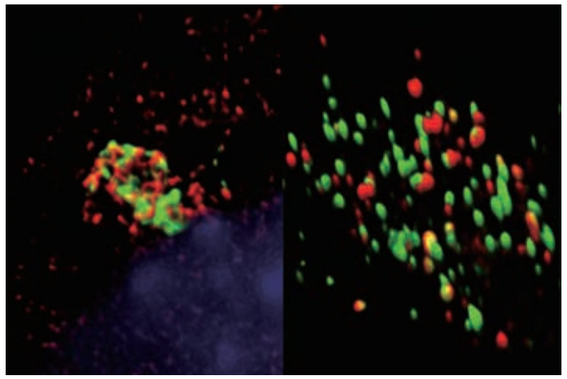


Fig. 3. Gate-16 (red) is required for antimicrobial host defense through cytosolic distribution of GTPase (green).
GTPase punctae are colocalized with Gate-16 (right fig.) throughout the cell. However, expression of mutant Gate-16 leads to GTPase aggregation and hampers immune response against pathogens.

Division of Infectious Disease

# Department of Infection Microbiology

Bacteria-gut interplay and the host immune response are the most critical issues in determining the fate of bacterial infections and the severity of the diseases. Our group has been studying the pathogenesis of mucosal infectious bacteria, such as *Helicobacter pylori*, *Shigella*, enteropathogenic *Escherichia coli*, and *Streptococcus pyogenes*, by defining the molecular and cellular mechanisms of infections and the roles of pathogenic factors as well as the host factors in infections.

**Staff**

Asst. Prof. : Ryota Otsubo ／
Postdoc : Takahito Sanada ／
Postdoc : Ryo D Kinoshita ／
Guest Researcher : Phawinee Subsomwong ／
Grad. Student 2

## Hitomi Mimuro

**Associate professor**

Dr. Mimuro received her Ph.D. from The University of Tokyo in 2004. She became Assistant professor at the Institute of Medical Science, The University of Tokyo in 2005 and worked for the same institution. She was appointed Associate Professor in RIMD in 2017.

## Publication


(1)Shigella effector IpaH4.5 targets 19S regulatory particle subunit RPN13 in the 26S proteasome to dampen cytotoxic T lymphocyte activation. Otsubo R., et al. ***Cell Microbiol***. (2018) e12974

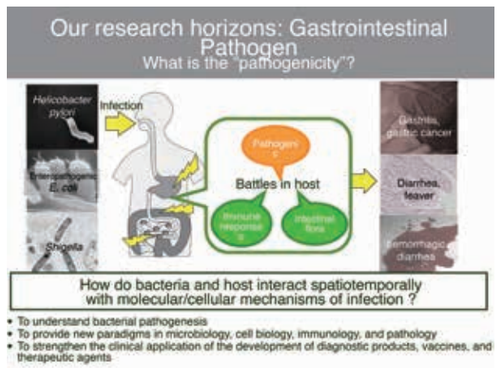
(2)Characterization of morphological conversion of Helicobacter pylori under anaerobic conditions. Hirukawa S., et al. ***Microbiol. Immunol***. (2018) 62(4);221-28

(3)Epigenetic silencing of miR-210 increases the proliferation of gastric epithelium during chronic Helicobacter pylori infection. Kiga K., et al. ***Nat. Commun***. (2014) 5:4497

(4)The immune receptor NOD1 and kinase RIP2 interact with bacterial peptidoglycan on early endosomes to promote autophagy and inflammatory signaling. Irving A.T., et al., ***Cell Host Microbe***. (2014) 15(5):623-35


In the Mimuro laboratory, researchers are exploring the pathogenesis of mucosal infectious bacteria, including *H. pylori*, *Shigella*, enteropathogenic *E. coli*, and *S. pyogenes*.

*H. pylori* transports effector proteins and other molecules into host epithelial cells via a Type IV secretion system and/or outer membrane vesicles. Researchers are studying how these effectors cause diseases such as gastritis, gastric ulcers, and cancer. They are also trying to elucidate the mechanisms that enable long-term infection of *H. pylori* in the stomach. In addition, researchers are focusing on the molecular mechanisms in the host that protect against infectious bacteria. The expected output of their research will not only shed further light on bacterial pathogenesis, but also provide a new paradigm in microbiology, cell biology, immunity, and pathology, and strengthen the molecular basis for developing diagnostic products, vaccines, animal models, and therapeutic agents.






Division of Infectious Disease

# Institute for Advanced Co-Creation Studies

Our research is focusing on pathogenesis of infection with hepatitis viruses such as Hepatitis C virus and Hepatitis B virus and with mosquito-borne flaviviruses such as Japanese encephalitis virus (JEV), Dengue virus (DENV) and Zika virus (ZIKV). It still remains unclear how these viruses induce a variety of diseases in hosts. We aim to study to understand molecular mechanisms of pathogenicity of virus infection through molecular biology and animal models.

**Staff**

Asst. Prof. : Tatsuya Suzuki

## Toru Okamoto

**Professor**

Dr. Okamoto received his Ph.D. from Osaka University in 2006. Thereafter he worked as a postdoctoral fellow at RIMD in 2006, a researcher at Walter and Eliza Hall Institute of Medical Research in 2008. He was appointed as a current position from 2019 after working as an assistant professor (2012) and an associate professor (2017) at Research Institute for Microbial Diseases.

## Publication


(1)USP15 Participates in Hepatitis C Virus Propagation through Regulation of Viral RNA Translation and Lipid Droplet Formation. Kusakabe S. & Okamoto T. et al., ***J Virol***. 2019 Mar 5; 93(6). pii:e01708-18

(2)Infection with flaviviruses requires BCLXL for cell survival. Suzuki T. & Okamoto T., et al. ***PLoS Pathog***. 2018 Sep 27; 14 (9):e1007299.

(3)Characterization of SPP inhibitors suppressing propagation of HCV and protozoa. Hirano J. & Okamoto T., et al. ***Proc Natl Acad Sci USA***. 2017 Dec 12;114 (50):E10782-E10791.

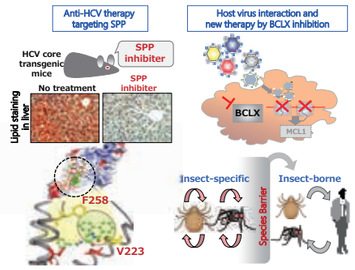
(4)TRC8-dependent degradation of hepatitis C virus immature core protein regulates viral propagation and pathogenesis. Aizawa S. & Okamoto T., et al. ***Nat. Commun***. 2016 May 6; 12 (5): e1005610


### ●Pathogenicity of hepatitis viruses

Infection with Hepatitis C virus (HCV) induces chronic infection and leads to develop steatosis, cirrhosis and hepatocellular carcinoma. Among 10 viral proteins, HCV core protein forms a viral particle and induces steatosis and hepatocellular carcinoma in transgenic mice model. It suggests that core is strongly associated with liver diseases in chronic hepatitis C. Our research is focusing on maturation of core protein by signal peptide peptidase (SPP) and its maturation is essential for formation of viral particle and development of liver diseases. We would like to clarify why maturation of HCV core is needed for its function, especially liver diseases.

### ●Pathology of mosquito-borne flavivirus and development of new antiviral drugs

Infectious diseases by infection with mosquito-borne flavivirus, one of which is microcephaly by infection with Zika virus, have become a serious problem worldwide. Mosquito-borne flavivirus spreads infection through blood feeding of virus-infected mosquito. While host ranges of virus infection are generally limited, mosquito-borne flavivirus can propagate mosquito and mammals. We study how mosquito-borne flavivirus infects mosquito and mammals and how transmission between mosquito and mammals is associate to development of diseases.



Division of Host Defense

# Department of Molecular Immunology

Our bodies are continuously exposed to external and internal insults caused by infection and tissue damage, most of which are primarily sensed by immune receptors to maintain tissue homeostasis. However, the molecular mechanism by which these receptors discriminate diverse insults to elicit suitable immune responses remains elusive. We have found that C-type lectin receptors can sense both damaged self and non-self pathogens (Figure 1). Recently, we also showed that clustered C-type lectin receptors, Mincle, MCL, Dectin-2 and DCAR, can recognize mycobacteria through their unique glycolipids possessing adjuvant activity (Figure 2). Our objective is to illustrate the principle behind the regulation of immune responses through C-type lectin receptors in physiological and pathological settings. Based on these results, we also aim to design new methods to efficiently elicit or modulate immune responses. To this end, our research is focusing on the following axes:

1) Immune sensing of pathogens and damaged-self via C-type lectin receptors.
2) Unique T cell responses induced by self peptides.
3) Atypical T cell subsets critical for autoimmune diseases.

## Staff

Asst. Prof. : Eri Ishikawa ／
Asst. Prof. : Chihiro Motozono ／
Postdoc. : Kenji Toyonaga ／
Postdoc. : Xiuyuan Lu ／ Guest Grad. Student 12

## Sho Yamasaki

**Professor**

Dr. Yamasaki received his Ph.D. from Kyoto University in 1999. After working at Mitsubishi Chemical Corporation and Chiba University Graduate School of Medicine, he worked for Research Center for Allergy and Immunology, RIKEN from 2004 to 2009. He was appointed as Professor in Medical Institute of Bioregulation, Kyushu University in 2009. He took his current position at RIMD from 2017.

## Publication


(1) Lipoteichoic acid anchor triggers Mincle to drive protective immunity against invasive group A Streptococcus infection.I mai T., et al. ***Proc. Natl. Acad. Sci USA***. (2018) 115:E10662-71.

(2) Intracellular metabolite β-glucosylceramide is an endogenous Mincle ligand possessing immunostimulatory activity. Nagata M., et al. ***Proc. Natl. Acad. Sci. USA***. (2017) 114:E3285-94

(3) Protein kinase D regulates positive selection of CD4(+) thymocytes through phosphorylation of SHP-1. Ishikawa E., et al. ***Nat. Commun***. (2016) 7:12756.

(4) C-type lectin receptor DCAR recognizes mycobacterial phosphatidyl-inositol mannosides to promote a Th1 response during infection. Toyonaga K., et al. ***Immunity.*** (2016) 45:1245-57.

(5) Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. Yonekawa A., et al. ***Immunity.*** (2014) 41:402-13.

(6) C-Type lectin MCL is an FcRγ-coupled receptor that mediates the adjuvanticity of mycobacterial cord factor. Miyake Y., et al. ***Immunity.*** (2013) 38:1050-62.




## ●C-type lectin receptors (CLRs) sense both “non-self pathogens” and “damaged self”.

CLRs are involved in innate immunity; however, this family exhibits higher diversity and ligand specificity than other innate immune receptor families such as Toll-like receptors due to the wider variety of genes [Fig.1]. CLRs recognize pathogen-associated molecular patterns. We showed that Mincle (macrophage-inducible C-type lectin) recognizes the glycolipid TDM (trehalose-6,6'-dimycolate), a component of the *Mycobacterium tuberculosis* cell wall. This CLR acts as a sensor to trigger the immune response through a signaling pathway that involves FcRγ and CARD9, among other components. We also identified the ligands through which the CLRs MCL (macrophage C-type lectin), Dectin-2 (dendritic cell-associated C-type lectin-2), and DCAR (dendritic cell immunoactivating receptor) recognize *M. tuberculosis* and other pathogens, and elucidated some of the mechanisms by which they induce immune responses.

In addition, in a recent study, our group shed light on the function of Mincle in the recognition of endogenous ligands. Mincle can bind to β-glucosylceramide, a glycolipid that is released by host cells after damage. Thus, Mincle not only detects pathogens or foreign ligands, but also endogenous molecules released by damaged cells to activate the immune system and the response to “danger” situations.

We are presently investigating in further detail the recognition of both self and non-self ligands by this family of receptors and their role in immunity [Fig.2].

## ●Self ligands are recognized by T cell receptors (TCRs) and play an important role in T cell persistence

T cells pass various selections of their TCRs before they are released from the thymus into the periphery. TCRs that weakly bind to self ligands cause T cell retention, and the signaling cascades induced by self ligands are important for T cell persistence but do not lead to T cell activation. Our objective is to clarify the recognition of “self” by TCRs and the distinct signals transduced by the same TCR upon stimulation with self and non-self ligands.

## ●Novel T cell subsets contribute to autoimmune diseases

Recent evidence has shown that novel T cell subsets are responsible for autoimmune phenotypes that are comparable with human disorders. We are interested in the ligands that are recognized by these pathogenic T cells and the mechanisms via which the diseases develop. Based on the analysis of the corresponding subsets in human, we aim to design new methods to diagnose and treat autoimmune diseases.

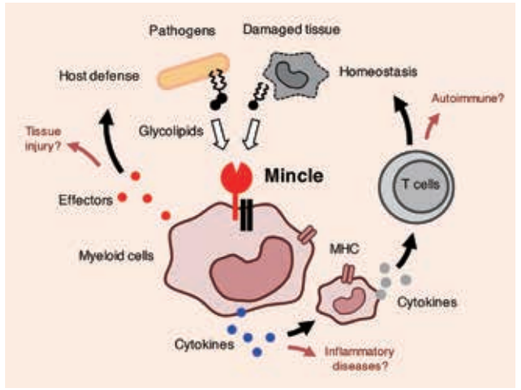



Fig. 1. Various immune responses triggered by Mincle

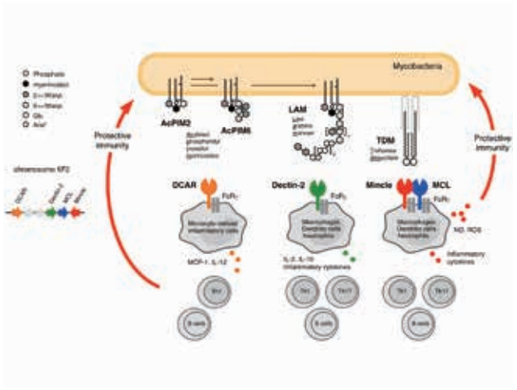



Fig. 2. Cooperative function of CLRs against mycobacteria

Division of Host Defense

# Dept of Immunochemistry

We have been studying interactions between pathogens and various paired receptors. In addition, we found that MHC class II molecules function as molecular chaperones to transport misfolded proteins to the cell surface. Analyses of misfolded proteins transported to the cell surface revealed that they are involved in autoimmune diseases by acting as a target for autoantibodies.

## Staff

Assoc. Prof. : Tadahiro Suenaga ／
Asst. Prof. : Masako Kohyama ／
Postdoc. : Kazuki Kishida ／
Undergrad. Student 3 ／
Grad. Student 9

## Hisashi Arase (concur.)

**Professor**

Prof. Arase received M.D. from Hokkaido University School of Medicine at 1990 and received Ph.D from Hokkaido University at 1994. Thereafter he worked as an assistant professor at Chiba University School of Medicine (1994), a research fellow at University of California San Francisco (2000), an associate professor at Chiba University (2002) and an associate professor at Research Institute for Microbial Diseases (2004). he is working as current position from 2006.

## Publication


(1) Immune evasion of Plasmodium falciparum by RIFIN via inhibitory receptors. Saito F et al. ***Nature*** (2017) 552:101–105. Saito F., et al.,

(2) LILRA2 is an innate immune sensor for microbially cleaved immunoglobulins. Hirayasu K., et al. ***Nature Microbiology.*** (2016) 6:16054. doi: 10.1038/nmicrobiol.2016.54.

(3) Autoantibodies to IgG/HLA class II complexes are associated with rheumatoid arthritis susceptibility. Jin H., et al. ***Proc. Natl. Acad. Sci. USA.*** (2014) 111: 3787-92.

(4) Neutrophil infiltration during inflammation is regulated by PILRα via modulation of integrin activation. Wang J., et al. ***Nat. Immunol.*** (2013) 14:34-40.

(5) Myelin-associated glycoprotein mediates membrane fusion and entry of neurotropic herpesviruses. Suenaga T., et al. ***Proc. Natl. Acad. Sci. USA*** (2010) 107:866-71.

(6) PILRα is a herpes simplex virus-1 entry co-receptor that associates with glycoprotein B. Satoh T., et al. ***Cell*** (2008) 132:935-44.




## Interaction between immune receptors and pathogens

Immune cells express “paired” activating and inhibitory receptors that are highly homologous. The inhibitory receptors recognize self-antigens and downregulate immune response to the self. On the other hand, we found that some inhibitory receptors are used by pathogens for immune evasion (Fig. 1). By contrast, we found that LILRA2, an orphan activating receptor expressed on human myeloid cells, recognizes abnormal immunoglobulins cleaved by microbial proteases but not normal immunoglobulins. Because immunoglobulins are important for host defense, their degradation is very dangerous in terms of immunity (Fig. 2). In this way, paired receptors play an important role not only in immune regulation but also in host defense against pathogens.

## Misfolded proteins complexed with MHC class II molecules trigger autoimmune disease

Allelic polymorphisms in MHC class II molecules are strongly associated with susceptibility to many autoimmune diseases. However, it is unclear how MHC class II molecules are involved in autoimmune disease susceptibility. We found that misfolded cellular autoantigens are rescued from protein degradation by MHC class II molecules. Furthermore, we found that misfolded proteins complex with MHC class II molecules and become targets for autoantibodies. Autoantibody binding to misfolded proteins that are transported to the cell surface by MHC class II molecules correlated strongly with susceptibility to autoimmune disease, suggesting that misfolded proteins, which normally would not be presented to the immune system, can be targets for autoantibodies by acting as “neo self” antigens, which are involved in the pathogenicity of autoimmune diseases (Fig.3).

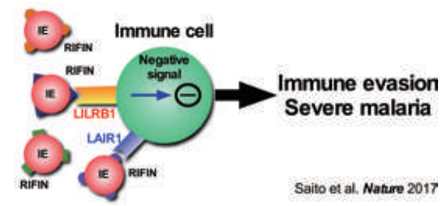



Fig. 1.
Inhibitory receptors play an important role in immune regulation, whereas pathogens exploit inhibitory receptors for immune evasion. We found malaria parasite has a mechanism to suppress the host immune response by using an inhibitory receptor, LILRB1, contributing to the pathogenesis of severe malaria.

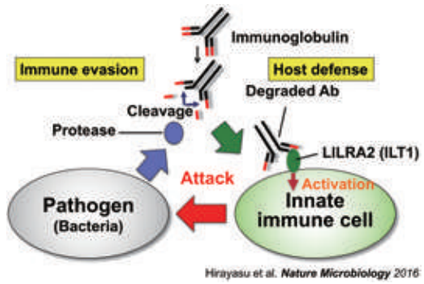



Fig. 2. Activating paired receptors play a role in host defense against bacterial infection
Activating paired receptor, LILRA2, recognizes immunoglobulin cleaved by bacterial protease activate innate immune cells (Hirayasu et al. *Nat. Microbiol.* 2016).

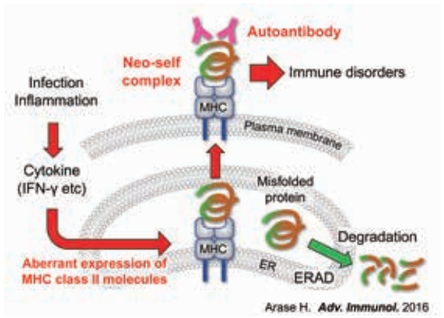
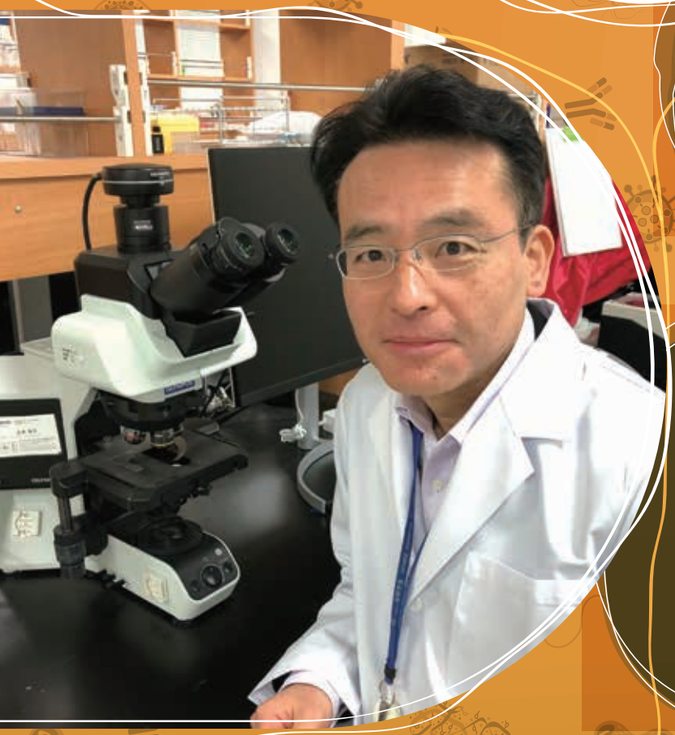



Fig. 3. Misfolded proteins complexed with MHC class II molecules are targets for autoantibodies.
Misfolded cellular proteins are transported to the cell surface without being processed to peptides by associating with MHC class II molecules in the ER. Furthermore, misfolded proteins complexed with MHC class II molecules encoded by disease-susceptible alleles are specifically recognized by autoantibodies. This suggests that misfolded proteins complexed with MHC class II molecules are natural autoantigens for autoantibodies, which affect susceptibility to autoimmune diseases (Arase *Adv. Immunol.* 2016).

Division of Cellular and Molecular Biology

# Department of Molecular Microbiology

It has become apparent that aging has a major impact on the incidence of cancers. However, the underlying mechanisms are unclear. We think that cellular senescence plays a key role. In our laboratory, we are aiming to understand the roles and mechanisms of cellular senescence *in vivo*. We believe that understanding the molecular mechanisms underlying cellular senescence *in vivo* will provide valuable insight into the development of aging-associated diseases such as cancer, and open up new possibilities for their control.



## Staff

Assoc. Prof. : Sugiko Watanabe ／
Asst. Prof. : Shimpei Kawamoto ／
Postdoc. : Masahiro Wakita ／
Postdoc. : Tatsuyuki Matsudaira ／
Postdoc. : Megumi Narukawa ／
Grad. Student 4

## Eiji Hara

**Professor**

Dr. Hara received his Ph.D. from Tokyo University of Science in 1993. After working at Imperial Cancer Research Fund Laboratories, U.K. (Postdoctoral Fellow), Cancer Research UK-Paterson Institute, U.K. (Group Leader) and the Institute for Genome Research, University of Tokushima (Professor), he was appointed Division Chief in the Cancer Institute, Japanese Foundation for Cancer Research in 2008. He took his current position at RIMD from 2015.

## Publication


(1)Downregulation of cytoplasmic DNases is implicated in cytoplasmic DNA accumulation and SASP in senescent cells. Takahashi A., et al. ***Nat Commun***. (2018) 9(1):1249

(2) Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Yoshimoto S., et al. ***Nature*** (2013) 499:97-101.

(3) DNA damage signaling triggers degradation of histone methyltransferases through APC/$C^{Cdh1}$ in senescent cells. Takahashi A., et al. ***Molecular Cell*** (2012) 45:123-31.

(4) Real-time in vivo imaging of $p16^{Ink4a}$ reveals cross-talk with p53. Yamakoshi K., et al. ***Journal of Cell Biology*** (2009) 186:393-407.

(5) Mitogenic signalling and the $p16^{INK4a}$-Rb pathway cooperate to enforce irreversible cellular senescence. Takahashi A., et al. ***Nature Cell Biology*** (2006) 8:1291-7.

(6) Opposing effects of Ets and Id proteins on $p16^{INK4a}$ expression during cellular senescence. Ohtani N., et al. ***Nature*** (2001) 409:1067-70.




## Exploring the physiological roles and mechanisms underlying cellular senescence *in vivo*

Cellular senescence is the state of irreversible cell cycle arrest that can be induced by a variety of potentially oncogenic stimuli and has, therefore, long been considered to suppress tumorigenesis. We reported that $p16^{INK4a}$ and $p21^{Waf1/Cip1}$, both cyclin-dependent kinase inhibitors, play crucial roles in both the onset and establishment of cellular senescence in cell culture and in mouse models. Recently, we generated transgenic mice expressing firefly luciferase under the control of the *$p16^{INK4a}$* or *$p21^{Waf1/Cip1}$* gene promoters. Using these senescence response reporter mice in combination with knockout mice, we are investigating the timing and, hence, the likely roles and mechanisms, of cellular senescence *in vivo*.

## Understanding the molecular mechanisms underlying inflammatory diseases induced by senescence-associated secretory phenotypes (SASPs)

In addition to stable cell cycle arrest, senescent cells also develop senescence-associated secretory phenotypes (SASPs), which contribute both positively and negatively to the onset of inflammatory diseases such as cancer (depending on the biological context). Despite considerable progress in understanding the biological roles of SASPs, far less is known about how they are induced.

Thus, a greater understanding of the underlying molecular mechanisms will lead to novel therapeutic strategies for various aging-associated diseases, including cancer.

Similar to aging, obesity is associated with cancer. However, the underlying mechanisms are not well understood. Recently, we traced the association between obesity and increased cancer risk to gut microbiota communities that produce DNA-damaging bile acid. We found that DNA-damaging bile acid promotes development of obesity-associated liver cancer by inducing SASPs in hepatic stellate cells. We are now focusing on the potential clinical implications of these findings.

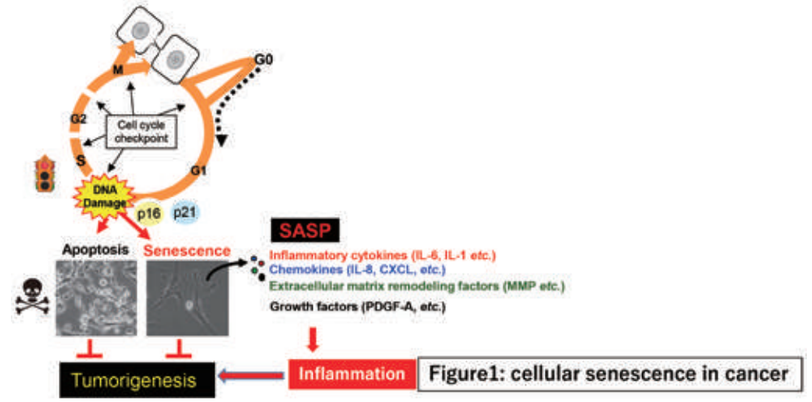
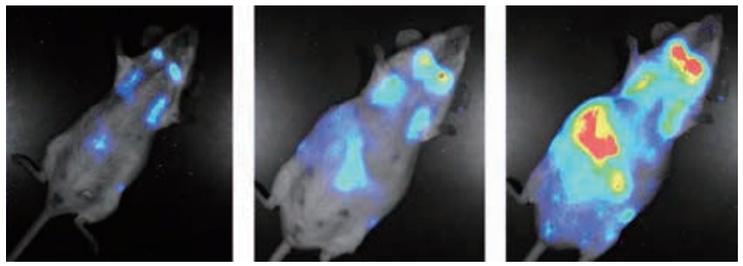



Fig. 1.
Cellular senescence initially inhibits proliferation of damaged cells, thereby acting as a fail-safe mechanism. However, in the long term, senescent cells may eventually promote tumorigenesis via SASPs.



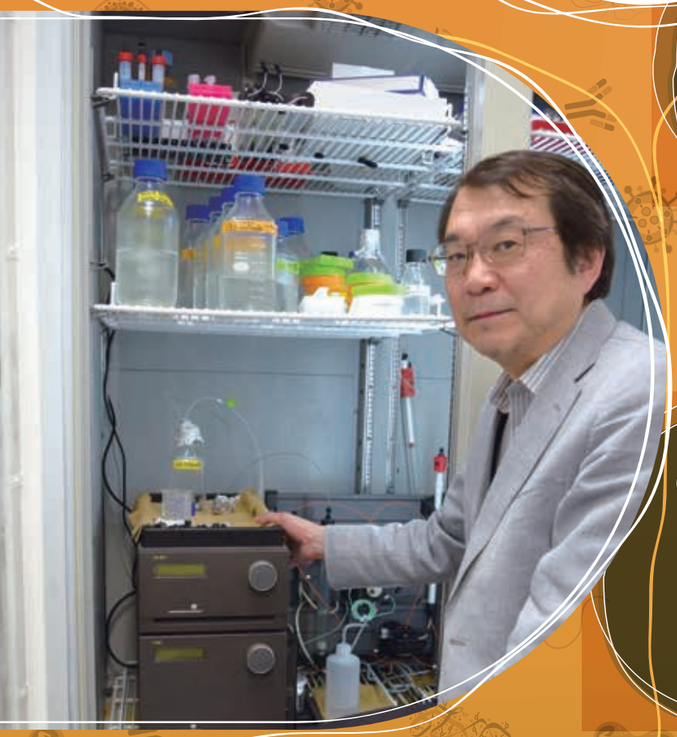
6 weeks　　12 months　　20 months

Fig. 2.Real-time bioluminescence imaging of *$p16^{INK4a}$* gene expression during aging (*Journal of Cell Biology* 186: 393-407. 2009).

Division of Cellular and Molecular Biology

# Department of Oncogene Research

Cancer develops due to accumulation of mutations within a cell, which can then become malignant through immortalization and transformation. The malignant traits of cancer cells occur as they evade cancer inhibitory mechanisms such as apoptosis and senescence and acquire capacity for autonomous proliferation. In addition, cancer cells acquire invasive and metastatic characteristics through the loss of intercellular communication and altered cell morphology.



## Staff

Assoc. Prof. : Shigeyuki Nada ／
Assoc. Prof. : Norikazu Yabuta ／
Asst. Prof. : Kentaro Kajiwara ／
Postdoc. : Akira Ogawa ／
Undergrad. Student 3 ／ Grad. Student 10

## Masato Okada

**Professor**

Dr. Okada graduated Kyoto University School of Science in 1981 and received his Ph.D. from Osaka University in 1988. He worked as a Research Associate at the Institute for Protein Research in Osaka University and became an Associate professor in 1996 in the same institution. He was appointed current position as a Professor of RIMD in 2000.

## Publication


(1) Structural basis for the assembly of the Ragulator-Rag GTPase complex. Yonehara R., et al. ***Nature Commun*** (2017) 8:1625

(2) The Rho guanine nucleotide exchange factor ARHGEF5 promotes tumor malignancy via epithelial-mesenchymal transition. Komiya Y., et al. ***Oncognesis*** (2016) 5: e258

(3) p18/LAMTOR1: a late endosome/lysosome-specific anchor protein for the mTORC1/MAPK signaling pathway. Nada S., et al. ***Methods Enzymol*** (2014) 535:249-63

(4) The novel lipid raft adaptor p18 controls endosome dynamics by anchoring the MEK-ERK pathway to late endosomes. Nada S., et al. ***EMBO J.*** (2009) 28:477-89

(5) The lipid raft-anchored adaptor protein cbp controls the oncogenic potential of c-Src. Oneyama C., et al. ***Mol Cell*** (2008) 30:426-36

(6) Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. Kawabuchu M., et al. ***Nature*** (2000) 404:999-1003




## ●Src and cancer development

Src is a signaling molecule that localizes to the sub-membrane and was the first oncogene to be discovered. Normal tissues retain morphology by maintaining contact between neighboring cells via cell-cell junctions; however, cancer cells exhibit altered morphology (shown in Fig. 1) and undergo invasion and metastasis by secreting growth factors and proteases. Our laboratory has examined the role of Src as an activator of signaling pathways that control remodeling of the cytoskeleton, which contributes to motility by inducing morphological changes. In addition, Src is involved in cell membrane-mediated signaling pathways that promote expression of genes encoding proteases, thereby leading to malignancy. We aim to further elucidate the detailed molecular mechanisms by which Src affects cancer cell invasion and metastasis.

Interestingly, unlike other oncogenes, Src harbors no mutations. We found that Src is involved in a phenomenon called “cell competition,” in which cells interact and compete with each other, producing a “winner” based on their relative fitness. We anticipate that revealing the relationship between Src and cell competition will increase our understanding of the function of Src in cancer development; these studies are ongoing.

## ●The molecular mechanism underlying p18/Ragulator and mTOR nutrient signaling

mTOR is responsible for nutrition- and growth-related signaling in cells, and is involved in various biological phenomena. Our laboratory discovered that the p18 protein acts as an adaptor for molecules involved in regulating mTOR, and that it plays a crucial role in activating mTOR (Fig.1). We will continue our research into the molecular mechanism by which p18 regulates mTOR using protein structural analysis and by studying the molecular interactions between other factors involved in mTOR regulation.

In addition, we are studying the molecular mechanisms underlying cancer defense in naked mole rats (NMR). NMR are rodents that are similar to mice; however, they are unique in that they have an exceptionally long life span (up to 10 times that of mice). They also exhibit significant resistance to aging and cancer. Our laboratory focuses on identifying the molecular mechanisms that allow NMR to acquire these traits.

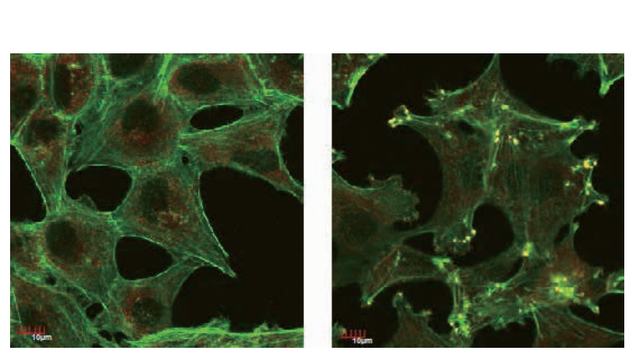



Fig. 1. Src activation induces morphological changes and increases cell mobility.

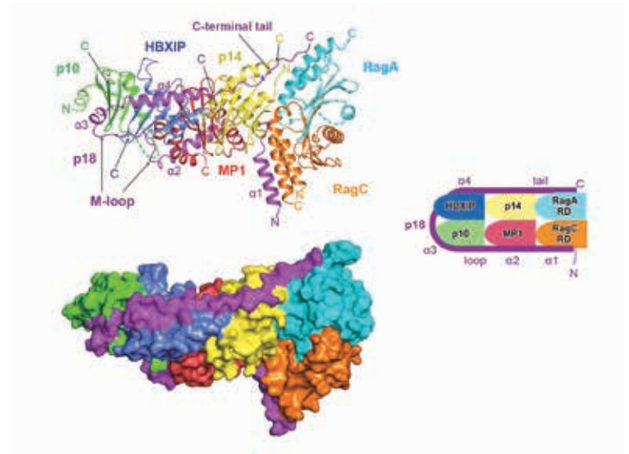
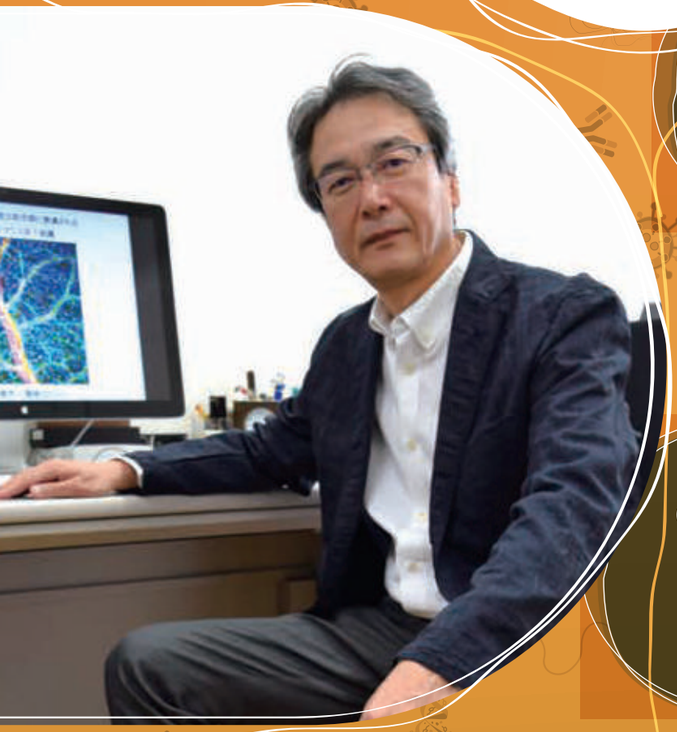



Fig. 2. Protein Structure for Ragulator complex

Division of Cellular and Molecular Biology

# Department of Signal Transduction

Tissue-specific stem cells continuously produce terminally differentiated functional cells and maintain organ integrity. Blood vessels supply oxygen and nutrients to all tissues; tissues and organs cannot develop without blood vessel formation. Our aim is to elucidate the cellular and molecular mechanisms underlying vascular formation (particularly those involving stem cells) and to develop strategies to manage patients with vascular diseases.



## Staff

Assoc. Prof. : Hisamichi Naito ／
Asst. Prof. : Hiroyasu Kidoya ／
Postdoc. : Wei-Zhen Jia ／
Postdoc. : Fumitaka Muramatsu ／
Postdoc. : Yumiko Hayashi

## Nobuyuki Takakura

**Professor**

Dr. Takakura obtained his Ph.D in Graduate School of Medicine, Kyoto University in 1997. He was appointed Professor of RIMD in 2006 after working at Kumamoto University as an assistant professor for 4 years and Kanazawa University as a professor for 5 years.

## Publication


(1) Regnase-1-mediated post-transcriptional regulation is essential for hematopoietic stem and progenitor cell homeostasis. Kidoya H. et al., ***Nat Commun***. (2019) 10 (1):1072.

(2) TAK1 prevents endothelial apoptosis and maintains vascular integrity. Naito H., et al., ***Dev Cell***. (2019) 48(2):151-166.e7.

(3) CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. Wakabayashi T., et al. ***Cell Stem Cell*** 22(3):384-397, 2018.

(4) APJ regulates parallel juxtapositional alignment of arteries and veins in the skin. Kidoya H., et al. ***Dev Cell*** (2015) 33 (3):247-59.

(5) A role for hematopoietic stem cells in promoting angiogenesis. Takakura N., et al. ***Cell*** (2000) 102(2):199-209.

(6) Critical role of the TIE2 endothelial cell receptor in the development of definitive hematopoiesis. Takakura N., et al. ***Immunity*** (1998) 9(5):677-86.




## ●Mechanism of vascular formation

Tissue homeostasis in all organs is maintained via a highly hierarchal architecture of blood vessels, which is precisely regulated in an organ-specific manner. We are examining how blood vessel diversity is regulated, focusing on the processes of angiogenesis and blood vessel maturation. Our recent studies clarified that arterial-venous alignment is regulated by the apelin/APJ system and is critical for thermoregulation (Kidoya, Dev Cell 2015).

## ●Stemness and vascular niche

Stem cells localize in perivascular areas in many organs. Cells that comprise such a vascular niche regulate the “stemness” of stem cells. In our cancer stem cell (CSC) model based on PSF1 promoter activity, we found that CSCs proliferate and survive in the vascular niche (Nagahama, Cancer Res 2010, Kinugasa, Stem Cells 2014). Regulation of the vascular niche is a promising approach to inhibiting tumor growth. Because blood vessels developing in the tumor microenvironment are immature and abnormal, normalization of blood vessel development must control CSCs in the vascular niche. Vascular normalization also improves anti-tumor immunity and drug delivery. Therefore, we are seeking ways to normalize blood vessels within tumors.

## ●Development of tissue regeneration methods based on endothelial stem cells

We have identified endothelial stem cells in pre-existing blood vessels and showed their utility for vascular regeneration (Naito, EMBO J 2012). Recently, we found that such endothelial stem cells affect the resistance of cancer cells to anti-angiogenic therapy (Naito, Cancer Res 2016). We are examining how endothelial stem cells develop and how they are maintained during development with a view to using this cell population to treat vascular disease.

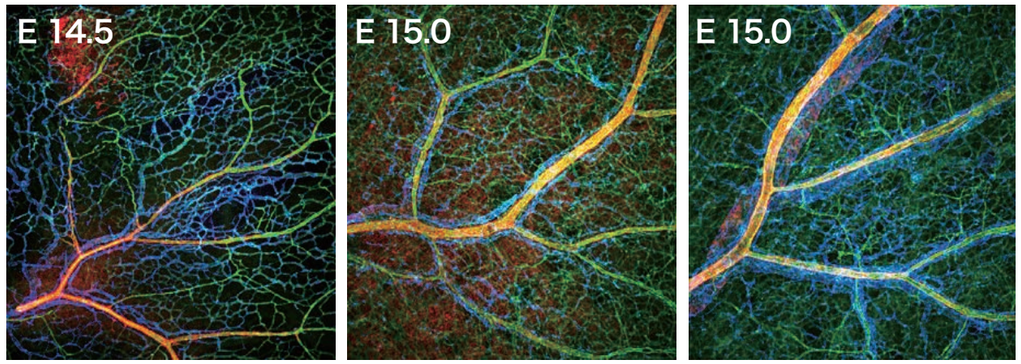



Fig. 1. Vascular development in mouse embryos. Hierarchal architecture of blood vessels accompanied by arterial (yellow)–venous (blue) alignment. Green; endothelial cells.

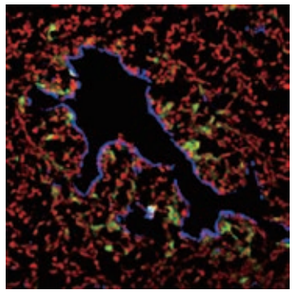


Fig. 2. Endothelial cells (blue) and CSCs (green) in a tumor.
CSCs localize at the perivascular area, the so called “vascular niche.”

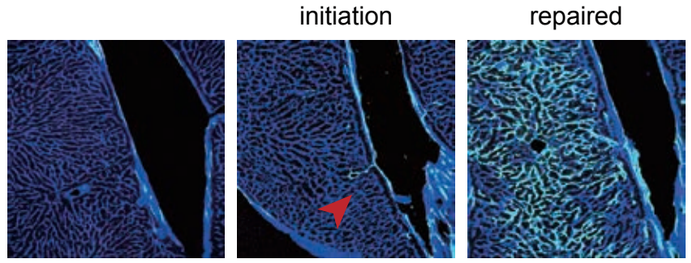



Fig3. Linage tracing of VESC. New blood vessels emerged from VESC (shown in green) Most of the endothelial cells (ECs) are replaced by ECs drived from VESC.

Division of Cellular and Molecular Biology

# Department of Cellular Regulation

Most cancers originate from epithelial cells. Normal epithelial cells form a sheet-like tissue structure in which cells are tightly attached to each other and to the basement membrane. Through malignant progression, cells proliferate and expand by invading surrounding tissues. Furthermore, cells metastasize to distant organs via blood vessels, forming often incurable tumors. Our aim is to elucidate the mechanism underlying this mysterious process of cancer development.

## Staff

Assoc. Prof. : Daisuke Yamazaki /
Asst. Prof. : Yosuke Funato /
Postdoc. : Osamu Hashizume /
Grad. Student 8

## Hiroaki Miki

**Professor**

Dr. Miki received his Ph.D from University of Tokyo in 1998. He was appointed Professor in RIMD after working at the Institute of Medical Science, Universuty of Tokyo and at the Institute for Protein Research, Osaka University.

## Publication


(1) Phosphocysteine in the PRL-CNNM pathway mediates magnesium homeostasis. Gulerez et al. ***EMBO Rep***. (2016) 17(12):1890-1900.

(2) $Mg^{2+}$ Extrusion from Intestinal Epithelia by CNNM Proteins Is Essential for Gonadogenesis via AMPK-TORC1 Signaling in Caenorhabditis elegans. Ishii T., et al. ***PLoS Genet.*** (2016) 12 (8):e1006276.

(3) Membrane protein CNNM4-dependent $Mg^{2+}$ efflux suppresses tumor progression. Funato Y., et al. ***J Clin Invest***. (2014) 124(12):5398-5410.

(4) Basolateral $Mg^{2+}$ extrusion via CNNM4 mediates transcellular $Mg^{2+}$ transport across epithelia: a mouse model. Yamazaki D., et al. ***PLoS Genet***. (2013) 9(12):e1003983.

(5) Thioredoxin mediates oxidation-dependent phosphorylation of CRMP2 and growth cone collapse. Morinaka A., et al. ***Sci Signal.*** (2011) 4 (170):ra26.

(6) Nucleoredoxin sustains Wnt/β-catenin signaling by retaining a pool of inactive dishevelled protein. Funato Y., et al. ***Curr Biol.*** (2010) 20(21):1945-52.




## ●Role of PRL in malignant progression of cancers

PRL is highly expressed in malignant tumors and promotes cancer metastasis. We discovered that PRL associates with CNNM4, a $Mg^{2+}$ transporter, and inhibits its $Mg^{2+}$ transporting activity. Moreover, we also found that intestinal polyps became malignant and invaded the surrounding muscle tissue when *CNNM4* was disrupted. At present, we are investigating the functional relationship between cancer malignancy and $Mg^{2+}$ dyshomeostasis caused by CNNM4 inhibition.

In normal epithelial tissues, cells are attached to each other and collectively maintain their structure; these characteristics are disrupted in cancer tissues. Forced expression of PRL in epithelial cells cultured on matrix gels induced a marked change in their morphology; some cells invaded into the matrix only when PRL-expressing cells were surrounded by non-expressing cells. These results suggest that physical interaction between PRL-expressing cells and non-expressing cells stimulates invasive behavior during malignant progression. We are trying to clarify the molecular mechanism underlying this function of PRL.

## ●Functional analyses based on organoid culture of intestinal epithelia

A method of culturing intestinal epithelia in 3D matrix gels that mimic *in vivo* situations was recently developed; the system is called organoid culture. In this system, intestinal epithelial cells differentiate and form a structure comprising a monolayer sheet of cells. We are using this organoid culture system to investigate the role of PRL/CNNM in cell proliferation, differentiation, and cancerous transformation.

Many oncogenes and anti-oncogenes involved in regulating cell proliferation and survival have been identified. By contrast, characteristics involved in transformation of epithelial cells in a 3D space, which accompany architectural changes (such as invasion and metastasis) in tissues, remain unclear. For example, how do cancer cells exit the epithelial tissue in which they are “born” and expand their territory by invading surrounding tissues? We are tackling these problems and trying to identify the mechanisms underlying cancer development.

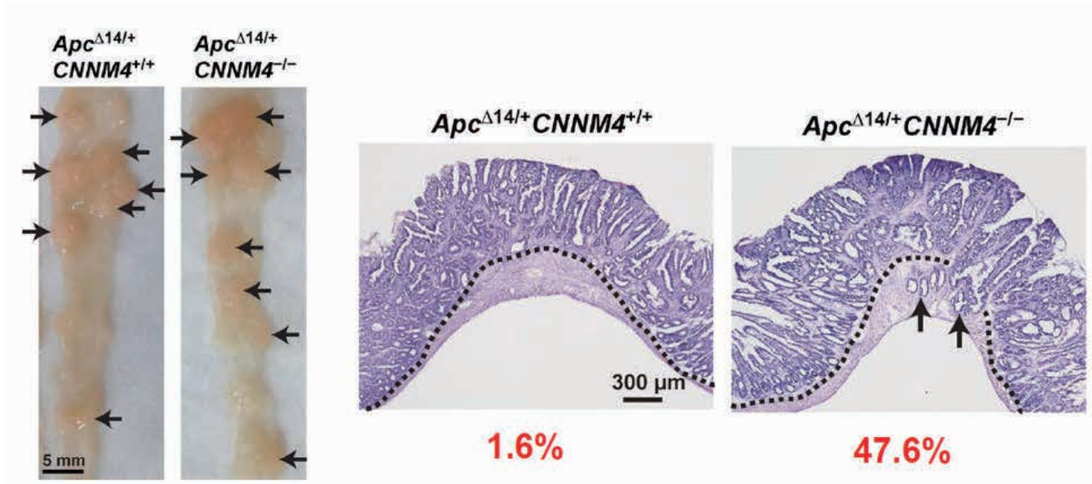



Fig. 1. Macroscopic images of the intestine (left) and histological images of polyps (right) in the indicated genetically engineered mice. *CNNM4*-deficient mice develop adenocarcinomas that invade the muscle layer (arrows).

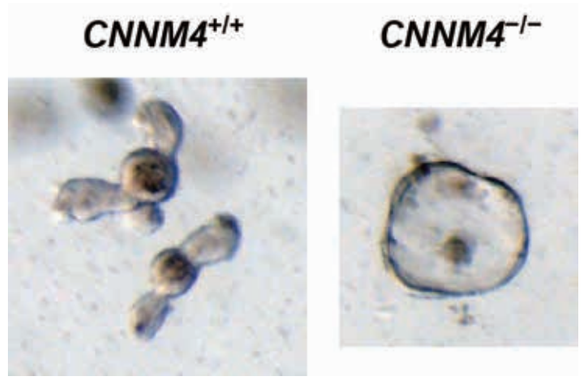
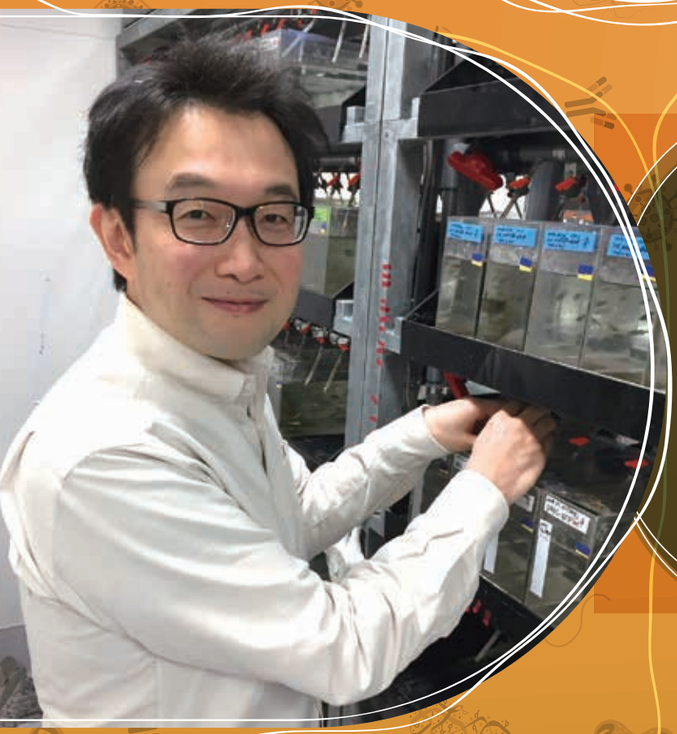



Fig. 2. Organoid culture of intestinal epithelia from the indicated genetically engineered mice. *CNNM4* deficiency causes morphological abnormalities.

Genome Information Research Center

# Department of Homeostatic regulation

In our body, cells recognize its position and roles via cell-cell communication and behave appropriately. Such cell behavior supports tissue morphogenesis and homeostasis, and its dysregulation is involved in congenital malformation, cancer, degenerative diseases, and aging. We focus especially on the cell-cell communication and behavior supporting tissue homeostasis and explore unknown molecular systems controlling embryonic development, organogenesis, regeneration, aging, and disease, using *in vivo* imaging, model animal genetics, molecular and cell biology, and biochemistry.



## Tohru Ishitani

**Professor**

Prof. Ishitani received his Ph.D. from Nagoya University in 2002. After working as postdoctoral fellow in the same university, he became Associate Professor in the Medical Institute of Bioregulation, Kyushu University in 2006. He was appointed Professor in the Institute for Molecular and Cellular Regulation, Gunma University in 2017. He took his current position at RIMD in 2019. He received Young Scientists' Prize of The Commendation for Science and Technology by MEXT in 2009, and Samuro Kakiuchi Memorial Award by the Japanese Biochemical Society in 2014.

## Publication


(1) Hipk2 and PP1c cooperate to maintain Dvl protein levels required for Wnt signal transduction. Shimizu N., et al. ***Cell Reports*** (2014) 8(5) 1391-1404

(2) Visualization and exploration of Tcf/Lef function using a highly responsive Wnt/β-catenin signaling-reporter transgenic zebrafish. Shimizu N., et al. ***Developmental biology*** (2012) 370(1) 71-85

(3) NLK positively regulates Wnt/β-catenin signalling by phosphorylating LEF1 in neural progenitor cells. Ota S., et al. ***EMBO Journal*** (2012) 31:1904-15

(4) Nemo-like kinase suppresses Notch signalling by interfering with formation of the Notch active transcriptional complex. Ishitani T., et al. ***Nat. Cell Biol***. (2010) 12:278-85

(5) Nrarp functions to modulate neural-crest-cell differentiation by regulating LEF1 protein stability. Ishitani T., et al. ***Nat. Cell Biol***. (2005) 7:1106-12

(6) The TAK1-NLK-MAPK-related pathway antagonizes signalling between beta-catenin and transcription factor TCF. Ishitani T., et al. ***Nature*** (1999) 399:798-802

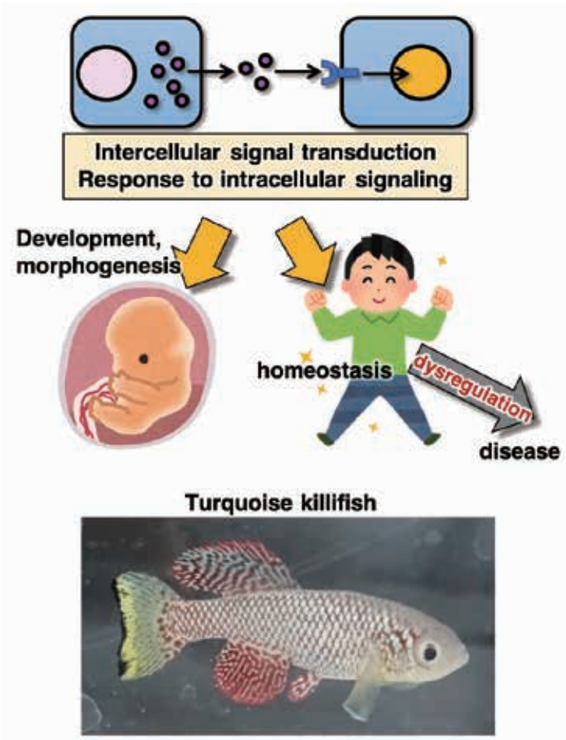


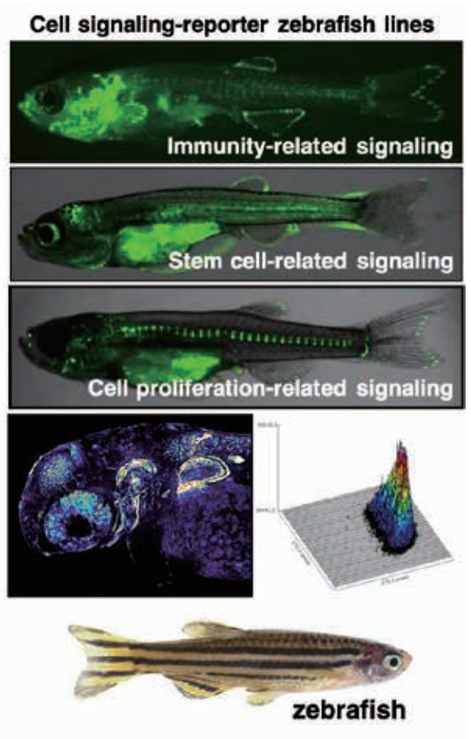

## ●A new concept of tissue homeostasis "Morphostasis"

Developing animal tissues are reproducibly formed in the same shape even in the presence of internal fluctuations and external perturbations (developmental robustness). Adult tissues also maintain a stable morphology while replacing old or damaged cells with new healthy cells (tissue homeostasis), but its dysregulation is involved in various diseases. We are focusing common ground between "developmental robustness" and "tissue homeostasis" and regard it as "Morphostasis". Specifically, using a zebrafish as a model animal which is suitable for *in vivo* imaging analysis of cell-cell communication and tissue dynamics and genetic analysis, we are exploring unknown molecular systems supporting developmental robustness and testing their potential roles in adult tissue homeostasis and their dysregulation in disease. We try to combine developmental biology and disease study to establish a new concept of tissue homeostasis.

## ●Aging program and its regulation

We are tackling the exploration of the molecular mechanisms underlying individual aging. Aging mechanisms have been studied using worm (*C.elegans*) and fly (*Drosophila*)as model animals because their life spans are very short. However, their organs are quite different from those of human. In addition, the life spans of mouse and zebrafish, which are well used as human disease model, are very long (3~4years). So, researchers have been searching for short-lived vertebrates. Our lab is using a short-lived fish "turquoise killifish" (the life span of which is 3~6months) as a new aging model. This fish shows age-dependent decline of motility, fertility, and cognitive function, similar to human. We are challenging the clarification of human aging mechanisms and the development of new technique extending "healthy life expectancy", using turquoise killifish!



Genome Information Research Center

# Department of Experimental Genome Research

In the past, animals harboring natural mutations have been used to elucidate the mechanisms underlying various diseases. In the “post-genome project era,” genetically modified animals play a key role in basic molecular biological investigations and act as models of human disease. Our laboratory studies the mechanisms underlying mammalian reproductive systems through genetic manipulation of animal models.

## Staff

Asst. Prof.: Haruhiko Miyata / Asst. Prof.: Taichi Noda / Asst. Prof.: Keisuke Shimada / SA Asst. Prof.: Daiji Kiyozumi (concur.) / SA Asst. Prof.: Tsutomu Endo (concur.) / SA Asst. Prof.: Julio Castaneda / Postdoc: Lu Yonggang / JSPS Research Fellow: Nobuyuki Sakurai / JSPS Research Fellow: Yamauchi (Ishikawa) Yu / Guest Professor: Martin M. Matzuk / Guest Associate Professor: Yoshitaka Fujihara / Guest Researcher: Masaru Okabe / Undergrad. Student 1 / Grad. Student 10

## Masahito Ikawa (concur.)

**Professor**

Dr. Ikawa received his Ph.D. from Osaka University in 1997. After working as JSPS postdoctoral fellow and a Research Associate at Genome Information Research Center in Osaka University, he spent 2 years at The Salk Institute in the USA as a Research Associate. After returning to Osaka University in 2002, he became an Associate Professor in 2004 and was appointed to the current position in 2012. He was awarded JSPS Prize in 2013. His lifework is to study mammalian reproductive systems using genetically engineered mice.

## Publication


(1) New Insights into the Molecular Events of Mammalian Fertilization. Satouh Y., e al. (2018) ***Trends Biochem Sci***. 43 (10):818-828

(2) Sperm-borne phospholipase Czeta-1 ensures monospermic fertilization in mice. Nozawa K., et. Al., (2018) ***Sci Rep***. 8(1):1315.

(3) TCTE1 is a conserved component of the dynein regulatory complex and is required for motility and metabolism in mouse spermatozoa. Castaneda J.M., et al., ***PNAS*** (2018) 114 (27):E5370-E5378

(4) Structural and functional insights into IZUMO1 recognition by JUNO in mammalian fertilization. Kato K., et al. ***Nat Commun.*** (2016) 7:12198.

(5) Genome engineering uncovers 54 evolutionarily conserved and testis-enriched genes that are not required for male fertility in mice. Miyata H., et al. ***Proc Natl Acad Sci USA.*** (2016) 113(28):7704-10.

(6) Sperm calcineurin inhibition prevents mouse fertility with implications for male contraceptive. Miyata H., et al. ***Science.*** (2015) 350(6259):442-5.




## ●Analysis of molecular mechanisms involved in mammalian gametogenesis, fertilization, and implantation

We were the first laboratory in the world to produce genetically modified mice that express green fluorescent protein (GFP) throughout the body (Fig. 1). These green fluorescent mice are useful for many types of research projects. Indeed, we used these animals to label sperm with fluorescent protein and visualize the fertilization process (***Exp Anim.*** 2010; ***JCS.*** 2010, 2012; ***PNAS.*** 2012, 2013) (Fig. 2).

Recently, we found that calcineurin (PPP3CC/PPP3R2) is essential for sperm motility and male fertility (***Science.*** 2015). Inhibiting sperm calcineurin may lead to the development of a reversible male contraceptive.

## ●Development of new technologies for producing genetically modified animals

Another tool improved by work in our laboratory is lentiviral (LV) vector-mediated genetic manipulation *in vivo*. We developed the technique of placenta-specific gene manipulation by transducing blastocyst stage embryos with LV vectors (***Nat Biotechnol.*** 2007; ***PNAS.*** 2011). Using this technique, we are trying to elucidate the mechanism underlying implantation and placentation.

We also established rat embryonic stem (ES) cells and generated mouse↔rat chimeric animals. We would like to use this animal model to study body/organ size control *in vivo*; indeed, this method may enable derivation of various organs from ES or iPS cells (***Genes Cell.*** 2011; ***Sci Rep.*** 2016).

Our recent interest is using the CRISPR/Cas9 system to generate genetically modified animals to study fertilization, implantation, and placentation. We have had success in mice and rats using sgRNA/Cas9-expressing plasmids (***Sci Rep.*** 2013,2016; ***DGD.*** 2014; ***PNAS.***2016; ***Nat. Commun.*** 2016).

Our laboratory and the Animal Resource Center for Infectious Diseases (http://www.arcid.biken.osaka-u.ac.jp/) offer support services such as generation of genetically modified animals, *in vitro* fertilization, and cryopreservation of mouse strains. For more information, please visit our homepage (http://www.egr.biken.osaka-u.ac.jp/index.php).

Fig. 1. GFP-expressing mice are useful for many types of research project. Genetically modified animals play a key role in basic molecular biology-based investigations and are good models for studying human disease.

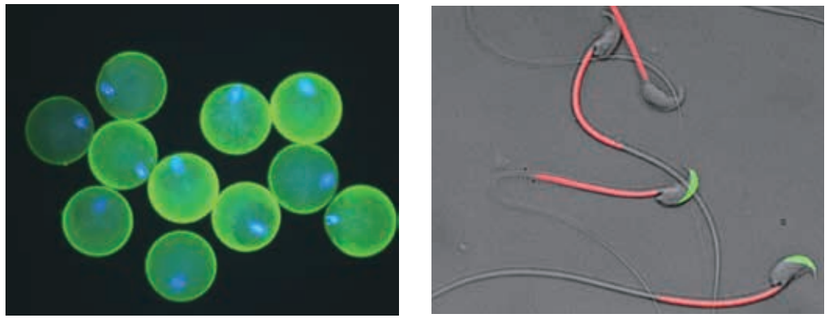


Fig. 2. Mouse eggs (left) and sperm (right) labeled with fluorescent proteins. These gametes are used to visualize the fertilization process. (Figs from Exp. Anim. 59(1), 105-7, 2010)

Genome Information Research Center

# Department of Genome Informatics

We use single cell sequencing along with computational methods to study problems that are difficult or impossible to observe by experimental methods alone. Some of the problems we work on include: analysis of B and T cell receptor repertoires, protein-nucleotide interactions and multiple sequence alignment of protein and nucleotide sequences. These themes are described in more detail below.

## Staff

Assoc. Prof. : Kazutaka Katoh ／
Assoc. Prof. : Shunsuke Teraguchi (concur.) ／
Asst. Prof. : Songling Li ／
Asst. Prof.: Floris J.Van Eerden ／
Postdoc : John Rozewicki ／
Postdoc : Jan Wilamowski

## Daron M. Standley

**Professor**

Prof. Standley received his PhD in Chemistry from Columbia University in 1998. He then joined Schrodinger, Inc. where he worked as a scientific software developer for five years. In 2003 he moved to the Institute for Protein Research, Osaka University as a Senior Scientist. He joined the Immunology Frontier Research Institute (IFReC) as a Principal Investigator in 2008 and, after a two-year cross-appointment at Kyoto University's Institute for Virus Research, became a Professor full time at the Research Institute for Microbial Diseases in 2016.

## Publication


(1) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Katoh, K., et al. ***Mol Biol Evol*** (2013) 30(4) 772-80

(2) Integrated sequence and structural alignment using MAFFT and DASH. Rozewicki, J., et al. (in prep) https://sysimm.org/dash/

(3) Repertoire Builder: High-throughput structural modeling of B and T cell receptors. Schritt, D., et al. (in prep) https://sysimm.org/rep_builder/

(4) Functional clustering of B cell receptors using sequence and structural features. Xu, Z., et al. (in prep) https://sysimm.org/interclone/

(5) Structural modeling of lymphocyte receptors and their antigens. Li, S., et al. ***Methods Mol Biol*** (2019) In press

(6) Regnase-1 and Roquin Regulate a Common Element in Inflammatory mRNAs by Spatiotemporally Distinct Mechanisms. Mino, T., et al. ***Cell*** (2015)161, 1058-1073




## ●Multiple sequence alignment

Multiple sequence alignment (MSA) is an important step in many computational biology pipelines and MAFFT is one of the most popular programs for building MSAs[1]. Since the first release of MAFFT in 2002, we have been continuously improving its accuracy, speed and utility in practical situations, and have provided different options for newly emerging types of data and analyses. Recent features include: inclusion of secondary structural information of non-coding RNAs and proteins, interactive selection of sequences for phylogenetic tree inference, and integration of protein sequences with comprehensive structural alignments[2]. The latter feature plays a central role in structural modeling methods in our lab.

## ●Sequencing B and T cell receptors

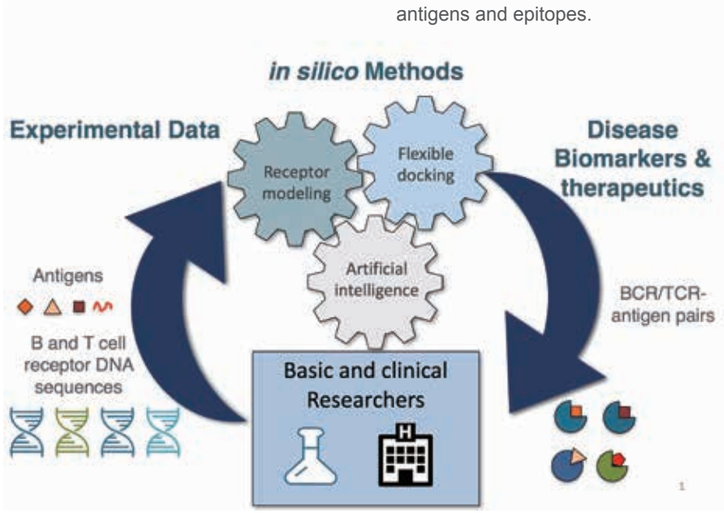
A healthy immune system maintains a vast repertoire of B and T cells that can recognize a wide range of molecules. B and T cell receptors are produced by the combination of two highly variable polypeptide chains which are encoded by different mRNA transcripts. Due to the high variability of each polypeptide and the combination of two different molecules, each individual's repertoire of receptors is in general unique. Several immunological and molecular methodologies have been employed to study the immune repertoire with a rather low-resolution. Nevertheless, the advent of next generation sequencing (NGS) has allowed to analyze millions of immune receptor sequences in one sample (bulk sequencing). This has been of great value to the study of immune repertoires, but cannot reveal the pairing of receptor sequences. In the past few years, single cell sequencing technologies have emerged and have made it possible to study paired polypeptide chains from thousands of individual B and T cells. We are currently making use of both bulk and single cell sequencing techniques to study immune cell repertoires in health and disease.

## ●Protein-nucleotide interactions

Protein-nucleotide interactions play a central role in the flow of biological information in all living systems. In the immune system, the importance of DNA-binding proteins in the regulation of transcription has been studied extensively. More recently, the importance of RNA-binding proteins (RBPs) in maintenance of homeostasis as well as in shaping the strength and duration of immune responses post-transcriptionally has been noted[6]. In order to gain further insight into the mechanisms of RBP-mediated immune regulation, we are developing tools for nucleotide binding site prediction and flexible protein-nucleotide docking which have been validated in a number of experimental studies.

## ●Analysis of B and T cell receptor specificity and repertoires

Prediction of B and T cell receptor antigen specificities from sequence is currently an important and open problem. Our lab is approaching this challenge using a combination of B and T cell receptor sequencing, structural modeling and artificial intelligence. We have developed a tool for generating BCR and TCR 3D models in a high-throughput and accurate manner[3]. We have further extended this technology to cluster such models according to their antigen and epitope specificity[4]. We have also developed a tool to build TCR-epitope-MHC structural models from sequence[5] and are working on new BCR epitope prediction methods that make use of structural information. The immediate goals of this research are to identify antigens and epitopes that are associated with specific diseases along with the B or T cell receptors that recognize these antigens and epitopes.



Genome Information Research Center

# Department of Infection Metagenomics

Next-generation sequencing (NGS) is a technology that can generate an enormous amount of genomic information in a short time and has allowed huge progress in genomic science and infectious disease research. Staffs at the Department of Infection Metagenomics who specialized in bioinformatics, microbiology, and infectious diseases gather to conduct research on pathogens and infectious diseases using NGS-based genomic/metagenomic analysis.





## Staff

Assoc. Prof. : Naohisa Goto (concur.) ／
SA Assoc. Prof. : Shota Nakamura (concur.) ／
SA Asst. Prof. : Daisuke Motooka (concur.) ／
Postdoc. : Yuki Matsumoto ／
Postdoc. : Hiroya Oki

**Tetsuya Iida (concur.)**
Professor

## Publication


(1) Non-Ischemic Heart Failure With Reduced Ejection Fraction Is Associated With Altered Intestinal Microbiota. Katsimichas T. et al., ***N Circ J.*** (2018) Mar 30. doi: 10.1253/circj.CJ-17-1285.

(2) A case of severe soft tissue infection due to Streptococcus tigurinus diagnosed by necropsy in which genomic analysis was useful for clarifying its pathogenicity. Yoshizawa H., et al., ***Pathol Int.*** (2018) doi: 10.1111/pin.12656.

(3) Fungal ITS1 Deep-Sequencing Strategies to Reconstruct the Composition of a 26-Species Community and Evaluation of the Gut Mycobiota of Healthy Japanese Individuals. Motooka D., et al., ***Front Microbiol.*** (2017) 8:238.

(4) The cell envelope-associated phospholipid-binding protein LmeA is required for mannan polymerization in mycobacteria. Rahlwes K.C., et al., ***J Biol Chem.*** (2017) 292 (42):17407-17417.

(5) The clinical and phylogenetic investigation for a nosocomial outbreak of respiratory syncytial virus infection in an adult hemato-oncology unit. Nabeya D., et al., ***J Med Virol.*** (2017) 89(8):1364-1372.




## ●Development of methods for pathogen detection based on metagenomic analysis

A metagenome is the sum of all genomes of all organisms inhabiting a particular environment. The emergence of NGS has enabled comprehensive analysis of genomic information from large numbers of organisms, thereby leading to significant advances in metagenomic analysis. For example, comprehensive analysis of microbial genomes in blood or nasopharyngeal samples from patients suffering from diseases of unknown cause makes it possible to identify the pathogens causing these symptoms and the genetic factors responsible for pathogenesis. This method, unlike conventional pathogen-specific methods, is applicable to various types of sample (e.g., blood, nasal swab, stool). It can also detect multiple pathogens in a single sample. Our laboratory uses metagenomic analysis to develop new methods for the diagnosis of infectious diseases.

## ●Study of gut flora during onset of infectious disease

It is becoming clear that the gut flora is microbiota that is involved in various diseases and plays an important role in host defense. By performing metagenomic analysis of changes in and recovery of bacterial gut flora over time in cases of diarrhea, our laboratory is studying the relationship between human gut flora and pathogens. Furthermore, not only is bacterial gut flora related to disease, but it is also closely related to lifestyle factors. Our research is focused on how bacterial gut flora is affected by environmental factors and the physiological state of the individual.

NGS technology had made remarkable progress. New hardware platforms are being developed. NGS itself reads only nucleic acid sequences, and further analysis is required to handle the enormous amount of data obtained. It is important to have a broad knowledge of bioinformatics, microbiology, and genomics in order to select the appropriate model based on the characteristics of each sequencing platform. At our laboratory, we carry out co-operative research with specialists in the fields of bioinformatics, microbiology, and infectious diseases.

## ●Genomic analysis of microbial pathogens

The molecular mechanisms underlying the pathogenicity of many infectious diseases remain unclear. Our laboratory conducts genomic analysis-based research to identify genes responsible for pathogenicity and to identify the molecular mechanisms by which infectious diseases develop.

Fig. 1.Large scale computer system for NGS data analysis.

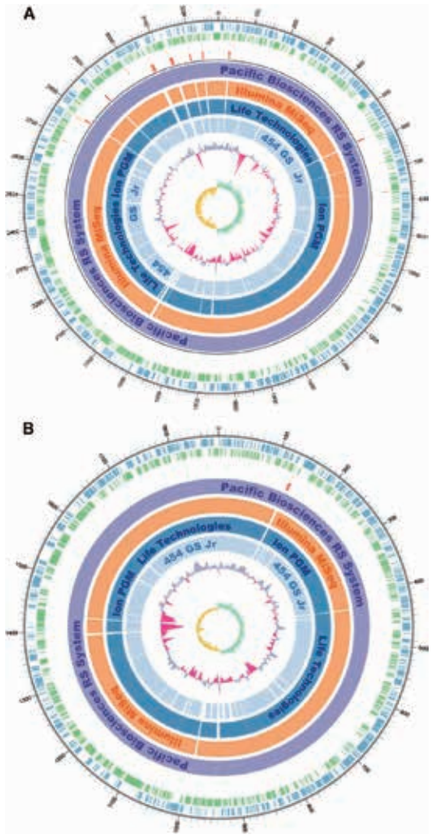



Fig. 2.
Genomic analysis of *Vibrio parahaemolyticus* using four models of next-generation sequencer: ■454 GS Jr (Roche)、■IonPGM (Life Technologies)、■MiSeq (Illumina)、■Pacific Biosciences RS System (PacBio)
GS Jr, MiSeq, and IonPGM produce short reads. Therefore, they require assembly of short fragments. The third generation sequencer, PacBio, on the other hand can produce long reads and assemble them into two long sequences with lengths equivalent to two chromosomes. However, PacBio has low accuracy with respect to sequence information. Although the read length of MiSeq is far shorter than that of PacBio, it has a much higher yield. Thus, to conduct a proper analysis it is necessary to understand the characteristics of each sequencing platform.

Genome Information Research Center

# Next-Generation Sequencing (NGS) Core Facility

## Staff

Prof. (concur.) : Tetsuya Iida, Ph.D.
SA Assoc. Prof. (concur.) : Shota Nakamura, Ph.D.
Asst. Prof. : Daisuke Okuzaki, Ph.D.
SA Asst. Prof. (concur.) : Daisuke Motooka, Ph.D.

To prevent and control infectious diseases, it is essential to understand both the mechanisms of pathogenicity as well as host immune responses. The NGS Core Facility of the Genome Information Research Center was founded to support and provide genomic technologies for research on infectious diseases and immunology. We are supporting researchers in analyzing large volumes of data obtained from NGS by combining bioinformatics approaches with large computing systems designed for big data. Recently, we have begun supporting activities outside of infectious disease research for researchers from Osaka University as well as other universities.

In the last decade, as a result of the remarkable technological innovation of NGS systems, which can read a massive number of sequences simultaneously and at high speed, we are now able to analyze genomic information quickly and at low cost. Various NGS instruments including MiSeq, HiSeq (Illumina), and MinION (Oxford Nanopore) are available in our Core Facility. We provide genomics applications according to researchers' needs in addition to training courses covering topics such as NGS procedures as well as other related experimental technologies. Furthermore, we are expanding research with the aim of improving bioinformatics analysis in collaboration with the Department of Genome Informatics and the Department of Infection Metagenomics.


Next Generation Sequencer HiSeq

Large-scale computer system for NGS

## Publication


(1) Clinical implications of monitoring nivolumab immunokinetics in non-small cell lung cancer patients. Osa A., et al. ***JCI Insight*** (2018) Oct 4;3(19).

(2) Heme ameliorates dextran sodium sulfate-induced colitis through providing intestinal macrophages with noninflammatory profiles. Kayama H., et al., ***Proc Natl Acad Sci U S A***. (2018) Aug 14;115 (33):8418-8423.

(3) Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. Kang S., et al. ***Nat Immunol***. (2018) Jun;19(6):561-570.

(4) Phenotype-genotype correlations of PIGO deficiency with variable phenotypes from infantile lethality to mild learning difficulties. Tanigawa J., et al. ***Hum Mutat***. (2017) Mar 23. 38;7::805-815.




Genome Information Research Center

# Laboratory of Genome Research

We are currently using animal models to investigate the molecular biological mechanisms involved in human disease, particularly cardiovascular disease.


## Takeshi Miwa

**Associate Professor**

Dr.Takeshi Miwa received his Ph.D. from Osaka University in 1983. He appointed as a Assoc. prof. in RIMD after working at The University of Tokyo and Stanford University.

## Publication


(1) Connexin45 contributes to global cardiovascular development by establishing myocardial impulse propagation. Nishii K., et al. ***Mech Dev.*** (2016) 140:41-52

(2) A novel heart failure mice model of hypertensive heart disease by angiotensin II infusion, nephrectomy, and salt loading. Tsukamoto Y., et al. ***Am J Physiol Heart Circ Physiol.*** (2013) 305:1658-67

(3) Interleukin-16 promotes cardiac fibrosis and myocardial stiffening in heart failure with preserved ejection fraction. Tamaki S., et al. ***PLoS One*** (2013) 8(7):e68893

(4) L-Carnitine prevents the development of ventricular fibrosis and heart failure with preserved ejection fraction in hypertensive heart disease, Omori Y., et al. ***J Hypertens.*** (2012) 30:1834-44


1) We have established a diastolic heart failure model using Dahl salt-sensitive rats. This model showed that left ventricular (LV) fibrosis and stiffening play crucial roles in the development of heart failure with preserved ejection fraction (HFpEF). Digitalis-like factors and the subsequent activation of the Na+/Ca2+ exchanger may play important roles in the development of hypertensive HFpEF and also regulate the effect of carnitine when administered to the HFpEF model. In addition, serum interleukin-16 (IL-16) levels are elevated both in patients with HFpEF and in the rat model. Increased cardiac expression of IL-16 in transgenic mice induces cardiac fibrosis and LV myocardial stiffening, which is accompanied by increased macrophage infiltration (Fig. 1).

2) To understand the cellular and molecular aspects of vascular smooth muscle (SM) cell growth in atherosclerotic plaques, we characterized the mechanisms responsible for transcription of SM-specific genes, particularly the human SM alpha-actin (SmαA) gene (Fig. 2). Several cis-acting DNA elements and transcriptional nuclear factors essential for SmαA expression have been identified. Since SmαA is also expressed in many tissues during acute inflammation, we are examining expression of the SmαA and its function(s).

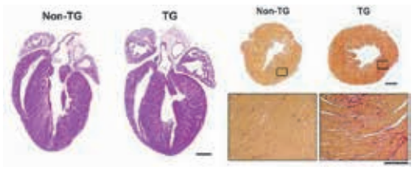



Fig. 1. Increased cardiac expression of IL-16 in mice under control of the α-MHC promoter causes increased myocardial fibrosis and stiffness. (Left) Four-chamber view of hearts from non-transgenic and transgenic mice. (Right) Sirius Red-stained sections of the LV from fibrotic areas.

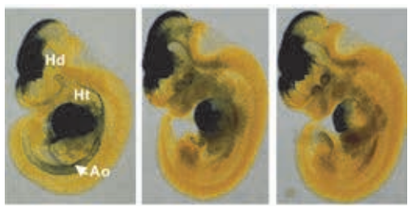



Fig. 2. Embryonic aorta (Ao) express the human vascular SM α-actin promoter (left), but those with -1M (center) and 4M (right) point mutations in the transcriptional nuclear factor-binding regions do not.

Research Center for Infectious Disease Control

# Department of Bacterial Infections

In our laboratory, we are conducting research and collecting genomic information to understand how bacterial pathogens infect the host and cause disease. In addition, by developing new pathogen detection methods using high-throughput DNA sequencers, we aim to identify novel pathogens and reveal the pathogenesis of unknown infectious diseases.

## Staff

Assoc. Prof. : Toshio Kodama /
Asst. Prof. : Shigeaki Matsuda /
SA Asst. Prof. : Pranee Somboonthum
/ Undergrad. Student 1 /
Grad. Student 3

## Tetsuya Iida

**Professor**

Dr. Iida graduated Faculty of Science, Kyoto University in 1984 and received his Ph.D. from Osaka university in 1991. He was appointed SA Professor at the International Research Center for Infectious Diseases in RIMD after working as a Research Associate for six years and an Associate Professor for seven years. He took his current position as a Professor of RIMD in 2015.

## Publication


(1) Export of a Vibrio parahaemolyticus toxin by the Sec and type III secretion machineries in tandem. Matsuda S. et al., ***Nat. Microbiol*** (2019) doi: 10.1038/s41564-019-0368-y. in press

(2) A repeat unit of *Vibrio* diarrheal T3S effector subverts cytoskeletal actin homeostasis via binding to interstrand region of actin filaments. Nishimura M., et al. ***Sci Rep.*** (2015) 5:10870.

(3) Interaction between the type III effector VopO and GEF-H1 activates the RhoA-ROCK pathway. Hiyoshi H., et al. ***PLoS Pathog.*** (2015) 11(3):e1004694.

(4) A cytotoxic type III secretion effector of *Vibrio parahaemolyticus* targets vacuolar $H^+$-ATPase subunit c and ruptures host cell lysosomes. Matsuda S., et al. ***PLoS Pathog.*** (2012);8(7):e1002803.

(5) VopV, an F-actin-binding type III secretion effector, is required for *Vibrio parahaemolyticus*-induced enterotoxicity. Hiyoshi H., et al. ***Cell Host Microbe.*** (2011) 10 (4):401-9. doi: 10.1016/j.chom.2011.08.014.

(6) Metagenomic diagnosis of bacterial infections. Nakamura S., et al. ***Emerg Infect Dis.*** (2008) 14(11):1784-6.




## ●Identifying the mechanism(s) underlying bacterial infection and pathogenesis

We performed whole genome sequencing of *Vibrio parahaemolyticus*, a bacterium that causes acute gastroenteritis in humans, and revealed that the type III secretion system T3SS2 is essential for pathogenicity. T3SS2 directly injects bacterial proteins (effectors) into target host cells. We demonstrated that injection of those effectors by T3SS2 from *V. parahaemolyticus* leads to inflammation of the intestinal mucosa and diarrhea. Currently, we are analyzing the molecular mechanism by which those effectors cause the symptoms of acute gastroenteritis.

Also, we revealed that expression of the genes encoding T3SS2 is induced by bile. In fact, chemical substances that adsorb and remove bile suppressed symptoms caused by *V. parahaemolyticus* in animal models, suggesting that these substances may be new therapeutic agents for *V. parahaemolyticus* infection. This is an example of “anti-virulence therapy” rather than antimicrobial therapy. This kind of approach is expected to provide novel therapeutic strategies for various bacterial infections.

Furthermore, based on findings obtained from our research on pathogenicity, we aim to explore the life cycle of bacterial pathogens in their natural environments.

## ●Development of methods to diagnose bacterial infections based on genomics and metagenomics

Emerging and re-emerging infectious diseases cause many problems worldwide. In many cases of such infection, the causative agent is unknown and/or the pathogenic mechanism is not yet clear. To identify the agents that cause such infections, and to understand the underlying pathogenesis, we are developing a high-throughput DNA sequencing-based system to detect pathogens and analyze their virulence traits.

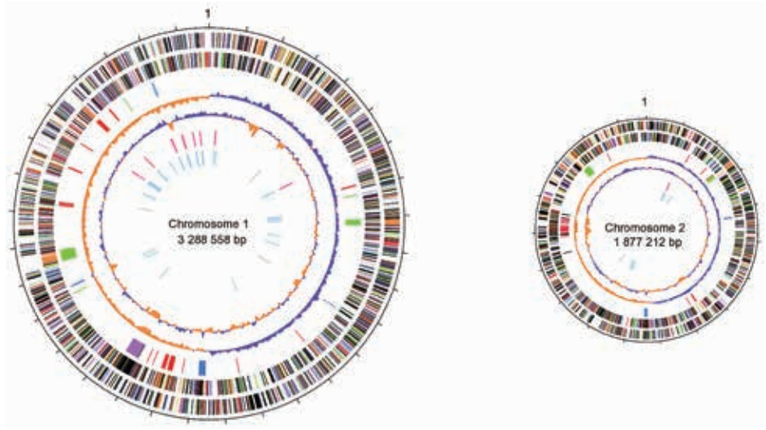



Fig. 1. The genomes of bacteria belonging to genus *Vibrio* comprise two distinct circular chromosomes.（Lancet, 2003）

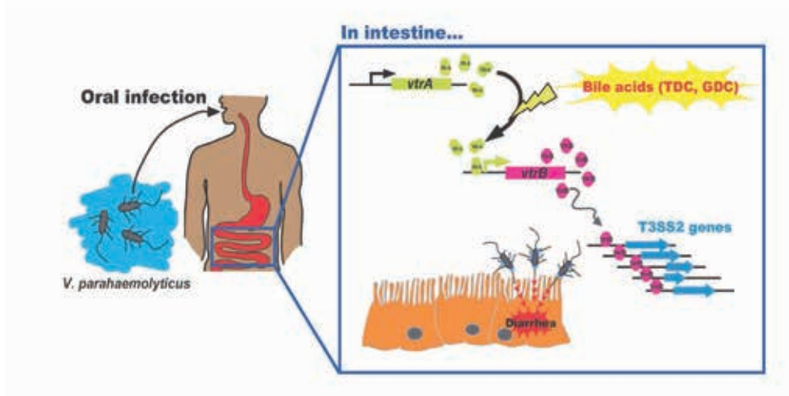



Fig. 2. Bile in the small intestine induces expression of genes encoding T3SS2, which is the major virulence factor produced by *Vibrio parahaemolyticus*, the causative agent of diarrhea.

Research Center for Infectious Disease Control

# Department of Virology

**Staff**

Assis. Prof. : Yuta Kanai ／
Postdoc. : Takahiro Kawagishi ／
Grad. Student 2

## Takeshi Kobayashi

**Associate Professor**

Dr. Kobayashi received his Ph.D. from Osaka University in 2000. He became a Research associate at RIMD in the same year. He spent for five years as a postdoctoral fellow in Vanderbilt University, USA before returning to Japan to work at the Institute for Virus Research in Kyoto University. He was appointed SA Associate Professor of RIMD in 2012. He is in his current position from 2016.

## Publication


(1) Development of stable rotavirus reporter expression systems. Kanai Y., et al. (2019) ***J. Virol***. 93:e01774-18

(2) Lethal murine infection model for human respiratory disease-associated Pteropine orthoreovirus. Kanai Y., et al., ***Virology*** (2018) 514:57-65.

(3) Entirely plasmid-based reverse genetics system for rotaviruses. Kanai Y., et al. (2017) ***Proc Natl Acad Sci U S A***. 114:2349-2354.

(4) Reverse genetics for fusogenic bat-borne orthoreovirus associated with acute respiratory tract Infections in humans: role of outer capsid protein sigmaC in viral replication and pathogenesis. Kawagishi T., et al. ***PLoS Pathog.*** (2016) 12:e1005455.


**1) Rotaviruses (RVs)**

RVs are highly important pathogens that cause severe diarrhea in infants and young children worldwide. Understanding of the molecular mechanisms underlying the replication and pathogenesis of RVs has been hampered by the lack of a reverse genetics system that allows the synthesis of recombinant viruses from artificial genes. Recently, we developed a long-awaited plasmid-based reverse genetics system for RVs. This technique opens up new horizons for studying the replication and pathogenesis of RVs. We are investigating RV biology and developing vaccines and therapeutics using a combination of genetic, biochemical, and biophysical approaches.

**2) Oncolytic viral therapy using reoviruses**

Mammalian orthoreoviruses (reoviruses) are members of the family *Reoviridae* and have a genome containing 10 segments of double-stranded (ds) RNA. Reoviruses are highly tractable experimental models for studying the replication and pathogenesis of dsRNA viruses. In the last decade, reoviruses have been evaluated as oncolytic agents against a variety of tumors, including head and neck, colon, breast, and pancreatic cancers, in animal models and humans. This is based on the observation that reoviruses induce cell death and apoptosis in tumor cells with an activated Ras signaling pathway. Wild-type reovirus-based oncolytic therapies are safe, but their efficacy is currently limited. We are developing safer and more effective reovirus-based cancer therapeutics by genetic modification.

**3) Highly pathogenic bat-borne reovirus**

Nelson Bay reovirus (NBV) was isolated from a flying fox in 1968 but had not been associated with any disease. However, NBVs were recently isolated from human patients suffering from acute respiratory tract infections in Malaysia, Indonesia, China, and Japan. These isolates have given rise to increasing concerns about bat-transmitted reovirus infections in humans. We are investigating how NBV replicates and causes disease *in vitro* and *in vivo*.

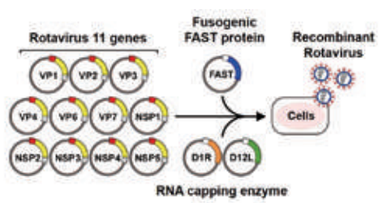



Fig. Generation of RVs from Cloned cDNAs



International Research Center for Infectious Diseases

# Laboratory of Clinical Research on Infectious Diseases

**Staff**

Grad. Student 1

## Wataru Kamitani

**SA Associate Professor**

Dr. Kamitani received his Ph.D. from Osaka University in 2003. After working at RIMD for one year, he spent the period from 2004 to 2009 as a postdoctoral fellow at the University of Texas Medical Branch at Galveston. He returned to the RIMD and became an Associate professor for Global COE program. He took his current position in 2013.

## Publication


(1) Middle East Respiratory Syndrome Coronavirus Spike Protein Is Not Activated Directly by Cellular Furin during Viral Entry into Target Cells. Matsuyama S., et al. ***J. Virol.*** (2018) 92(19). pii: e00683-18

(2) MERS coronavirus nsp1 participates in an efficient propagation through a specific interaction with viral RNA. Terada Y., et al. ***Virology.*** (2017) 511:95-105.

(3) Two-amino acids change in the nsp4 of SARS coronavirus abolishes viral replication. Sakai Y., et al. ***Virology.*** (2017) 510:165-174.

(4) Severe Acute Respiratory Syndrome Coronavirus nsp1 Facilitates Efficient Propagation in Cells through a Specific Translational Shutoff of Host mRNA. Tanaka T., et al. ***J. Virol.*** (2012) 86(20):11128-37


Coronaviruses infect many different animals, including human, and cause them to have respiratory and gastrointestinal diseases. Newly emerged Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) infect respiratory tract and cause severe pneumonia disease. Our research group focuses on these coronaviruses and studies the molecular biology and host cell-virus interaction of these coronaviruses.

SARS-CoV is the etiological agent of a newly-emerged human respiratory disease that originated in southern China in 2002 and spread worldwide in the 2003 epidemic. After 10 years of the epidemic of SARS-CoV, novel coronavirus, MERS-CoV, has been reported in Middle East region. MERS-CoV spreads to North America, Europe, China, and Korea. No effective treatment against MERS-CoV. Our research group studies about non-structural protein 1 (nsp1), that is one of pathogenicity factor in Coronavirus. Nsp1 of SARS-CoV induces host protein synthesis suppression through binding to 40S ribosome complex. The nsp1 enhance viral replication through binding to viral RNA. Our research group utilizes a Bacterial Artificial chromosome (BAC)-based revers genetics system for these coronaviruses. Our group try to understand the mechanism of Coronaviruses replication and pathogenesis for development therapeutic targets against Coronaviruses using the BAC-based revers genetics system.

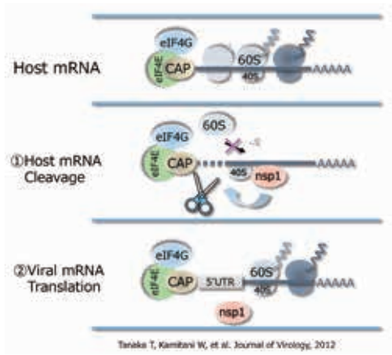



Fig. Gene expression control by SARS-CoV nsp1
①Nsp1 binds to 40S ribosome, and then induces translational shutoff. The nsp1-40S binding induces cleavage of mRNA.
②Nsp1 binds to viral mRNA, and then the interaction enhances viral replication.

International Research Center for Infectious Diseases

# Laboratory of Emerging Viral Diseases

Mammarenaviruses include highly pathogenic agents such as Lassa (West Africa) and Junin (South America) viruses, which cause viral hemorrhagic fever in humans and pose important public health problems within their regions of endemicity. In addition, the worldwide-distributed, prototypic mammarenavirus, lymphocytic choriomeningitis virus (LCMV) is a neglected human pathogen of clinical significance. Despite their substantial impact on human health, current therapeutic options for mammarenaviruses are very limited. Our research focuses on investigating the molecular and cellular biology of mammarenaviruses to facilitate the development of novel antivirals and vaccines.

## Masaharu Iwasaki

**SA Associate Professor**

Dr. Masaharu Iwasaki received his Ph.D. from Kyushu University for his work on measles virus RNA synthesis and virion assembly in 2010 and graduated from Kyushu University School of Medicine (MD-PhD program) in 2012. Thereafter, he worked as a Research Associate (2012), a Senior Research Associate (2015), and a Staff Scientist (2017) at The Scripps Research Institute, where he studied the molecular mechanisms underlying mammarenavirus multiplication. He was appointed to his current position in 2018.

## Publication


(1) Interactome analysis of the lymphocytic choriomeningitis virus nucleoprotein in infected cells reveals ATPase Na+/K+ transporting subunit Alpha 1 and prohibitin as host-cell factors involved in the life cycle of mammarenaviruses. Iwasaki M. et al., ***PLoS Pathog.*** (2018) 20;14(2):e1006892.

(2) The High Degree of Sequence Plasticity of the Arenavirus Noncoding Intergenic Region (IGR) Enables the Use of a Nonviral Universal Synthetic IGR To Attenuate Arenaviruses. Iwasaki M. et al., ***J Virol.*** (2016) 90(6):3187-97.

(3) General Molecular Strategy for Development of Arenavirus Live-Attenuated Vaccines. Iwasaki M. et al., ***J Virol.*** (2015) 89 (23):12166-77.

(4) Sodium Hydrogen Exchangers Contribute to Arenavirus Cell Entry. Iwasaki M. et al., ***J Virol.*** (2014) 88(1):643-54.


Mammarenaviruses are simple enveloped viruses with a bi-segmented ambisense RNA genome encoding four genes (Figure A). Each RNA segment, small (S) and large (L), directs the synthesis of two viral proteins from two open reading frames, which are separated by a non-coding intergenic region (IGR). Despite this simple genome organization, we know very little about the mechanisms by which these viruses multiply and cause disease in infected hosts. To better understand the complex biology of mammarenaviruses and to develop antiviral strategies that can combat these viruses, we use reverse genetics systems to generate recombinant mammarenaviruses, which contain pre-determined mutations and/or additional foreign genes such as enhanced green fluorescent protein (eGFP), from cloned cDNAs. We used these technologies to generate a recombinant LCMV harboring a synthetic LCMV S-IGR-like IGR instead of the L-IGR [rLCMV(IGR/S-Ssyn)] (Figure B). rLCMV(IGR/S-Ssyn) was severely attenuated *in vivo* but elicited protective immunity against a lethal challenge with wild-type LCMV. This strategy can be used to generate live-attenuated vaccines for currently known and potentially newly emerging hemorrhagic fever-causing mammarenaviruses without the need to incorporate amino acid changes. In addition, we generated recombinant LCMVs expressing eGFP or an affinity-tagged viral protein to facilitate genetic and pharmacological screenings and proteomic analyses with the aim of identifying virus-host interactions required for efficient multiplication of mammarenaviruses that can be exploited as druggable targets.

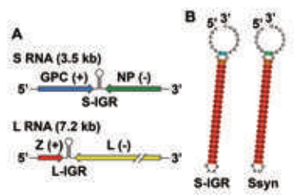



Fig. (A) Schematic diagram of the genome organization of mammarenaviruses. (B) Predicted RNA secondary structures of the LCMV S-IGR (left) and the synthetic LCMV S-IGR-like IGR (right).



International Research Center for Infectious Diseases

# Pathogenic Microbes Repository Unit

The facility is part of the National BioResource Project (NBRP) directed by the Ministry of Education, Culture, Sports, Science and Technology, Japan, and is a member of the Japan Society for Culture Collections (JSCC); therefore, we collect and preserve pathogenic bacterial strains. These strains are distributed to investigators in and outside this country upon request. Our collection is listed on the website for this facility (http://rceid.biken.osaka-u.ac.jp) and that of the NBRP.

## Staff

Head (concur.): Tetsuya Iida, Ph.D.
Associate Professor (concur.): Toshio Kodama, Ph.D.

## Collection list:

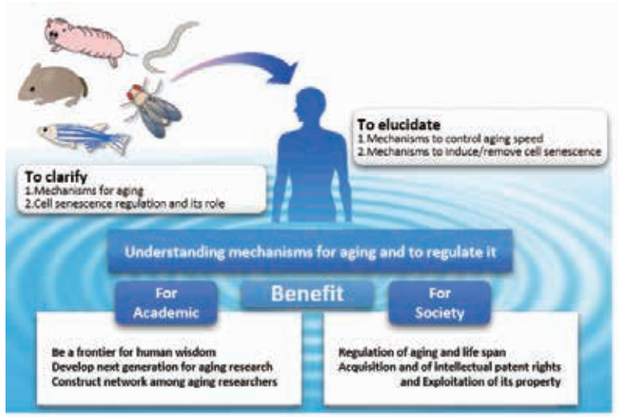
http://rceid.biken.osaka-u.ac.jp

# Research Center for Mechanism and Regulation of Aging

## Staff

Director : Eiji Hara

The life expectancy of humans has increased markedly over recent decades. Ironically, this has resulted in a startling rise in the incidence of aging-associated diseases, resulting in serious social problems such as increased medical expenses and nursing care costs. To solve these problems, the Research Center for Mechanism and Regulation of Aging was established in 2017. The center aims to clarify the fundamental mechanisms that regulate aging and to understand the role of cellular senescence in aging and aging-associated diseases.




■ Division of Aging Model Organism

| | |
|---|---|
| Department of Aging Rate Biology | Head: Invited Professor Eisuke Nishida<br>RIKEN Center for Biosystems Dynamics Research |
| Department of Research of Signals Regulating Aging | Head: Invited Professor Naoki Hisamoto<br>Group of Signaling Mechanisms,<br>Graduate School of Science, Nagoya University |
| Department of Genetics and Metabolism | Head: Invited Professor Masayuki Miura<br>Dept. of Genetics, Graduate School of Pharmaceutical Sciences, University of Tokyo |
| Department of Cell-cell Communication | Head: Invited Professor Tatsushi Igaki<br>Lab of Genetics, Graduate School of Biostudies, Kyoto University |
| Department of Organismal Aging Research | Head: Cocurrent Professor Tohru Ishitani<br>Dept. of Homeostatic regulation, Research Institute for Mcrobial Diseases, Osaka University |
| Department of Autophagy and Aging | Head: Concurrent Professor Tamotsu Yoshimori<br>Lab of Intracellular Membrane Dynamics, Graduate School of Frontier Biosciences, Osaka University |
| Department of Sleep and Aging Regulation | Head: Invited Researcher Akiko Sato<br>National Center for Geriatrics and Gerontology |
| Department of Reproductive Aging | Head: Concurrent Professor Masahito Ikawa<br>Dept. of Experimental Research, Research Institute for Microbial Diseases, Osaka University |
| Department of Animal Longevity and Aging Research | Head: Invited Associate Professor Kyoko Miura<br>Laboratory for Molecular Biology of Aging and Longevity, Faculty of Life Sciences, Kumamoto University |

■ Division of Cellular Senescence

| | |
|---|---|
| Department of Cell Senescence Mechanism | Head: Concurrent Professor Eiji Hara<br>Dept. of Molecular Microbiology, Research Institute for Microbial Diseases, Osaka University |
| Department of Aging-associated Stress Signaling | Head: Invited Professor Hidenori Ichijo<br>Lab of Cell Signaling, Graduate School of Pharmaceutical Sciences, The University of Tokyo |
| Department of Senescent Cell Morphology & Motility | Head: Invited Professor: Yasuhiro Minami<br>Dept. of Physiology and Cell Biology,<br>Graduate School of Medicine, Kobe University |
| Department of Senescence Regulation | Head: Invited Professor Nakanishi Makoto<br>Div. of Cancer Cell Biology, The Insitute of Medical Science, The University of Tokyo |
| Department of Transposon-Mediated Processes | Head: Invited Professor Haruhiko Shiomi<br>Dept. of Molecular Biology,<br>Keio University School of Medicine |
| Department of Senescent Metabolism | Head: Invited Researcher Tomonori Kimura<br>KAGAMI Project, National Institutes of Biomedical Innovation, Health and Nutrition |
| Department of Immune Aging | Head: Invited Professor Yoko Hamazaki<br>Dept. of Life Science Frontiers, Center for iPS Cell Research and Application, Kyoto University |
| Department of Brain Aging | Head: Invited Researcher Kiyohito Mizutani<br>Div. of Pathogenic Signaling, Graduate School of Medicine, Kobe University |



# Germ Cell Group

## Masami Nozaki

**Associate Professor**

Dr.Nozaki received his Ph.D. from Osaka University in 1986. He took his current position at RIMD in 2007 after working as a Research Associate and an Associate Professor in the same institution.

## Publication


(1) LATS1/2 kinases trigger self-renewal of cancer stem cells in aggressive oral cancer. Nozaki M. et al. ***Oncotarget*** (2019) 10 (10): 1014-30.

(2) Migration of oral squamous cell carcinoma cells are induced by HGF/c-Met signalling via lamellipodia and filopodia formation. Yasui H. et al. ***Oncol Rep*** (2017) 37 (6): 3674-80

(3) Interaction between basigin and monocarboxylate transporter 2 in the mouse testes and spermatozoa. Chen C., et al. ***Asian J Androl*** (2016) 18 (4):600-6.

(4) Isolation and propagation of neural crest stem cells from mouse embryonic stem cells via cranial neurospheres. Minamino Y., et al. ***Stem Cells Dev*** (2015) 24 (2):172-81.


Our body is derived from a single fertilized egg. During development, germ cells (and sometimes cancer cells), as well as many types of tissues, are generated. We in the germ cell group have been conducting research focusing on germ cells, the early embryo, stem cells, and cancer cells. Currently, we study osteogenesis from pluripotent stem cells and are characterizing cancer stem cells.

Neural crest cells are derived from neuroepithelium via epithelial-mesenchymal transition (EMT) at the early developmental stage. These cells migrate extensively and differentiate into various tissues in the whole embryo. Bone is generated from mesoderm; however, most of the skull is derived from neural crest cells. Recently, we established a method of differentiating ES cells into maxillofacial bone via neural crest cells. We are conducting research aimed at applying our findings to regenerative medicine using iPS cells, adult tissue stem cells, and ES cells.

EMT is a phenomenon also observed in malignancy. Epithelial cell-derived cancer cells acquire migration and invasive potency after EMT, leading to metastasis. Some malignant cancer cells have stem cell-like properties. Differences in the characteristics of cancer stem cells and other cancer cells make the disease difficult to treat. Thus, our aim is to understand the characteristics of cancer stem cells and establish new treatment strategies.

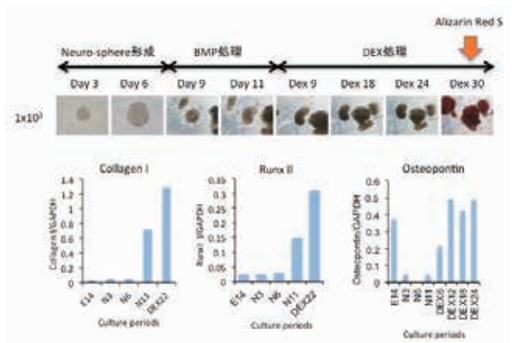



Fig. Neuro-spheres derived from ES cells are induced to undergo osteogenesis by treatment with BMP4 and DEX. Calcium deposition is shown by red-staining of alizarin, and upregulation of osteogenic marker genes during osteogenesis is detected by semi-quantitative RT-PCR.

# Yabumoto Department of Intractable Disease Research

Glycosylphosphatidylinositol (GPI) is a glycolipid attached to proteins and anchors them onto the plasma membrane. GPI-anchored protein has various and important physiological functions in our body. Why proteins have this peculiar structure like GPI? Our research goal is to elucidate biogenesis, transport and remodeling of GPI-anchored proteins and understand its physiological significance in our body.

**Staff**

Undergrad. Student 1 ／
Grad. Student 1

## Taroh Kinoshita

**Endowed Chair Professor**

Dr. Kinoshita received Ph.D. from Osaka University in 1981. After working at Department of Bacteriology, Osaka University Medical School and Department of Pathology, New York University School of Medicine, he appointed as a Professor in RIMD in 1990. He served as a Director of RIMD from 2003 to 2007. He concurrently serves as a Professor in Osaka University Immunology Frontier Research Center from 2007. From 2017, he is in the current position.2017 Takeda Medical Prize, 2018 Medalwith Purple ribbon

## Yoshiko Murakami

**Endowed Chair Professor**

Dr. Murakami received Ph.D. from Osaka University in 2001. After working at Osaka University Hospital and Hyogo Prefectural Nishinomiya Hospital, she joined Dept. of Immunoregulation at RIMD in 1998. She became an Associate professor for Office of Combined Program on Microbiology and Immunology (concurrently serve for Dept. of Immunoregulation and Immunoglycobiology in IFReC) in 2009. She is in the current position from 2017.

## Publication


(1) Identification of a Golgi GPI-N-acetylgalactosamine transferase with tandem transmembrane regions in the catalytic domain. Hirata, T., et al. ***Nat. Commun.*** (2018) 9:405.

(2) N-Glycan dependent protein folding and endoplasmic reticulum retention regulate GPI-anchor processing. Liu, Y.-S., et al. ***J. Cell Biol.*** (2017) 217: 585-599.

(3) Phenotype-genotype correlations of PIGO deficiency with variable phenotypes from infantile lethality to mild learning difficulties. Tanigawa, J., et al. ***Hum. Mutat.*** (2017) 38:805-815.

(4) A GPI processing phospholipase A2, PGAP6, modulates Nodal signaling in embryos by shedding CRIPTO. Lee, G-H., et al. ***J. Cell Biol***. (2016) 215:705-718.

(5) Pathogenic variants in PIGG cause intellectual disability with seizures and hypotonia. Makrythanasis, P., et al. ***Am. J. Hum. Genet.*** (2016) 98:615-626.

(6) Post-Golgi anterograde transport requires GARP-dependent endosome-to-TGN retrograde transport. Hirata, T., et al. ***Mol. Biol. Cell*** (2015) 26:3071-3084.



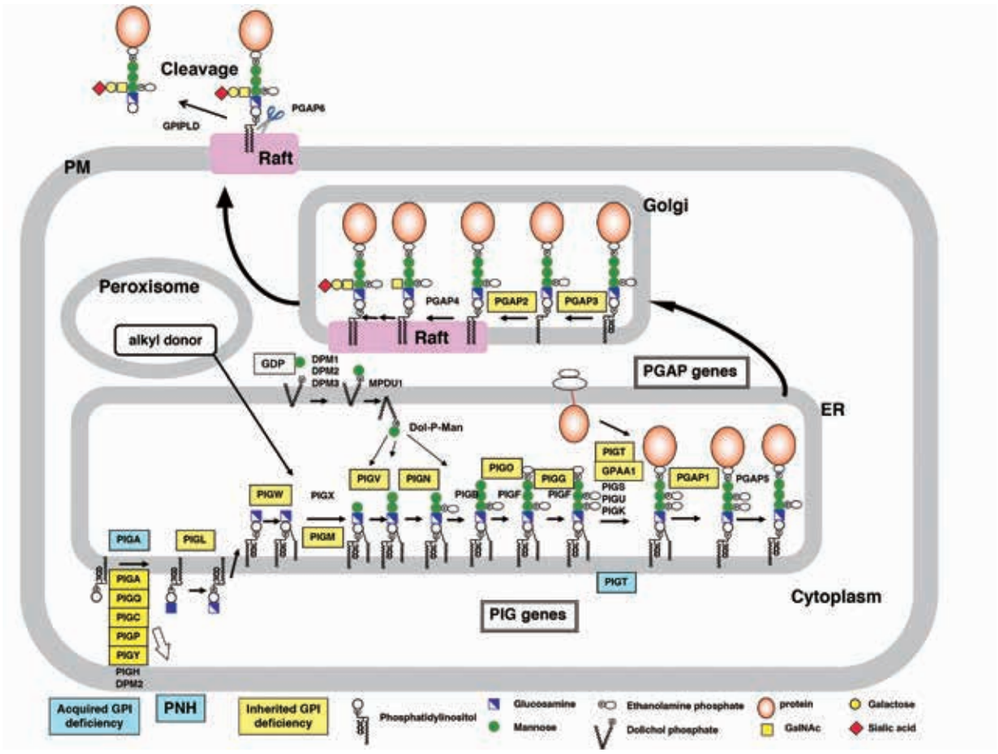

### ●How are GPI-anchored proteins (GPI-APs) regulated?

GPI anchors are synthesized in the endoplasmic reticulum and attached to the C terminus of proteins during posttranslational modification. GPI-anchored proteins are transported from the endoplasmic reticulum to the Golgi and further to the cell surface in a way that is regulated according to the features of GPI. Recently, we identified the enzyme that can cut GPI-anchors, and showed GPI-APs can be secreted and work in the tissues distant from its origin. This result indicates that GPI anchors enable our body system to regulate where and when the protein works in a various way. We are currently studying the molecular mechanism to control the functions of GPI-APs. In addition, GPI anchor has specific carbohydrate side-chains and intriguingly, the chain varies among cells and proteins. We are interested in the physiological significance of this carbohydrate chain and asking how this chain is synthesized in our cells.

### ●Molecular mechanisms of GPI deficiencies.

We found that paroxysmal nocturnal hemoglobinuria (PNH) is caused by somatic mutation of the X-linked PIGA gene, a gene for GPI-anchor biosynthesis. A recent report showed that atypical PNH is caused by somatic mutation of one allele of the PIGT gene, a gene for GPI-anchor attachment, in combination with a germline mutation in the other allele. Now, we are studying how unused GPI-anchor is involved in pathogenesis of atypical PNH and try to find a cure for this disease.

We also identified a disease called inherited GPI deficiency (IGD) caused by the mutation of the GPI-anchor synthesizing enzyme, PIGM. The recent whole exome sequencing analysis using the next generation sequencer revealed 16 GPI-related gene mutations responsible for IGD. To elucidate the molecular mechanisms of the pathogenesis of this disease, we developed the system to analyze GPI biosynthesis and modification. This system contributes to the IGD research in all over the world. Our aim is to elucidate how GPI-anchors are involved in IGD and find the way to overcome this disease.




GPI-anchor biosynthesis and the transport/remodeling of GPI-APs.

Endwed Chair

# Department of Malaria Vaccine Development

Malaria is widespread in tropical and subtropical regions, and millions of people, particularly in Africa, remain at risk of disease and death despite substantial progress in malaria control. No effective malaria vaccine has been developed or licensed. Our laboratory is undertaking basic research and conducting clinical trials on our own candidate vaccine antigen gene.

**Staff**

Prof. : Nirianne Marie Querijero Palacpac

## Toshihiro Horii

**Professor**

Dr. Horii received his Ph.D. from Osaka University in 1981. After working at the Faculty of Science in Osaka University as Research Associate, he was appointed Associate Professor at RIMD in 1991 and promoted Professor in 1999. He moved to the current department in 2019.

## Publication


(1) Molecular Camouflage of *Plasmodium falciparum* Merozoites by Binding of Host Vitronectin to P47 Fragment of SERA5. Tougan T., et al. ***Sci Rep.*** (2018) 8:5052. doi: (5)10.1038/s41598-018-23194-9.

(2) Antibody titres and boosting after natural malaria infection in BK-SE36 vaccine responders during a follow-up study in Uganda. Yagi M., et al. ***Sci Rep.*** (2016) 6:34363. doi: 10.1038/srep34363.

(3) Protective Epitopes of the *Plasmodium falciparum* SERA5 Malaria Vaccine Reside in Intrinsically Unstructured N-Terminal Repetitive Sequences.Yagi M., et al. ***PLoS One.*** (2014) 9:e98460. doi: 10.1371/journal.pone.0098460.

(4) Phase 1b randomized trial and follow-up study in Uganda of the blood-stage malaria vaccine candidate BK-SE36. Palacpac N.M.Q., et al., ***PLoS ONE.*** (2013) 8: e64073. doi:10.1371/journal.pone.0064073

(5) *Plasmodium falciparum* serine repeat antigen 5 (SE36) as a malaria vaccine candidate. Palacpac N.M., et al., ***Vaccine.*** (2011) 29:5837-45. doi: 10.1016/j.vaccine.2011.06.052.

(6) Evidences of Protection Against Blood-stage Infection of *Plasmodium falciparum* by the Novel Protein Vaccine SE36. Horii T., et al., ***Parasitol. Int.*** (2010) 59:380-6. doi: 10.1016/j.parint.2010.05.002.




## ●Malaria vaccine targeting SERA5

The treatment of malaria patients is completely dependent on the efficacy of anti-malaria drugs; however, drug-resistant parasites are emerging. Although a malaria vaccine is the ideal weapon against this pathogen, vaccine development is hampered by genetic polymorphisms in candidate antigen genes.

We have been focusing on the SERA5 molecule of *P. falciparum* and developed malaria vaccine NPC-SE36 by utilizing a recombinant SE36 protein. SE36 is a protein that is highly expressed/produced in large amounts during parasite growth in red blood cells. Epidemiological studies in malaria hyper-endemic areas showed that children with antibodies against SERA5 experienced few or no symptomatic/clinical malaria, albeit such children are a minority.

It was surprising that Ugandan adults that suffered numerous malaria infections did not respond to vaccination with BK-SE36. By contrast, malaria-naïve Japanese adults produced high levels of antibodies. Moreover, in young Ugandan children that experienced few malaria episode, we observed good antibody response. We obtained 72% protective efficacy 1 year post-2nd-vaccination in a follow-up study of 6-20 years old in the phase Ib trial. This suggests that our vaccine provides better protection in younger individuals. We have conducted Phase Ib clinical trial of NPC-SE36 in Burkina Faso in west Africa in 2015-2017. Vaccine was well tolerated, and it was found that the immune response in 1 year infants group was much higher than 2-5 years child group. Currently we are conducting Phase Ib clinical trial of NPC-SE36 with CpG adjuvant that stimulates innate immunity.

## ●Molecular strategy for malaria parasite survival and a function of SE36 protein

The malaria parasite develops highly sophisticated strategies to evade the human immune system. One of the most difficult phenomena encountered by those developing vaccines is genetic polymorphism of vaccine candidate genes; that is, field-isolated parasites harbor different sequences from the vaccine candidate genes. Fortunately, SERA5 is highly homologous among malaria parasites worldwide. We have analyzed protective epitopes on SE36 protein. Recently we have shown that SE36 protein tightly binds to host vitronectin as cytoadherence molecule on the surface of parasite cell, merozoite, and vitronectin further binds to over 30 different host proteins for molecular camouflage from host immune system.


Fig. 1. Clinical trial of the NPC-SE36 malaria vaccine.
The vaccine was produced under GMP (Good Manufacturing Practice) conditions at the Kanonji Institute of The Research Foundation for Microbial Diseases of Osaka University.
(NPC-SE36 malaria vaccine was previously called BK-SE36 malaria vaccine.)

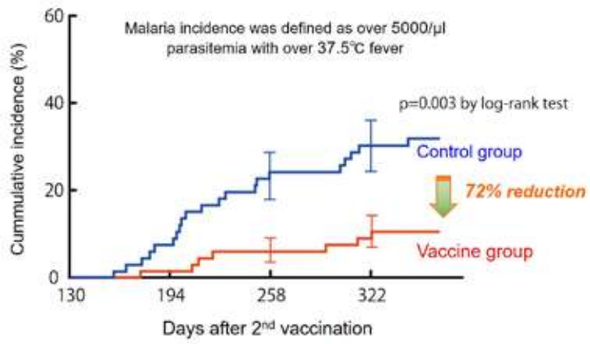



Fig. 2. Protective efficacy of NPC-SE36 malaria vaccine
Palacpac et al., ***Plos ONE.*** 2013; 8(5): e64073

# Thailand-Japan Research Collaboration Center

Until recently, it was believed that infectious diseases could be conquered by developing chemotherapy regimens and vaccines; however, the recent worldwide emergence of new infectious diseases and the re-emergence of infectious diseases once considered to be under control have seriously challenged this notion. Since many infectious diseases spread rapidly across national borders, it is clear that they cannot be controlled by the efforts of individual countries.

To overcome this, Osaka University founded the Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI) at the Thai National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health of Thailand, in 2005. The program is now in the third phase (2015–2020) and is sponsored by the Japan Agency for Medical Research and Development, which succeeded to the second phase program named “the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID).”

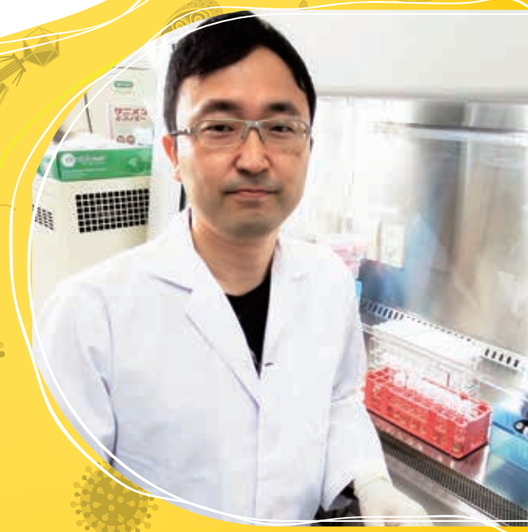
In addition to basic and applied research into emerging and re-emerging infections, we aim to develop human resources. We also aim to establish an effective system that would (i) provide information that would help prevent the spread of emerging and re-emerging infections, and (ii) promptly activate a variety of countermeasures should such a disease emerge, including the development of therapeutics and/or vaccines. Finally, we wish to enter into collaboration with laboratories from nations that neighbor Thailand so that we can be on the “frontline,” with the capacity to respond quickly to the global spread of infectious disease.

The collaboration center is located in the campus of the Ministry of Public Health.

BSL-2 and BLS-3 laboratories in the center.

Thailand-Japan Research Collaboration Center

## Section of Bacterial Infections



**Tetsuya Iida** (concur.)

Professor

**Kazuhisa Okada**

SA Associate Professor

Dr. Okada received his Ph.D. from Osaka university in 2005 and joined RIMD as a postdoctoral fellow in that same year. He was appointed Lecturer of Thailand-Japan Research Collaboration Center on Emerging and Re-emerging infections in 2015 after working as a Postdoctoral fellow from 2005 to 2011 and an Asistant Professor from 2011 to 2015 in the same institution.

## Publication


(1) Simultaneous detection and quantification of 19 diarrhea-related pathogens with a quantitative real-time PCR panel assay. Wongboot W. et al., ***J Microbiol Methods.*** (2018) 151:76-82

(2) Vibrio cholerae embraces two major evolutionary traits as revealed by targeted gene sequencing. Okada K., et al. ***Sci. Rep.*** (2018) 8(1):1631

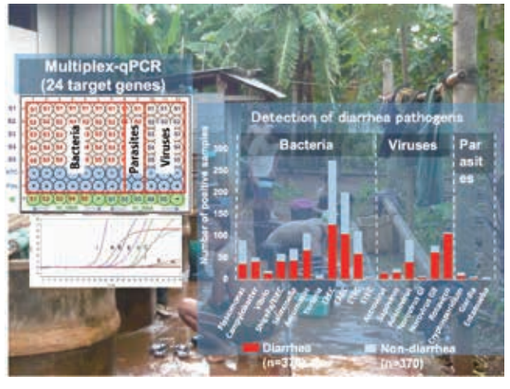
(3) Characterization of 3 Megabase-Sized Circular Replicons from *Vibrio cholerae.* Okada K., et al. ***Emerg Infect Dis.*** (2015) 21(7):1262-3.

(4) Cholera in Yangon, Myanmar, 2012-2013. Aung WW., et al. ***Emerg Infect Dis.*** (2015) 21(3):543-4.

(5) *Vibrio cholerae* O1 isolate with novel genetic background, Thailand–Myanmar. Okada K., et al. ***Emerg Infect Dis.*** (2013) 19:1015-7.


Enteric infectious diseases caused by various microbes occur frequently in Thailand. However, there are no large-scale epidemiological studies of the etiology of gastroenteritis in Thailand. In the Section of Bacterial Infections, we are trying to develop effective diagnostic tools to detect bacterial pathogens and devise measures to prevent enteric infections, including those mediated by Vibrio cholerae O1.

We collect and analyze fecal specimens from patients with severe diarrhea admitted to leading hospitals located in different parts of Thailand. We then try to detect specific pathogens by real-time PCR analysis of bacterial genes and culture analysis. We also attempt to identify unknown pathogens using TOF-MS or NGS. In cases of cholera in Thailand or Myanmar, we will co-operate with governmental authorities to analyze *V. cholerae* isolates to elucidate the route of transmission. We will also undertake molecular and/or genomic characterization of any identified pathogen. Our research goals are to develop methods for rapid diagnosis and to prepare countermeasures against what is still a challenging pathogen.




Detection of "pathogenic" agents from stool specimens of inpatients with acute diarrhea and control subjects using multiplex real-time PCR. Eight hospitals in different parts of Thailand participate in this study.

Thailand-Japan Research Collaboration Center

# Section of Viral Infections

## Staff

SA Assoc. Prof. : Atsushi Tanaka ／
SA Assoc. Prof. : Hiroto Mizushima

## Masashi Tatsumi

**SA Professor**

Dr. Tatsumi received his Ph.D. from The University of Tokyo. After working at National Institute of Health in Japan, Pasteur Institute and INSERM (French National Institute of Health and Medical Research) in France, he spent 12 years in National Institute of Infectious Diseases. After retirement, he served as a chief advisor for JICA until taking his current position at RIMD in 2016.

## Publication

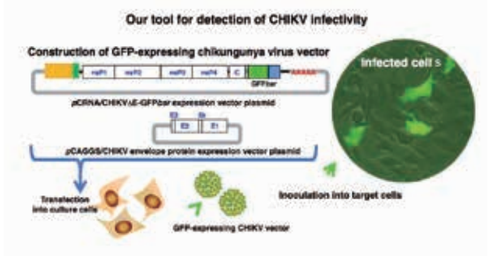

(1) Genome-Wide Screening Uncovers the Significance of N-sulfation of Heparan Sulfate as a Host Cell Factor for Chikungunya Virus Infection. Tanaka A. et al ***J.Virol.*** 2017, 91 e00432-17

(2) Distribution of norovirus genotypes and subtypes in river water by ultra-deep sequencing-based analysis. Boonchan M et al. ***Lett Appl Microbiol.*** 2017 65:98-104.

(3) Evolutionary constraints on the norovirus pandemic variant GII.4_2006b over the five-year persistence in Japan. Sato H et al ***Frontiers in Microbiology*** 2017 8:410


A variety of arboviruses are prevalent in Thailand located in the tropics, of which we investigate chikungunya fever from an epidemiological, molecular biological, and immunological points of view. We focus on exploring the cellular factors necessary for viral replication with the experimental system of knock-out and -in cell libraries of susceptible cells, and chikungunya pseudovirus. In addition, we try to isolate virus from clinical samples and establish the reverse genetics system to clarify the characteristics of zika virus, another arbovirus which has been endemic long-termed in Thailand.

Another target is norovirus, a major cause of both sporadic cases and outbreaks of nonbacterial acute gastroenteritis in all age groups worldwide every year. We investigate whether or not it is possible to predict the genotypes of norovirus which are involved in new epidemics by evolutionary phylogenic analysis with special reference to genotyping of epidemic strains. In addition, recent epidemiological analysis has revealed that "asymptomatic persons" who do not develop acute gastroenteritis even when infected with norovirus, might play a significant role as reservoirs in new outbreaks. Noroviruses highly evolve with diversification of their genome through mutation and recombination, which allows them to protect themselves from human host immunity and sustain their transmission in human communities. We try to clarify the retention and transmission of norovirus in asymptomatic persons, especially involvement in genome diversification to elucidate the actual condition of asymptomatic carriers. On the other hand, we try to explore cellular factors necessary for virus propagation in cells to establish a culture system of norovirus, which remains still difficult to grow in vitro yet.






Thailand-Japan Research Collaboration Center

# Section of Bacterial Drug Resistance Research

## Staff

Head, Prof. (concur.) : Tetsuya Iida ／
Assoc. Prof. : Yukihiro Akeda (concur.) ／
SA Asst. Prof. : Yo Sugahara ／
SA Researcher : Noriko Sakamoto

## Shigeyuki Hamada

**Guest Professor**

Dr. Hamada received D.D.S. and Ph.D. degrees from Osaka university in 1967 and 1971, respectively. He became the Director of Dental Research, National Institute of Health in 1980. Then he served as Professor of Microbiology in Osaka University School of Dentistry from 1986 to 2005 and Professor of Nihon University from 2005 to 2009. He joined RIMD as SA professor in 2009 and later as guest professor.

## Publication


(1) Spreading patterns of NDM-producing Enterobacteriaceae in clinical settings in Yangon, Myanmar. Sugawara Y. et al. ***Antimicrob Agents Chemother*** (2019) 63(3): e01924-18.

(2) PCR-dipstick-oriented surveillance and characterization of mcr-1- and carbapenemase-carrying Enterobacteriaceae in a Thai hospital. Shanmugakani R.K. et al. ***Front Microbiol*** (2019) 10:149.

(3) Genomic characterization of carbapenemase-producing Klebsiella pneumoniae with chromosamally encoded bla NDM-1. Sakamoto N. et al. ***Antimicrob Agents Chemother*** (2018) 62(12): e01520-18.

(4) Establishment of a dual-wavelength spectrophotometric method for analyzing and detecting carbapenemase-producing Enterobacteriaceae. Takeuchi D. et al. ***Sci Rep*** (2018) 8:15689.

(5) PCR-dipstik chromatography for differential detection of carbapenemase genes directly in stool specimens. Shanmugakani R.K. et al. ***Antimicr Agents Chemother*** (2017) 61(6): e00067-17.


Carbapenem-resistant Enterobacteriaceae (CRE), including *Klebsiella pneumoniae* and *Escherichia coli*, are highly resistant to carbapenems and many other antibiotics. The rapidly increasing prevalence of CRE over the past decade has increased concern in healthcare facilities and public health communities worldwide. Japan is no exception, even though the prevalence of CRE at this time remains low. Our aim is to examine the epidemiological dissemination of CRE in the Southeast Asian countries. Carbapenem resistance is usually carried by plasmid(s) that harbor genes encoding carbapenemases, i.e., class A KPCs, class B metallo-β-lactamases (including IMP, VIM, or NDMs), or class D OXA-type enzymes. We have attempted to isolate CRE from patients admitted to leading hospitals in Thailand and Myanmar. CRE isolates are identified by biochemical characterization or MALDI-TOF-MS, followed by profiling using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). We then determine the whole genome sequence of CRE isolates to identify the full plasmid and construct a comprehensive image of the relationships between isolates based on MLST and phylogeny. By undertaking these genomic epidemiological studies, we increase our understanding of how CRE spread and may be able to identify potential reservoirs.

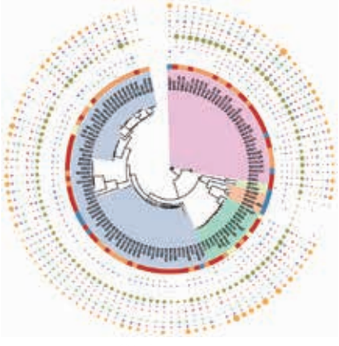


Figure. A whole genome SNP-based phylogenetic tree of CRE isolates from Myanmar. The inner colored regions define bacterial species. Next outer colored regions denote the origins of isolates. Colors and sizes of outer dots denote classes and numbers of antimicrobial resistance genes harbored by each isolate.

Thailand-Japan Research Collaboration Center

# Section of Antiviral Research

## Staff

SA Asst. Prof. : Akatsuki Saito (concur.)

## Tatsuo Shioda (concur.)

**Professor**

Dr. Shioda obtaind his B. Sc. from the University of Tokyo in 1982 and his Ph.D. form the same institution in 1990. After working at the Institute of Medical Science in the University of Tokyo and University of California San Francisco, he was appointed as Associate Professor in 1997. He took his current position as a Professor of RIMD in 2000.

## Publication

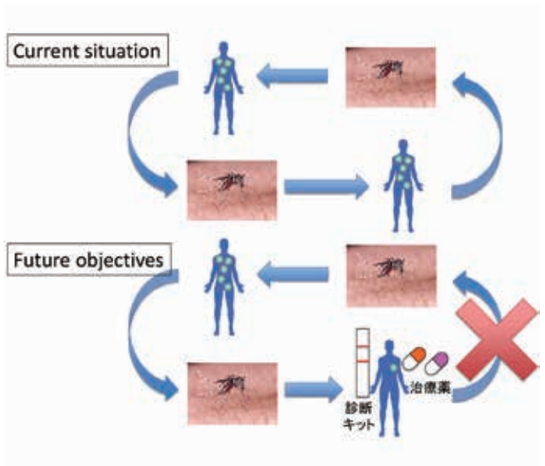

(1) Capsid-CPSF6 Interaction Is Dispensable for HIV-1 Replication in Primary Cells but Is Selected during Virus Passage In Vivo. Saito A., ***J Virol.*** (2016) 11;90(15):6918-35.

(2) Roles of Capsid-Interacting Host Factors in Multimodal Inhibition of HIV-1 by PF74. Saito A., ***J Virol.*** (2016) 27;90 (12):5808-23

(3) Sequence diversity of dengue virus type 2 in brain and thymus of infected interferon receptor ko mice: implications for dengue virulence. Dhole P., et al., ***Virol J.*** (2016) 13(1):199.


Recently, climate change and urbanization have increased the risk of vector-borne diseases. Dengue and chikungunya viruses cause dengue fever/hemorrhagic fever and chikungunya fever, respectively. Both viruses are transmitted by *Aedes* mosquitoes. In 2014, there was an outbreak of dengue fever in Tokyo. However, no antiviral drugs are available to treat these infections. Our aim is to develop antiviral drugs against dengue and chikungunya viruses.

Four serotypes of dengue virus are distributed across the world, and re-infection with different serotypes of dengue virus leads to a more severe infection. We aim to develop novel diagnostic tools that can differentiate between the four serotypes of dengue virus.





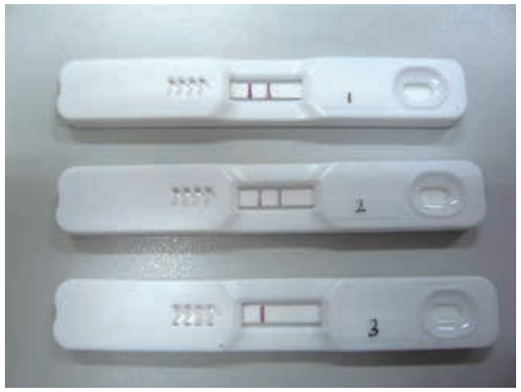

Thailand-Japan Research Collaboration Center

# Mahidol-Osaka Center for Infectious Diseases

The Mahidol-Osaka Center for Infectious Diseases (MOCID) focuses on several tropical infectious diseases that are of importance to human health in Thailand. Mosquito-borne viral infectious diseases such as dengue fever/dengue hemorrhagic fever and chikungunya fever are of particular interest. We are currently developing rapid diagnosis kits and are examining factors that affect disease severity. We would like to improve the research skills of young scientists and increase their interest in infectious diseases by collaborating with Mahidol University, which provides clinical samples.

## Staff

Director of MOCID (concur.) : Prof. Tatsuo Shioda
SA Asst. Prof. : Atsushi Yamanaka



Diagnostic kit developed by the MOCID.

## Publication


(1) Variation at position 350 in the Chikungunya virus 6K-E1 protein determines the sensitivity of detection in a rapid E1-antigen test. Aekkachai T. et al., ***Sci. Rep.*** (2018) 8:1094

(2) Diagnostic accuracy of a rapid E1-antigen test for chikungunya virus infection in a reference setting. Huits R., et al., ***Clinical Microbiology and Infection*** 24 (2018) 78-81.

(3) Circulation of HIV-1 Multiple Complexity Recombinant Forms Among Female Sex Workers Recently Infected with HIV-1 in Thailand. Saeng-Aroon S., et al., ***AIDS Res Hum Retroviruses.*** (2016) 32(7):694-701.

(4) Detection of chikungunya virus antigen by a novel rapid immunochromatographic test. Okabayashi T., et al. ***Clin Microbiol.*** (2015) 53(2):382-8.


Evaluation of CHIKV detection kit at Safdarjung Hospiral, Dehli, India

Evaluation of CHIKV detection kit at the Institute of Tropical Medicine Antwerp, Belgium

# BIKEN Innovative Vaccine Research Alliance Laboratories

As the recent Ebola virus outbreak in Africa and the worldwide influenza pandemic have powerfully demonstrated, society demands the development, production, and distribution of vaccines against infectious diseases. To meet this strong demand, The Research Foundation for Microbial Diseases of Osaka University (BIKEN) and the Research Institute for Microbial Disease, Osaka University (RIMD), have established a new research organization, called The BIKEN Innovative Vaccine Research Alliance Laboratories, to promote the co-operative development of vaccines. Here, we are developing new technologies to acquire basic information required to design next-generation vaccines.

## Staff

Director, SA Professor
**Koichi Yamanishi**

A regular meeting between laboratories.
All members for this research project attend the meeting. Researchers address their research topics in depth and take part in active discussions.

Experimental laboratories.
Researchers can move freely among three laboratories. All three laboratories have common equipment.



BIKEN Innovative Vaccine Research Alliance Laboratories

# Vaccine Creation Project

## Staff

Postdoc. : Kazuki Misato ／
Undergrad. Student 4 ／
Grad. Student 3

## Yasuo Yoshioka

**SA Associate Professor**

Dr. Yoshioka received his Ph.D. from Osaka University in 2004. He took his current positon at RIMD from 2015 after working at National Institute of Health Sciences, the Center for Advanced Medical Engineering and Informatics and Grad. School of Pharmaceutical Sciences in Osaka University.

## Publication


(1) Carbonate Apatite Nanoparticles Act as Potent Vaccine Adjuvant Delivery Vehicles by Enhancing Cytokine Production Induced by Encapsulated Cytosine-Phosphate-Guanine Oligodeoxynucleotides. Takahashi H, et al. ***Front Immunol***. (2018) Apr 18;9:783.

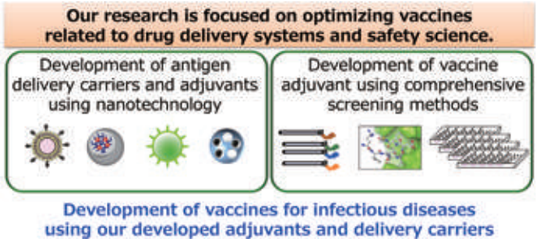
(2) Distribution of silver nanoparticles to breast milk and their biological effects on breast-fed offspring mice. Morishita Y, Yoshioka Y, et al. ***ACS Nano***. (2016) Aug 15.

(3) Metal nanoparticles in the presence of lipopolysaccharides trigger the onset of metal allergy in mice. Hirai T, Yoshioka Y, et al. ***Nat Nanotechnol***. (2016) 11(9):808-16.

(4) Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. Yamashita K, Yoshioka Y, et al. ***Nat Nanotechnol***. (2011) 6(5):321-8.

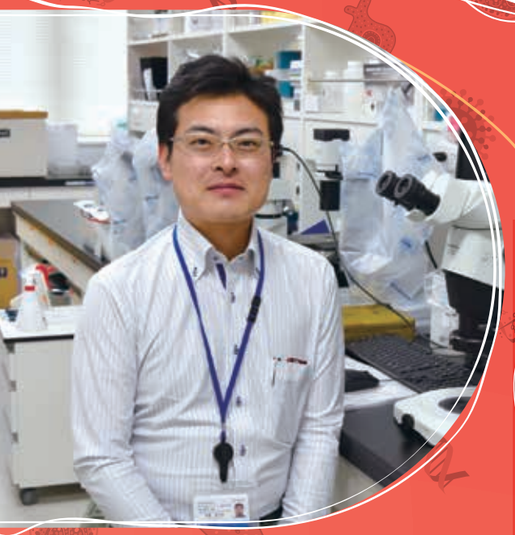

Most protein antigens such as non-living macromolecules or protein-subunit antigens evoke weak or undetectable adaptive immune responses. Therefore, to develop effective vaccines it is necessary to develop vaccine adjuvants and antigen delivery carriers. In addition, to develop optimal (in terms of efficacy and safety) vaccines for clinical application, it is important to understand the mechanism by which vaccines act on the immune system. In this regard, our research is focused on optimizing vaccines through drug delivery systems and safety science. Our specific research projects are:

1) Development of vaccine adjuvants using comprehensive screening methods.
2) Development of antigen delivery carriers and adjuvants using nanotechnology.
3) To use these adjuvants and delivery carriers to develop vaccines for infectious diseases.



BIKEN Innovative Vaccine Research Alliance Laboratories

# Mucosal Vaccine Project



## Shintaro Sato

**SA Associate Professor**

Dr. Sato received his master's degree from Hokkaido university in 1999 and his Ph.D. from Osaka University in 2003. He took his current position in 2015 after working at RIMD as a Postdoctoral fellow and at University of Tokyo as an Assistant Professor.

## Publication


(1)Human Norovirus Propagation in Human Induced Pluripotent Stem Cell-Derived Intestinal Epithelial Cells. Sato S., et al. ***Cell Mol. Gastroenterol. Hepatol.*** (2018) pii: S2352-345X (18)30164-4

(2) A Refined Culture System for Human Induced Pluripotent Stem Cell-Derived Intestinal Epithelial Organoids. Takahashi Y., et al. ***Stem Cell Rep.*** (2018) 10(1):314.

(3) Allograft inflammatory factor 1 is a regulator of transcytosis in M cells. Kishikawa S., et al. ***Nat. Commun.*** (2017) 8:14509.

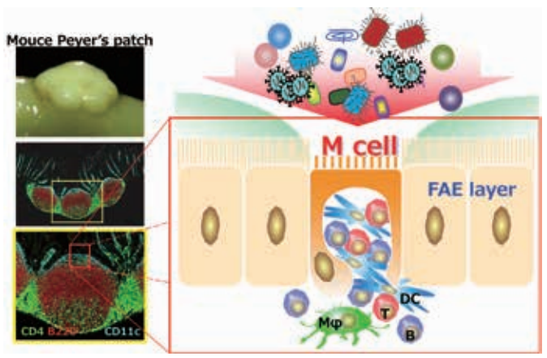
(4) Innate lymphoid cells regulate intestinal epithelial cell glycosylation. Goto Y., et al. ***Science*** (2014) 345 (6202):1254009.


Because most pathogens invade and infect their host via mucosal tissues, mammals have established a strictly organized and dynamic immune system at mucosal surfaces. This system, named the mucosal immune system, combats infectious pathogens. The development of mucosal vaccines, which activate mucosal and systemic immune responses, is receiving increasing attention. We have focused on epithelial cells, particularly M cells, which are professional antigen uptake cells located in areas that come into contact with non-self-antigens. Our main research themes are:

1)To identify new M cell-specific genes and elucidate the mechanism of M cell antigen uptake.

2)To understand the aged mucosal immune system and develop effective mucosal vaccines for older people.

3)To establish an *in vitro* culture system for normal and functional primary mucosal epithelial cells and use it to screen for candidate mucosal vaccine antigens and adjuvants.






BIKEN Innovative Vaccine Research Alliance Laboratories

# Vaccine Dynamics Project

**Staff**

Postdoc. : Yasunari Haseda

## Taiki Aoshi

**SA Associate Professor**

Dr. Aoshi received his M.D. from Hamamatsu University School of Medicine in 1999 and his Ph.D. from the same institution in 2006. He was appointed current positon in 2015 after working at Washington University in St. Louis, NIBIOHN, and IFReC in Osaka University.

## Publication


(1) Quantifying the relative immune cell activation from whole tissue/organ-derived differentially expressed gene data. Wijaya E., et al. ***Sci Rep.*** (2017) 7(1):12847.

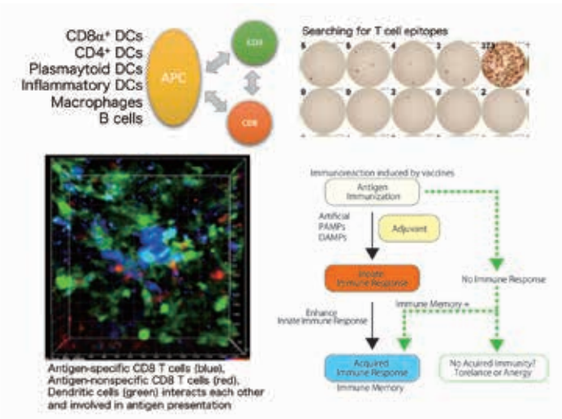
(2) Development of non-aggregating poly-A tailed immunostimulatory A/D-type CpG oligodeoxynucleotides applicable for clinical use. Aoshi T., et al. ***J Immunol Res.*** (2015) 2015:316364. doi: 10.1155/2015/316364.

(3) Plasmacytoid dendritic cells delineate immunogenicity of influenza vaccine subtypes. Koyama S., et al. ***Sci Transl Med.*** (2010) Mar 31; 2(25):25ra24.

(4) Bacterial entry to the splenic white pulp initiates antigen presentation to CD8+ T cells. Aoshi T., et al. ***Immunity*** (2008) Sep 19;29(3):476-86.


Vaccination, which utilizes the built-in "immune system" in the body, is one of the most successful medicine for controlling microbial infections. The immune system comprises many different immune cells. T cells are one of the most important immune cells that help to establish protective immunity against many pathogens. In addition, T cells are also involved in many non-infectious conditions such as autoimmunity and cancer.

Although T cells are critically involved in many diseases, current vaccines (with the exception of attenuated live vaccines) cannot induce sufficiently strong T cell responses. We are developing "T cell inducing vaccine" through the studies of T cell epitope and T cell/antigen presenting cell interaction. We are also developing clinically applicable "nucleic acid adjuvants" that not only induce strong Th1/CTL responses but also retain good safety profiles. We believe that the understanding of T cell epitope, T cell/antigen presenting cell interactions, and the development of new adjuvants will enable us to make safer and more effective T cell inducing vaccines in the near future.



# Animal Resource Center for Infectious Diseases

## Staff

Head Prof.: Masahito Ikawa
Asst. Prof.: Keisuke Shimada
Asst. Prof. (concur.): Haruhiko Miyata
Asst. Prof. (concur.): Taichi Noda
SA Asst. Prof. (concur.): Daiji Kiyozumi
SA Asst. Prof. (concur.): Tsutomu Endo

To study infectious diseases and cancer, it is important to analyze interactions between pathogenic factors and the human body. Animal models are indispensable for biomedical research, particularly since molecular biology and biotechnology methods can be used to generate genetically modified mice that aid our understanding of the molecular mechanisms underlying such diseases. Experimentally infected animals and genetically engineered animals used for these purposes should be managed in a suitable, safe, and controlled manner. The Animal Resource Center for Infectious Diseases is a unique facility that was established in 1967 to meet these requirements.

The center is equipped with pass-through-type autoclave systems and HEPA filtered air exchange systems to minimize the risk of contamination so that infected or genetically engineered animals are maintained in a safe environment. The animals are housed in three areas: SPF (specific pathogen free), BSL (biosafety level) 2, and BSL3. Before gaining access to restricted areas, researchers must take an official orientation and submit a research plan for committee review. The condition of the animals is monitored regularly.

Our facility provides the following services: generation of genetically manipulated animals, *in vitro* fertilization, and cryopreservation of mouse strains (Table 1). The facility provides these services in co-operation with the Department of Experimental Genome Research.

Table 1 *Tg, Transgenic; KO, Knockout; IVF, *in vitro* Fertilization; ET, Embryo transfer.

| | IVF/ET | TG | KO, KI |
|---|---|---|---|
| -2003 | 261 | 228 | 50 |
| 2001-2003 | 443 | 104 | 57 |
| 2004-2006 | 331 | 43 | 69 |
| 2007-2009 | 216 | 22 | 74 |
| 2010-2012 | 388 | 55 | 152 |
| 2013-2015 | 580 | 50 | 242* |
| 2016-2018 | 505 | 21 | 191 |

* Includes lines generated using the CRISPR/Cas9 genome editing system.

Biosafety level 3 room.
The room is used for research involving disease model animals at biosafety level 3. Hemorrhagic fever with renal syndrome-causing virus (HFRSV) was isolated in this area. In addition, animal experiments involving Zika virus, SARS, and Acquired Immune Deficiency Syndrome (AIDS), can be handled in this facility.

Buildings at the Animal Resource Center.
Building A (in front of the chimney, built in 1967, two-story).
Building B (rear right of the chimney, built in 1978, four-story).
Building C (on the right side of Building A, built in 2009, four-story).



# Office for Research Promotion

## Staff

Head, Prof. (concur.) : Masato Okada
Assoc. Prof. : Ryo Iwamoto
Asst. Prof. : Saya Nakagomi

The researchers at RIMD are supported by administrative functions provided by the Office for Research Promotion. The aim of this office is to promote communication among researchers and to develop human resources with expertise in scientific research. The office also communicates RIMD research achievements to the general public.

**The office provides the following services:**

**1. Manages of International Forums:**
- **Awaji International Forum on Infection and Immunity**
- **International Symposium of the Institute Network for Biomedical Sciences**

**2. Organizes seminars and lectures at RIMD.**

**3. Organizes lecture programs for undergraduate and graduate students.**

**4. Organizes orientation and Lab Tours for RIMD and IFReC for candidates looking to fill graduate school/post-doctoral positions.**

**5. Analyzes institutional research activity and performance.**

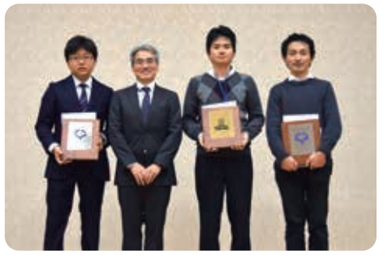
**6. Covers public communications and outreach.**

**7. Oversees the Taniguchi Fellowship Program for ASEAN students.**

Awaji International Forum on Infection and Immunity

Advanced Seminar Series

Poster session in RIMD Result Presentation



RIMD Result Presentation Academic prize awardee

Winterschool for High school teachers


RIMD booklets and newsletters

# Common Research Facilities

## Central Instrumentation Laboratory

### Staff

Head, Prof. (concur.) :Hiroaki Miki
Assoc. Prof. :Shinji Higashiyama
Assoc. Prof. :Naohisa Goto

The Central Instrumentation Laboratory was established in 1959. When equipment was lacking in many laboratories, researchers brought their machines together and co-operated with each other. Now, various pieces of precision apparatus and high performance machines are available in the laboratory at all times. These include ultracentrifuges, transmission and scanning electron microscopes, a Biacore system, cell analyzer/sorters, an DNA sequencers, and mass spectrometers. Also, large cell storage tanks equipped with automatic liquid nitrogen supply systems and a specified chemical treatment room are also present. In addition, professional technicians are employed to maintain and manage these devices, as well as to provide services, education, and training for newcomers. They also provide in-house services such as cell sorting, mass spectrometry-based protein identification, electron microscope image capture, and DNA sequencing. As experimental machines become more and more complicated, the services provided by specialist staff are essential for ongoing research at the institute.


Central Instrumentation Laboratory staffs

## Radioisotope Labolatory

### Staff

Head, Prof. (concur.) :Hiroaki Miki

The radioisotope (RI) laboratory was established in 1967 and was designed for biomedical experiments involving RIs. Now, RIMD researchers perform RI experiments in the RI Laboratory at the Immunology Frontier Research Center at 9F, the Central Laboratory for Biological Hazardous Microbes at 1F, and the radiation exposure room in the North building at 1F. Facilities include a RI stockroom, a distribution room, a tissue culture room, and an area for RI measuring equipment. Safety requirements are met by a stringent security system that involves the use of ID cards and the computerized management of RIs.



Common Research Facilities

## Central Laboratory for Biological Hazardous Microbes

Central Laboratory for Biological Hazardous Microbes

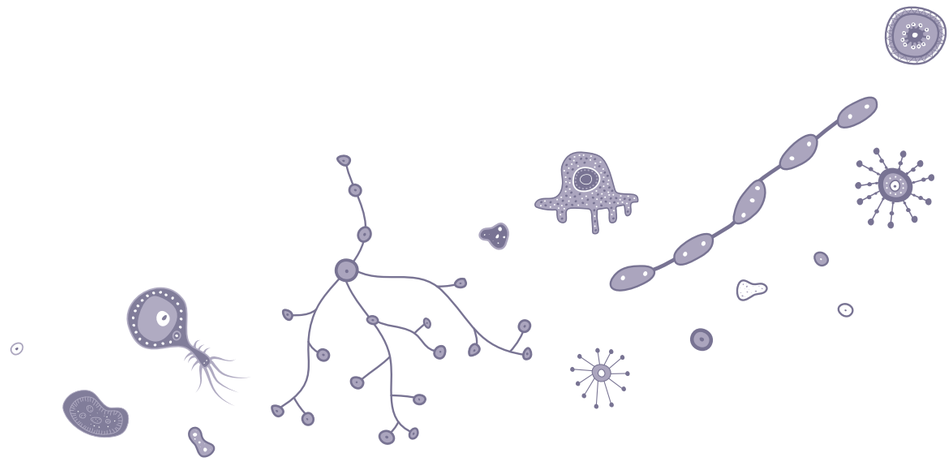
### Staff

Head, Prof. (concur.) :Tatsuo Shioda

This BSL-3 laboratory was set up in 1983 to ensure the safe handling of biologically hazardous microbes such as hemorrhagic fever with renal syndrome (HFRS) virus. All experimental studies using hazardous microbes, such as HIV, should be handled in this laboratory. The laboratory is a three-story building with 550 $m^2$ of floor space. The facilities are designed to protect researchers from pathogenic infection and to prevent the spread of biohazardous pathogens outside the building. The supply of fresh air is regulated to keep the room interiors at negative pressure. High-quality filters are installed on the exhaust outlet to minimize microbial contamination of the environment. Each room is equipped with safety cabinets and autoclaves to sterilize used material before disposal. Researchers must be approved by the Biosafety Committee before they use this laboratory. Various microbes, including HIV, SARS corona virus, and scrapie agent, are studies in this facility.

## Administration

General Affairs Section
Accounting Section
Research Cooperation Section



# RIMD History

The Research Institute for Microbial Diseases (RIMD) was established in 1934 to study microbial and infectious diseases, the immune system, and cancer. The RIMD of today is the result of the hard work and amazing achievements of many researchers over the years.

## KEY PERSON

**Tenji Taniguchi**

Professor of Bacteriology at the Osaka Medical school. He played a huge role in the foundation of RIMD as he emphasized the need for a research institute in the KANSAI area that focused on microbial or infectious diseases.

**Gendo Yamaguchi**

A successful businessman in the KANSAI area. He gave back to the community by offering his property for public benefit services and temples. He donated 200,000 yen to establish RIMD.

RIMD buildings in 1934

**RIMD History**

HISTORY

KEY PERSON

### 1934
**Research Institute for Microbial Diseases opened**

RIMD was founded through a merger of the Research Center for Communicable Diseases (Osaka Medical School), the Takeo Tuberculosis Institute (donated by Mr. Jiemon Takeo), and the Osaka Leprosy Institute (donated by an anonymous benefactor).

RIMD buildings in 1934

The Takeo Tuberculosis research Center

The main RIMD building at Dojima in 1934

The Osaka Leprosy Institute

### 1950s
Discovered *Vibrio parahaemolyticus*

Tsunesaburo Fujino

### 1960s
Discovered cell fusion

Yoshio Okada

Developed a measles vaccine

Yoshiomi Okuno

### 1967
RIMD moved to the Suita Campus

RIMD buildings in 1967

The Suita Campus, Osaka University

### 1970s
Discovered a viral oncogene

Kumao Toyoshima

### 1980s
Developed a chickenpox vaccine

Michiaki Takahashi

### 2000s
Elucidation of the Innate Immune System

Shizuo Akira

### 2005
Three centers for specialized research on infectious disease and genome information launched. The Research Collaboration Center on Emerging and Re-emerging Infections in Thailand was founded

### 2008
Selected for funding by the Global COE programs on the theme of "Frontier Biomedical Science Underlying Organelle Network Biology"

### 2015
BIKEN Innovative Vaccine Research Alliance Laboratories was launched

### 1993
RIMD Hospital was merged with Osaka University Hospital

### 2003
Selected for funding by the 21st Century COE programs on the theme of "Combined program on microbiology and immunology"

### 2007
Immunology Frontier Research Center (IFReC) was founded

### 2010
Approved as a Joint Usage / Research Center by Ministry of Education, Culture, Sports, Science and Technology

**The Epochs in Biology**

HISTORY

### 1798
Development of Smallpox vaccine (The first successful vaccine developed)

E. Jenner

### 1870-1880
Establishment of the germ theory of disease

L. Pasteur, R. Koch

### 1919
Proved chemical carcinogenesis

K. Yamagiwa

### 1928
Discovery of Penicillin (The first antibiotics)

A. Fleming

### 1953
Discovery of the DNA structure

J. Watoson, F. Crick

### 1957
Clonal selection theory in immunology

F.M. Burnet

### 1965
Revealed Genetic code

H. Khorana

### 1975
Production of monoclonal antibodies using cell fusion technique.

C. Milstein

### 1977
Discovery of the genetic mechanism to produce antibody diversity

S. Tonegawa

### 1979
Discovery of oncogene, c-Src

J.M. Bishop, H.E. Varmus

### 1980
Eradication of smallpox was declared by WHO

### 1981
Establishment of Embryonic Stem Cells (ES cells)

M. Evans, M. Kaufman

### 1996
Dolly the sheep was born (The first cloned mammal)

### 2003
Human Genome Project completed

### 2006
Establishment of Induced Pluripotent Stem Cells (iPS cells)

S. Yamanaka

# RIMD Awards 2018

| | |
|---|---|
| **Suita City Mayor's Commendation Excellent Hazardous Materials Security Superintendent Award** | |
| Shinji Higashiyama　Central Instrumentation Laboratory | 2018.4 |
| **The Commendation by the Minister of Education, Culture, Sports, Science and Technology The Young Scientists' Prize** | |
| Miwa Sasai　Dept. of Immunoparasitology | 2018.4 |
| **The 34th Annual Meeting of the Japan Society of Drug Delivery System Outstanding Presentation Award** | |
| Kazuki Misato　Vaccine Creation Project | 2018.6 |
| **JVBMO Young Investigator Travel Award for The 20th International Vascular Biology Meeting(IVBM2018)** | |
| Tomohiro Iba　Dept. of Signal Transduction | 2018.6 |
| **JVBMO Young Investigator Travel Award for The 20th International Vascular Biology Meeting(IVBM2018)** | |
| Yohei Tsukada　Dept. of Signal Transduction | 2018.6 |
| **JVBMO Young Investigator Travel Award for The 20th International Vascular Biology Meeting(IVBM2018)** | |
| Fumitaka Muramatsu　Dept. of Signal Transduction | 2018.6 |
| **Best International Abstract Award, 51st Annual Meeting of the Society for the Study of Reproduction** | |
| Yoshitaka Fujihara　Dept. of Experimental Genome Research | 2018.7 |
| **Best International Abstract Award, 51st Annual Meeting of the Society for the Study of Reproduction** | |
| Julio Castaneda　Dept. of Experimental Genome Research | 2018.7 |
| **Poster Presentation Award, JVBMO** | |
| Hisamichi Naito　Dept. of Signal Transduction | 2018.9 |
| **Poster Presentation Award, JVBMO** | |
| Hiroyasu Kidoya　Dept. of Signal Transduction | 2018.9 |
| **Best Talk Award, Grant-in-Aid for Scientific Research on Innovative Areas - Platforms for Advanced Technologies and Research Resources, Platform of Advanced Animal Model Support** | |
| Hiroyasu Kidoya　Dept. of Signal Transduction | 2018.9 |
| **Excellence Award, 2018 Uehara H. pylori Award** | |
| Hitomi Mimuro　Dept. of Infection Microbiology | 2018.9 |
| **2018 International Symposium of Innovative Research and Graduate Education in Biomedical Sciences Best Presentation Award** | |
| Woei-Yaw Chee　Dept. of Oncogene Research | 2018.9 |
| **Medal with Purple Ribbon** | |
| Taroh Kinoshita　Yabumoto Department of Intractable Disease Research | 2018.11 |
| **The 3rd Study Group for Mycobacteria Best Presentation Award** | |
| Naoya Nishimura　Dept. of Molecular Immunology | 2018.11 |
| **The 21st JSI Award ( Japanese Society for Immunology )** | |
| Sho Yamasaki　Dept. of Molecular Immunology | 2018.12 |
| **Japan Medical R&D Grand Prize.** | |
| Masahiro Yamamoto, Dept of Immunoparasitology | 2018.12 |
| **15th JSPS Prize** | |
| Masahiro Yamamoto, Dept of Immunoparasitology | 2018.12 |

Miwa Sasai
(Dept. of Immunoparasitology)
The Commendation by the MEXT,
The Young Scientists' Prize

Masahiro Yamamoto (3rd from left, in the back raw)
Japan Medical R&D Grand Prize.

# Collaboration with relevant institutes and universities

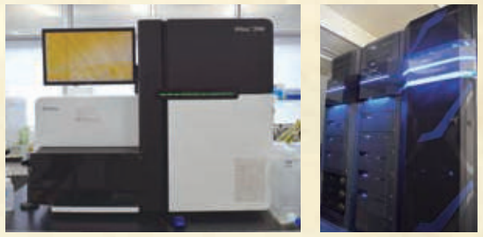
## ●MEXT Joint Usage / Research Center

RIMD was certified as a “Joint Usage/Research Center” by the Ministry of Education, Culture, Sports, Science and Technology in Japan (MEXT) in 2010. The “Joint Usage/Research Center” is a sharing system first organized by MEXT in 2008 and which allows Japanese researchers to use facilities, equipment, and databases at inter-university research institutes.

The aims of RIMD as “Joint Usage/Research Center” are to promote research and develop human resources in the fields of immunology, bacteriology, and oncology. We provide specialized facilities equipped with BSL2 and 3 laboratories in Animal Resource Center and Infectious Diseases and Central Laboratory for Biological Hazardous Microbes. In addition, Genome Information Center is for genome research with Next Generation Sequencers and support researchers to analyze genomes of organisms.

We also hosts international symposiums such as Awaji International Forum on Infection and Immunity to share research achievements and facilitate communication among researchers.

Animal Facility



Next Generation Sequencer and server

Awaji International Forum on Infection and Immunity

## ●International Collaborations

RIMD researchers conduct international collaborative Projects that involve researchers from various contries. FIMD also concludes academic agreements with four institutes and universities.

### International collaborative projects

| Year | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 |
|---|---|---|---|---|---|---|---|
| Projects | 24 | 42 | 49 | 42 | 32 | 35 | 43 |

### Academic agreements

| Country | Institute / University | Starting date |
|---|---|---|
| Thailand | Bamrasnaradura Infectious Diseases Institute | 2013/2/15～ |
| Indonasia | Airlangga University (Faculty of Medicine) | 2013/7/31～ |
| Lithuania | Vilnius University (Faculty of Medicine) | 2017/6/16～ |
| U.S.A. | Baylor College of Medicine (Departments of Pathology & Immunology) | 2017/4/10～ |



## ●Collaboration with BIKEN foundation and Immunology Frontier Research Center (IFReC)

RIMD and IFReC conducts world-class researches in biological fields including microbiology and immunology. RIMD and IFReC collaborate each other to promote researches and foster talented human resources in these fields.

The BIKEN foundation develops vaccines, thereby to translate the research achievements in of RIMD to the society. In addition, the foundation is dedicated to promote basic research through the Taniguchi Memorial Scholarship program and the BIKEN Center for Innovative Vaccine Research Alliance Laboratories.

RIMD
Research Institute for Microbial Diseases
大阪大学微生物病研究所

Joint Usage/
Research Center

**Triangle Relationships**
**on Infectious Diseases and Immunology**

WPI Osaka University
iFReC

World Premier
International Research Center
on immunology

BIKEN

The Research Foundation for Microbial Diseases
of Osaka University

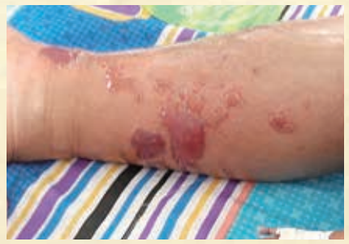
# To Develop Human Resources Globally

## ●Clinical Training Course on Tropical Infectious Diseases in the Thailand

The age of global travel means that people can spread pathogens worldwide. Infectious diseases are now a global problem that extends beyond national borders. In Japan, there is a compelling need for experienced specialists to study these infectious diseases.
Since 2009, RIMD, together with the School of Medicine at Osaka University, has provided a clinical training course on Tropical Infectious Diseases in Thailand. The course provides medical doctors with clinical training on the diagnosis and treatment of infectious diseases and is supported by hospitals in Thailand. This training course provides a valuable opportunity for Japanese clinicians to gain clinical experience from hospital staff operating in a high-incidence area. Over 50 doctors participated in this training course, and the alumni are conducting basic/clinical research into infectious diseases or working for Medicines Sans Frontieres and various governmental organizations.

http://tmtc.biken.osaka-u.ac.jp/intention/index.html

Hospitals participating in clinical training in Thailand.
Doctors can learn directly from local staff.



Doctors gain hands-on experience during the training course.


**<Hospitals for clinical training in Thailand>**

**Mae Sot：**
Mae Sot General Hospital
Mae La refugee camp
Mae Tao Clinic
Maeramad Hospital
Shoklo Malaria Research Unit

**Udon Thani：**
Udon Thani Genelas Hospital

**Bangkok：**
Ramathibodi Hospital, Mahidol University
Queen Sirikit National Institute of Child Health

**Khon kaen：**
Srinagarind Hospital, Khon Kaen University
Khon Kaen General Hospital

## ●Taniguchi Scholarship: International Students Scholarship Program

RIMD established a scholarship program for Students from ASEAN countries to study at RIMD as graduate students and provide leadership and support to become independent researchers. Particularly excellent candidates will be offered a regular faculty position at RIMD after they obtained Ph.D.. This new scholarship program aims to significantly contribute to the development of science by training world-leading researchers from the international students learned at RIMD.

# Seminars and Events

We organize conferences and seminars to facilitate communication among researchers. We also organize an outreach event to provide accurate information about our research findings and achievements.

## ●Events for Researches

### International Conferenes

Awaji International Forum on Infection and Immunity
（http://awaji-forum.com/）
International Symposium of the Institute Network
(http://awaji-forum.com/)

Awaji International Forum on Infection and Immunity

### BIKEN Monthly Seminar

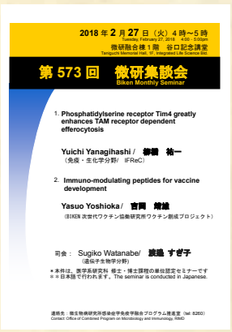
Held monthly, except August and December. Young researchers present their recent research findings.

### Advanced Seminar Series on Microbiology and Immunology

Lecture series hosted by the Office for Research Promotion. Leading researchers in the field of Microbiology and Immunology are invited to this lecture series.

### Bridge Seminar

Seminar series hosted by young researchers at RIMD.




Biken Monthly Seminar


Advanced Seminar Series

## ●Outreach Events

We organize outreach Events and Exhibitions for the non-scientific community. In addition, we try to encourage young students, including high school students and junior high school students, to take an interest in scientific research.

Osaka University ICHO Festival

Summer Seminar for high school studets

# For Students and Researchers who wants to study in RIMD

RIMD is one of the world's foremost institute in immunology, microbiology and cancer research. We also conduct research in various bioscience related fields including gene engineering, genomic science and bioinformatics. We welcome motivated grad-students and researchers from around the world.
The way to join RIMD would be different depends on the situation. Candidate for grad-school students or post-docs may need to decide the lab to join and then ask PIs how to belong to RIMD.

The Orientation and lab tour would be held in May every year. Please check our website for detail.

www.biken.osaka-u.ac.jp/en/recruit

## Information in Osaka University website

> Osaka University website for Global Affairs
http://www.osaka-u.ac.jp/en/international

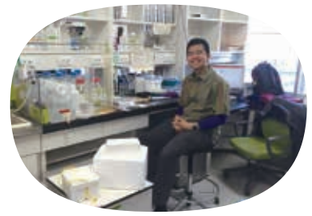
> Osaka University website for International Students
http://www.osaka-u.ac.jp/en/for-student

> Osaka University Support Office for international Students
https://iss-intl.osaka-u.ac.jp/supportoffice/

> Osaka University Brothers and Sisters Program (BSP)
An International-exchange circle organized by Osaka University Students to support international students.
http://www.bsp-ou.net/



> Osaka University COOP guidance book
The Osaka University COOP is a non-profitable organization for students and staffs in Osaka university. They provide stores, cafeterias and other life supports including housing and traveling.
http://www.osaka-univ.coop/english/index.html



## Information in Japanese Government or Organization

> Study in Japan Comprehensive Guide by The Ministry of Foreign Affairs of Japan
http://www.studyjapan.go.jp/en/index.html

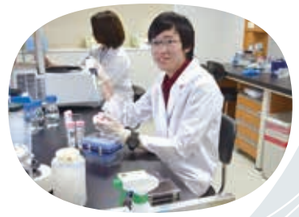
> Websites of Japanese Embassies in your country
http://www.mofa.go.jp/about/emb_cons/mofaserv.html

> Japan Student Services Organization (JASSO)
An independent administrative institution established under the MEXT, comprehensively administers support programs for international students including scholarship loan programs.
http://www.jasso.go.jp/en/index.html

> Gateway to study in Japan by JASSO
Information in Japanese, English, Chinese, Korean, Indonesia, Thai, Vietnamese
http://www.g-studyinjapan.jasso.go.jp

> Japanese Government scholarship by MEXT
http://www.studyjapan.go.jp/en/toj/toj0302e.html

> Suita International Friendship Association
A public interest Incorporated Foundation to support citizen's international exchange activities and provide assistance to foreigners living or Studying in Suita city.
http://suita-sifa.org/en/



RIMD and IFReC conducts world-class researches in biological fields including the fields of microbiology and immunology. RIMD and IFReC collaborate each other to promote researches and foster talented human resources in these fields.
The BIKEN foundation develops vaccines, thereby to translate the research achievements in of RIMD and IFReC to the whole of society. In addition, the foundation is dedicated to promote basic research by through the Taniguchi Memorial Scholarship program and the BIKEN Center for Innovative Vaccine Research Alliance Laboratories.

# Grad Students Studying in RIMD

## Why RIMD?

A few years ago, I was lucky to experience Japan's super advanced research as an exchange student at NIPS (National Institute for Physiological Sciences) and University of Tsukuba. Since then, my interest to Japan not only research but also its cultures and language has developed. Apart from that, during my bachelor course, I studied bacterial genetics and physiology which drove my interest to the mechanism of bacterial infections in human. After finishing study I thought my understanding on this field was insufficient. Therefore, I set the dream to pursue graduate study. Strong engagement with Japan, lead me to choose Japan as study destination. Fortunately, universe supported me. There was a scholarship opportunity from RIMD, one of the foremost research institution with one of the research field is bacterial infection. In addition, RIMD seems to offer very good environment, advanced facilities, and most importantly it has many world-level researchers on each field. With those reasons, I decided to join RIMD and I would say it was one of the best decision I have ever made.

## A day in the life

My experience as a graduate student at RIMD has been pleasant, mainly because of the limitless support and encouragement of the lab members and supervisor. Performing research becomes main activity to spend a day, it is always challenging yet rewarding. During that process, we are encouraged to come up with ideas, to think critically, to solve problems, to persist, and to learn other aspects necessary to become a competent researcher. RIMD also provides chances to interact with well-known researchers through various interesting seminars. Furthermore, I find that living in Osaka is fun and always been decent place to explore. Since I like sport and culinary, sometimes on the weekend I play badminton or travel around to try different foods and snacks. Lastly, as Japanese is mostly used during experiment or daily life, I keep forcing myself to speak Japanese everyday with other lab members, and I am grateful that they are extremely supportive to me.

## Research Interest

I was fortunate to be accepted into Prof. Horiguchi's team in which one of the big goal is to understand the entire picture of the bacterial infection from each particular steps. Various human or animal pathogens, such as *Bordetella* spp did not emerge suddenly, instead it underwent evolutionary steps which eventually developed their adaptation from environmental bacteria to human or animal pathogen. Since the focus of the pathogenic bacteria study is mostly done during host infection, the information regarding their life style outside the mammalian hosts is still limited. This issue becomes my current research topic. To be specific, I am trying to examine the nature of *B. bronchiseptica* (part of *Bordetella* spp) life style and determine the possibility of the bacteria interaction with protozoan predators, especially amoeba. Furthermore, understanding the molecular mechanism of this interaction will bring new insight on how the bacteria obtain the ability to establish their infection in human or animal hosts.

## Message for young students

Exploring the whole new environment will always be adrenaline-rush experience. However, without new challenges, we cannot see how far we have improved. It is the same with pursuing graduate study, it is challenging and is demanding high effort and persistence yet really worth doing. Therefore, I encourage to all young mind out there to tenaciously pursue your passion and work hard for it. If you have interest on basic research and may think to work as researcher in the future, bring that passion to RIMD and see how far you can advance yourself. Nothing better than turning the passion into real work because it always helps us to stay focus, feel excited, and become more passionate on what we do. Finally, I hope the best of luck for them who want to get in to RIMD and Osaka University.


**Dendi Krisna Nugraha**

Department of Molecular Bacteriology (Horiguchi Lab)
Doctoral Course in Graduate School of Frontier Biosciences, Osaka University

BA: Microbiology, Bandung Institute of Technology
MA: Graduate school of Frontier Biosciences, Osaka University from Bandung Institute of Technology, Indonasia




## Why RIMD?

I used to be a pediatrician in China and I wanted to help all of my lovely patients overcome their sickness. However, there are still a lot of incurable diseases that take many lives away. The mission of a clinical doctor is to help patients, but what if I become a researcher? Could I help more people and save more lives? After thinking about this, I decided that I wanted to make my own contribution to the medical development. I chose to focus on infectious diseases which are the most common but dangerous illnesses for children. RIMD is one of the most famous institutes which concentrates on microbiology and immunology. It can offer me many opportunities to learn cutting-edge knowledge and help me achieve my goal.

## A day in the life

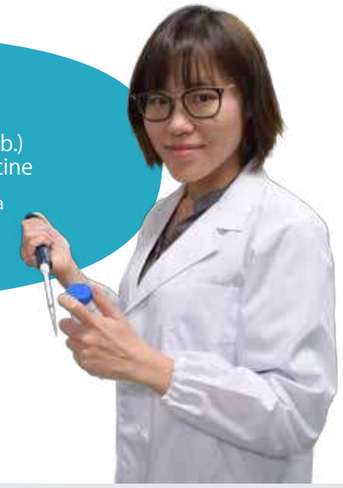
I was definitely a beginner at experiments when I first came to the lab. My professor and seniors in our lab taught me various techniques with great patience. Without their help, I wouldn't be able to adapt to a new study life in a foreign country that soon. I also attend lots of interesting seminars which help broaden my horizon. I'm working hard on weekdays and playing hard in the holidays. In my opinion, studying abroad is not only about doing experiments, but also about having real experiences with the world beyond my border. In my spare time, I'd like to travel around Japan and experience different languages, cultures and societies. I have been to many places such as Kyoto, Hiroshima, Gifu, Tokyo and so on. Every day I spend here is very rich and colorful, and all of these experiences have become precious treasures in my life.

## Research Interest

I was very lucky to be accepted by the department of Molecular Virology, which focus on Hepatitis viruses and flavivirus virus. Now I' m doing research about hepatitis B virus (HBV). Up until now, Chronic hepatitis B virus infection affects approximately 240 million people in the world. However, the present therapeutics fail to provide a cure for the vast majority of patients, because the Covalently closed circular DNA (cccDNA) in the nucleus of infected cells cannot be eliminated, and that may result in persistence and relapse. In HBV life cycle, cccDNA is transformed from rcDNA after it enter the nucleus. We could interrupt HBV replication if the nuclear transport step is blocked. Therefore, I' m trying to identify the host factor involved in HBV nuclear transport, which may lead to the development of novel antiviral strategies and drug targets.

## Message for young students

"Never forget why you started and don' t be afraid of failure." This is the sentence I usually use to motivate myself. Research sometimes can be boring. It is a roller coaster of ups and downs. We are working hard day and night, looking for a spark of light in the dark, while the future is unknown and all the effort might be in vain. It' s easy to give up in the harsh times, but the reason we started can ignite our passion again. We will achieve our goal sooner or later if we stick to it, whereas there is nothing but failure once we give up. Don' t be anxious to achieve quick success or *get overnight results*, be at peace with yourself, love the process and you' ll love what the process produces brings you.




**張　赫**

Department of Molecular Virology(Matsuura Lab.)
Doctral Course in the Graduate School of Medicine

BA : Clinical Medicine, the Capital Medical University, China

# RIMD STAFF

## Staffs

| | |
|---|---|
| Professor | 14 |
| Endowed Chair Professor | 2 |
| Associate Professor | 19 |
| Assistant Professor | 22 |
| SA Professor | 3 |
| SA Associate Professor | 4 |
| SA Assistant Professor | 9 |
| SA Researcher | 8 |
| SA staff | 40 |
| Educational Support Staff | 3 |
| Technical Staff | 20 |
| Part-time Gneral & Technical Staff | 36 |
| Administrative Staff | 22 |
| Total | 202 |

(SA: Specially Appointed)

## Graduate Students

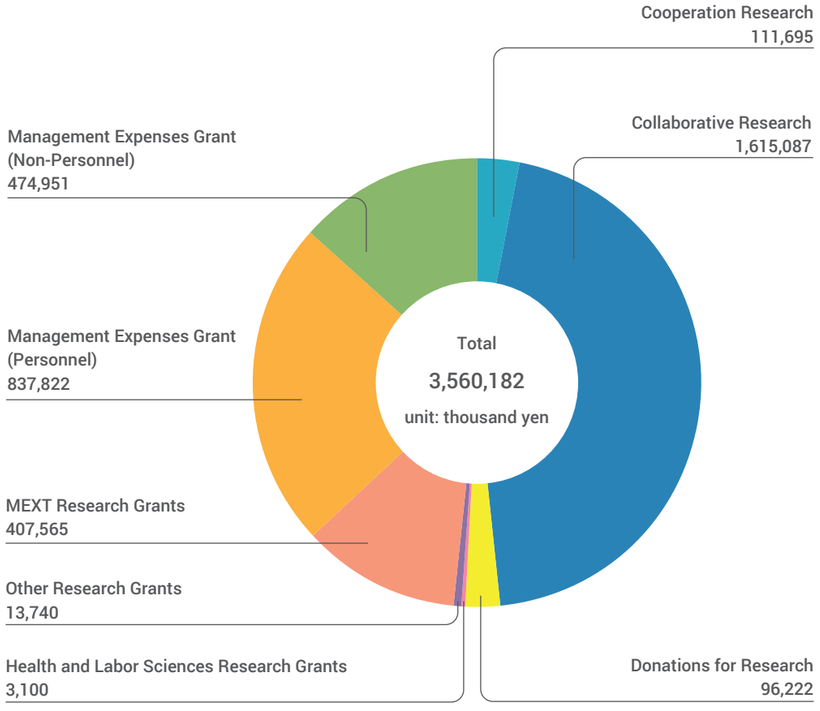
| | ① | ② |
|---|---|---|
| Graduate school of Medicine | 47 | 7 |
| Graduate school of Science | 6 | 10 |
| Graduate school of Pharmaceutical Science | 3 | 11 |
| Graduate school of Frontier Biosciences | 19 | - |

①Doctoral Program
②Master' s Program

## Research Fellows and Research Students

| | |
|---|---|
| Special research students | 5 |
| Research students | 7 |
| JSPS Research fellows | 1 |

# ACCOUNTS




# BUILDING AREA

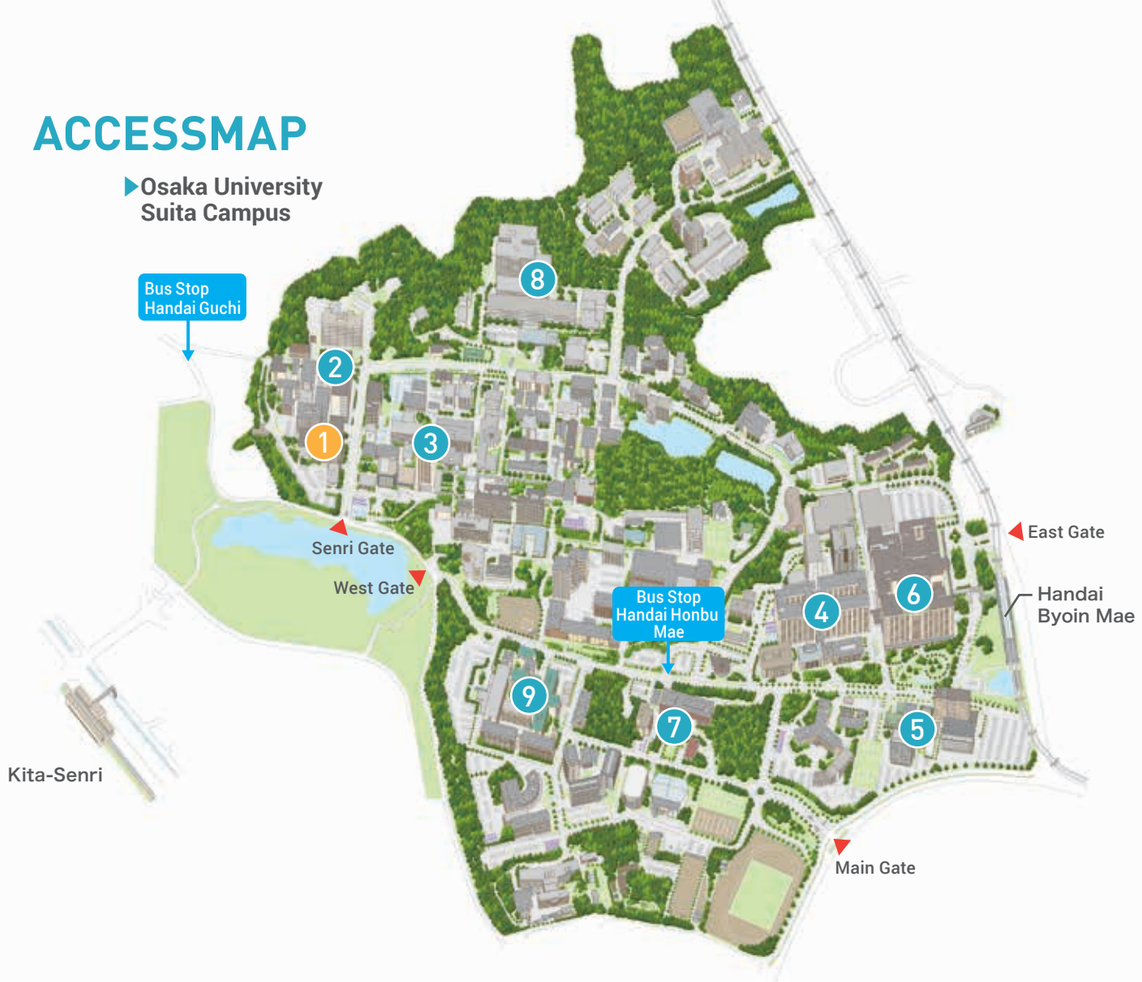


Site Area 36,036㎡
Building Area 8,702㎡ Gross Floor Area 39,945㎡

| Building name | Total floor numbers | Building area (㎡) | Total floor area(㎡) |
|---|---|---|---|
| ①Main Building | 7 | 1,706 | 6,397 |
| ②South Building | 2 | 409 | 945 |
| ③North Building | 3 | 492 | 1,252 |
| ④Annex | 2 | 768 | 1,548 |
| ⑤Animal Resource Center A | 2 | 640 | 1,391 |
| ⑥Animal Resource Center B | 4 | 355 | 1,425 |
| ⑦Central Laboratory for Biological Hazardous Microbes | 3 | 241 | 550 |
| ⑧Central Instrumentation Laboratory | 2 | 378 | 504 |
| ⑨Depository for Dangerous Chemicals | 1 | 160 | 160 |
| ⑩Integrated Life Science Building | 10 | 1,072 | 9,258 |
| ⑪Cutting-edge Research Building for Infectious Diseases | 9 | 973 | 7,448 |
| ⑫Animal Resource Center C (belonging to IFReC) | 4 | 738 | 2,482 |
| ⑬IFReC Building | 9 | 770 | 6,585 |

# ACCESSMAP

Osaka University
Suita Campus




1 Research Institute for Microbial Diseases
2 Immunology Frontier Research Center
3 Graduate School of Engineering
4 Graduate School of Medicine
5 Graduate School of Frontier Biosciences
6 Osaka University Hospital
7 Administration Bureau
8 The Institute of Scientific and Industrial Research
9 Osaka University Dental Hospital


### Train
12-minute walk from “Kita-Senri” Station on Hankyu Senri Line.

### Monorail
20-minute walk from “Handai Byoin Mae” Station on Osaka Monorail Saito Line.

### Bus
- From Senri-Chuo Station :
  5-minute walk from “Handai-Guchi” Bus Stop on Hankyu Buses heading to “Onohara Higashi” , “Toyokawa-Eki” , “Fujikasai” .
  12-minute walk from “Handai Honbu Mae” Bus Stop on Hankyu Buses heading to “Handai Honbu Mae” or “Ibaraki Mihogaoka” .

- From Hankyu Ibaraki-shi Station:
  12-minute walk from “Handai Honbu Mae” Bus Stop on buses heading to “Handai Honbu Mae” (via JR Ibaraki Station).



# Support RIMD Research

-Be part of the quest to find our more in science-

RIMD is the world’ s outstanding research institute in immunology, microbiology, oncology and biology. We have brought about drastic development in this field by identifying new pathogens and pathogenic mechanisms, vaccine development, oncogenic research. We work to support human resources development to promote advanced research in this field.
Your support will enable to fuel innovative research in RIMD. Please contact us to learn more about how you can help Science tomorrow by supporting our research.

## How your donations are utilized

- **Supporting RIMD researches overseas.**
- **Helping student to study in RIMD (Scholarships etc.)**
- **Helping international students to study in RIMD.**
- **Helping Training Course on Tropical Infectious Diseases for clinical doctors.**
- **Organizing scientific lectures and seminars for non-scientists**

## [How to donate]

Credit card, Bank transfer

For detail please check the website

**https://www.miraikikin.osaka-u.ac.jp/english/**

Please make your donation for following projects at the Website
<Institutes and Facilities>
■Research Institute for Microbial Diseases
■Research and Development of Malarial Vaccine Fund