Osaka University

Research Institute for Microbial Diseases

2014–2015

Contents

Photo by Hiroshi Nojima.

Research Institute for Microbial Diseases

The Research Institute for Microbial Diseases was originally established as a five-department "Research Center for Communicable Diseases" in February, 1934 after a donation from Mr. Gendo Yamaguchi at Dojima in Osaka City. At that time, the Institute was located on Nakanoshima campus of Osaka Medical School, but then became a part of Osaka University in September, 1934. It was moved to its present site on Suita campus in 1967, in accordance with the university's relocation program.

Until 1993, the Institute had grown to include nineteen departments spanning a wide range of diverse subjects, such as infectious disease, immunology, cancer and molecular biology. Additionally, it had three special research facilities, a central laboratory and a library. In 1997, the Research Center for Emerging Infectious Diseases was added to the Institute. In 2005, the Institute was reorganized to constitute three Research divisions; the "Division of Infectious Disease", the "Division of Host Defence" and the "Division of Cellular and Molecular Biology", which represent 15 departments together with three attached centers for specialized research on infectious disease and genome information. Moreover, the Research Collaboration Center on Emerging and Re-emerging Infections was founded in Bangkok in collaboration with the National Institute of Health, Thailand, to defend people against possible emerging and re-emerging infections.

Basic research on infectious disease, immunology, and cell biology is the Institute's principal focus. The results of research in these fields at the Institute have contributed considerably to the diagnosis, prevention, and treatment of infectious diseases, immunological diseases and cancer, as well as to progress in basic biomedical science. The Institute was selected as one of "the 21st century center of excellence programs" on the theme of "combined program on microbiology and immunology" in 2003. Also, it was certified as "joint usage / research center" by MEXT (the Ministry of education, culture, sports, science and technology) in 2009, and started the mission in April, 2010. Moreover, the institute newly established the "Center for Genetic Analysis of Biological Responses" to conserve gene resources and to protect intellectual property.

The Institute accepts and trains Master and Ph.D. candidates in the medical and biological sciences. At present, more than 200 full, associate, and assistant professors, research associates, graduate students and research fellows pursue studies in microbiology, oncology and molecular biology in state of the art facilities at the Institute.

The copper plate recalling that the Research Institute for Microbial Diseases was originally established by a donation from Mr. Gendo Yamaguchi. (At the entrance hall of the main building)

Mr. Gendo Yamaguchi

Born in Onomichi City in 1863 as the eldest son of a physician, he moved to Osaka at the age of 15 and became one of western Japan's most successful businessmen. He retired in 1917, and devoted the rest of his life to religion and the tea ceremony. He donated most of the proceeds of his estate to public enterprise, shrines and temples.

Organization

Director

Faculty Meeting — **Delegate Assembly**

Research Division

- Division of Infectious Diseases
  - Department of Molecular Bacteriology
  - Department of Viral lnfections
  - Department of Molecular Virology
  - Department of Pharmacotherapy
  - Department of Immunoparasitology
- Division of Host Defense
  - Department of Molecular Immunology
  - Department of Immunoregulation
  - Department of Host Defense
  - Department of Cell Biology
  - Department of Immunochemistry
- Division of Cellular and Molecular Biology
  - Department of Molecular Microbiology
  - Department of Molecular Genetics
  - Department of Oncogene Research
  - Department of Signal Transduction
  - Department of Cellular Regulation

Special Research Facilities

- Research Center for Infectious Disease Control
  - Department of Bacterial Infections
  - Department of Molecular Protozoology
  - Department of Virology
- Genome Information Research Center
  - Department of Experimental Genome Research
  - Department of Genome Informatics
  - Department of Infection Metagenomics
- International Research Center for Infectious Diseases
  - Department of Special Pathogens
  - Department of Infectious Disease Control
  - Pathogenic Microbes Repository Unit
- Animal Resource Center for Infectious Diseases
- DNA-chip Development Center for Infectious Diseases
- Center for Genetic Analysis of Biological Responses

Office of Combined Program on Microbiology and Immunology

- Research Promotion Group
- Education Promotion Group

Research Collaboration Center in Overseas

- Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections
  - Section of Bacterial Infections
  - Section of Viral Infections
- Mahidol-Osaka Center for Infectious Diseases
- BIKEN Endowed Department of Dengue Vaccine Development

Common Research Facilities

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

Administration

- General Affairs Section
- Accounting Section
- Research Cooperation Section

World Premier International Research Center

- Immunology Frontier Research Center

Former Directors

Yashiro Kotake, M.D., Professor	1934.9−1940. 6
Arao Imamura, M.D., Professor	1940.8−1943. 7
Tenji Taniguchi, M.D., Professor	1943.7−1955. 3
Tsunesaburo Fujino, M.D., Professor	1955.4−1958. 3
Juntaro Kamahora, M.D., Professor	1958.4−1964. 3
Tsunehisa Amano, M.D., Professor	1964.4−1968. 3
Yoshiomi Okuno, M.D., Professor	1968.4−1972. 3
Mitsuo Hori, M.D., Professor	1972.4−1976. 3
Junichi Kawamata, M.D., Professor	1976.4−1980. 3
Shiro Kato, M.D., Professor	1980.4−1984. 3
Michiaki Takahashi, M.D., Professor	1984.4−1986. 3
Toshio Miwatani, M.D., Professor	1986.4−1988. 3
Takeo Kakunaga, D.Pharm., Professor	1988.4−1988. 9
Hajime Fujio, M.D., Professor	1988.11−1990.10
Kumao Toyoshima, M.D., Professor	1990.11−1993.10
Akira Hakura, D.Sc., Professor	1993.10−1997.10
Yoshitake Nishimune, M.D., Professor	1997.10−2001.10
Takeji Honda, M.D., Professor	2001.10−2003.10
Taroh Kinoshita, D.Med.Sc., Professor	2003.10−2007.10
Hitoshi Kikutani, M.D., Professor	2007.10−2011.10
Eisuke Mekada Ph.D., Professor	2011.10−

Former Professors

Yashiro Kotake, M.D., Professor
Sadao Yoshida, M.D., Professor
Arao Imamura, M.D., Professor
Yukichi Satani, M.D., Professor
Tenji Taniguchi, M.D., Professor
Kota Sera, M.D., Professor
Tatsunori Masayama, M.D., Professor
Shohei Otani, M.D., Professor
Teishiro Seki, M.D., Professor
Masami Suda, M.D., Professor
Kaoru Morishita, M.D., Professor
Hisashi Yamaguchi, M.D., Professor
Tsunesaburo Fujino, M.D., Professor
Masakazu Ito, M.D., Professor
Juntaro Kamahora, M.D., Professor
Shinji Nishimura, M.D., Professor
Mitsuhiko Kato, M.D., Professor
Masahiko Yoneda, M.D., Professor
Shigeru Shiba, M.D., Professor
Shozo Inoki, M.D., Professor
Mitsuo Hori, M.D., Professor
Yoshiomi Okuno, M.D., Professor
Shigeyuki Ishigami, M.D., Professor
Tsunehisa Amano, M.D., Professor
Junichi Kawamata, M.D., Professor
Yoshio Okada, M.D., Professor
Mitsuo Torii, D.Sc., Professor
Konosuke Fukai, M.D., Professor
Tatsuo Mori, M.D., Professor
Tonetaro Ito, M.D., Professor
Takeo Kakunaga, D.Pharm., Professor
Shiro Kato, M.D., Professor
Toshio Nakabayashi, M.D., Professor
Takahisa Yamanouchi, M.D., Professor
Toshio Miwatani, M.D., Professor
Michiaki Takahashi, M.D., Professor
Hajime Fujio, M.D., Professor
Tetsuo Taguchi, M.D., Professor
Aizo Matsushiro, D.Sc., Professor
Atsuo Nakata, D.Sc., Professor
Hiroto Okayama, M.D., Professor
Kumao Toyoshima, M.D., Professor
Teruo Kitani, M.D., Professor
Shin-ichiro Takai, M.D., Professor
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Koichi Yamanishi, M.D., Professor
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Toru Nakano, M.D., D.Med. SC., Professor
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Fumio Imamoto, D.Sc., Professor
Atsushi Kumanogo, M.D., Ph. D., Professor
Kazunori Oishi, M.D., Ph. D., SA Professor
Masanori Kameoka, Ph. D., SA Professor
Masaru Okabe, Ph. D., Professor
Kazuyoshi Ikuta, Ph. D., Professor

Department Heads

2014,04,01

Director	Professor Eisuke Mekada Ph.D.
Vice Director	Professor Yoshiharu Matsuura D. V. M., Ph.D.
Division of Infectious Diseases	
Department of Molecular Bacteriology	Professor Yasuhiko Horiguchi D. V. M., Ph.D.
Department of Viral Infections	Professor Tatsuo Shioda Ph.D.
Department of Molecular Virology	Professor Yoshiharu Matsuura D. V. M., Ph.D.
Department of Pharmacotherapy	
Department of Immunoparasitology	Professsor Masahiro Yamamoto Ph.D.
Division of Host Defense	
Department of Molecular Immunology	Professor Hitoshi Kikutani M. D., Ph.D.
Department of Immunoregulation	Professor Taroh Kinoshita Ph.D.
Department of Host Defense	Professor Shizuo Akira M. D., Ph.D.
Department of Cell Biology	Professor Eisuke Mekada Ph.D.
Department of Immunochemistry	Professor Hisashi Arase M. D., Ph.D.
Division of Cellular and Molecular Biology	
Department of Molecular Microbiology	
Department of Molecular Genetics	Professor Hiroshi Nojima Ph.D.
Department of Oncogene Research	Professor Masato Okada Ph.D.
Department of Signal Transduction	Professor Nobuyuki Takakura M. D., Ph.D.
Department of Cellular Regulation	Professor Hiroaki Miki Ph.D.
Research Center for Infectious Disease Control	Head, Professor Toshihiro Horii Ph.D.
Department of Bacterial Infections	
Department of Molecular Protozoology	Professor Toshihiro Horii Ph.D.
Department of Viorogy	
Genome Infomation Research Center	Head, Professor Teruo Yasunaga Ph.D.
Department of Experimental Genome Research	Professor Masahito Ikawa Ph.D.
Department of Genome Informatics	Professor Teruo Yasunaga Ph.D.
Department of Infection Metagenomics	Professor Toshihiro Horii Ph.D.
International Research Center for Infectious Diseases	Head, Professor Toshihiro Horii Ph.D.
Department of Special Pathogens	
Laboratory of Clinical Research on Infectious Diseases	
Laboratory of Infection Cell Biology	SA Professor Yukako Fujinaga Ph.D.
Laboratory of Viral Infection	SA Associate Professor Eiji Morita Ph.D.
Department of Infectious Disease Control	
Laboratory of Genomic Research on Pathogenic Bacteria	SA Professor Tetsuya Iida Ph.D.
Laboratory of Viral Replication	SA Associate Professor Takeshi Kobayashi D.V.M., Ph.D.
Laboratory of Combined Research on Microbiology and Immunology	Associate Professor Hiroki Nagai Ph.D.
Pathogenic Microbes Repository Unit	SA Professor Tetsuya Iida Ph.D.
Animal Resource Center for Infectious Diseases	Head, Professor Masahito Ikawa Ph.D.
DNA-chip Development Center for Infectious Diseases	Head, Professor Hiroshi Nojima Ph.D.
Center for Genetic Analysis of Biological Responses	Head, Professor Masahito Ikawa Ph.D.
Office of Combined Program on Microbiology and Immunology	Head, Director Eisuke Mekada Ph.D.
Research Promotion Group	Associate Professsor Yoshiko Murakami M. D., Ph.D.
Education Promotion Group	Associate Professsor Hodaka Fujii M. D., Ph.D.
Thailand - Japan Research Collaboration Center on Emerging and Re-emerging Infections	Head, SA Professor Naokazu Takeda Ph.D.
Section of Bacterial Infections	SA Professor Shigeyuki Hamada D.D.S., Ph.D.
Section of Viral Infections	SA Professor Naokazu Tokeda Ph.D.
Mahidol-Osaka Center for Infectious Diseases	Head,Professor Yoshiharu Matsuura D.V.M., Ph.D. SA Associate Professor Tamaki Okabayashi D.V.M., Ph.D.
BIKEN Endowed Department of Dengue Vaccine Development	Endowed Chair Professor Eiji Konishi Ph.D.
Central Instrumentation Laboratory	Head,Professor Hiroaki Miki Ph.D.
Radioisotope Laboratory	Head,Professor Hiroaki Miki Ph.D.
Central Laboratory for Biological Hazardous Microbes	Head, Professor Tatsuo Shioda Ph.D.
Library	Head, Professor Hiroaki Miki Ph.D.
Administration	Head, Yoshinobu Ishikura

*SA : Specially Appointed

Staff

2014,04,01

Professor	16
Endowed Chair Professor	1
SA Professor	5
Associate Professor	14
SA Associate Professor	7
Lecturer	1
SA Lecturer	5
Assistant Professor	28
Endowed Chair Assistant Professor	1
SA Assistant Professor	7
Educational Support Staff	3
Technical Staff	7
Administrative Staff	22
SA Researcher	48
Part-time General & Technical staff	39
Total	203

SA：Specially Appointed

Graduate Students

2014,04,01

	Doctor Course	Master Course
Graduate School of Medicine	35	8
Graduate School of Science	1	1
Graduate School of Pharmaceutical Science	2	2
Graduate School of Dentistry	0	0
Graduate School of Frontier Biosciences	8	0
Total	46	11

Research Fellows & Research Students

2014,04,01

Special research students	4
Research Students	5
Visiting Research Scholars	3
JSPS Research Fellows	1
Total	13

Department of Molecular Bacteriology

Research Group

Professor	Yasuhiko Horiguchi, D.V. M., D. Agr. Sci.
Assistant Professor	Hiroyuki Abe, Ph.D.
Assistant Professor	Naoaki Shinzawa, Ph.D.
SA Researcher	Aya Fukui, Ph. D.
SA Researcher	Keiji Nakamura, D.V.M., Ph.D.

Research Projects

The objective of this department is to understand the molecular mechanisms by which pathogenic bacterial virulence factors affect host cell functions. Our present research interests include:

(1) Analysis of the structure and function of bacterial protein toxins

Bacterial protein toxins, which are the most poisonous substances on the earth, are known to act specifically on a particular cell and a particular biomolecule. To understand how bacterial toxins act so powerfully and specifically, we are analyzing their effects on the host at the systemic, tissue, cellular and molecular levels. The toxins currently under investigation are *Bordetella* dermonecrotic toxin, *Pasteurella* toxin, *Clostridium perfringens* enterotoxin, and *Escherichia coli* cytotoxic necrotizing factor. We are also analyzing the steric structure and molecular localization of the functional domains of the toxins. These approaches together will help to clarify the structure and function of these bacterial toxins.

(2) Analysis of the pathogenesis of whooping cough

Bordetella pertussis, a pathogenic bacterium, infects the human respiratory tract and causes whooping cough, which is characterized by paroxysmal cough. There are two significant questions about the pathogenesis of *B. pertussis* infection. First, why does *B. pertussis* only infect humans but not other mammals? Second, how do the bacteria cause the paroxysmal cough? We are examining the pathology of the disease and the function of bacterial virulence factors by using an animal model of the infection.

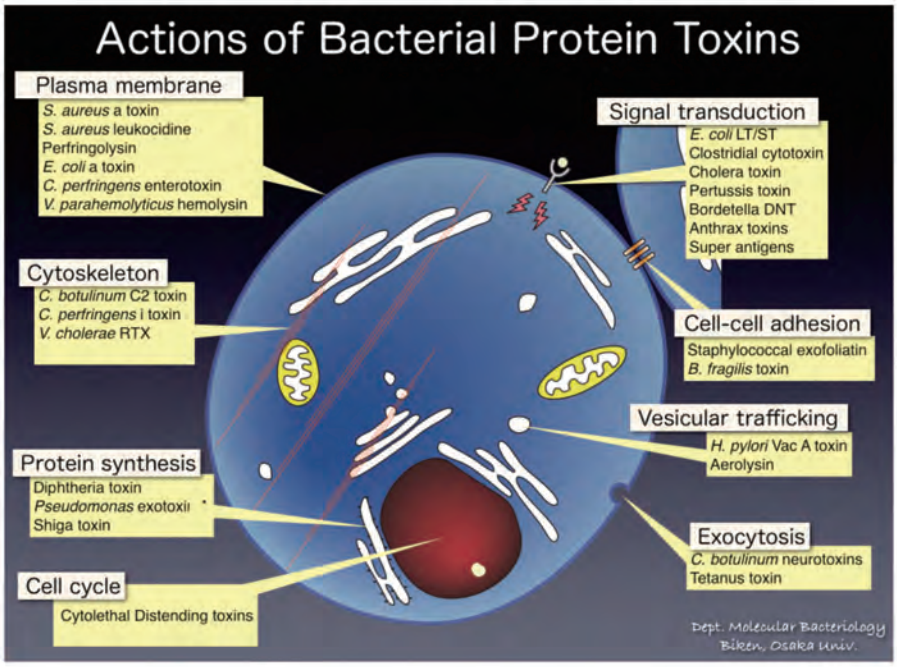


Fig. 1: Bacterial protein toxins with various activities that influence particular cellular functions. Many bacterial protein toxins exert their toxicity by modifying important functions of the host cells. The relevant physiological functions of the cells can be determined by dissecting the actions of the bacterial toxins.

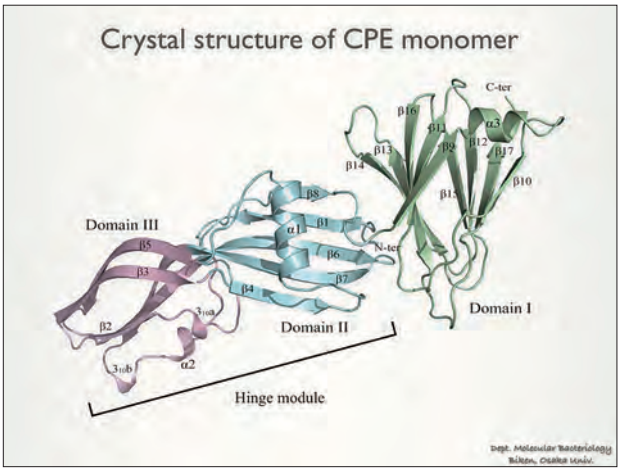


Fig. 2: Overall structure of *Clostidium perfringens* enterotoxin.

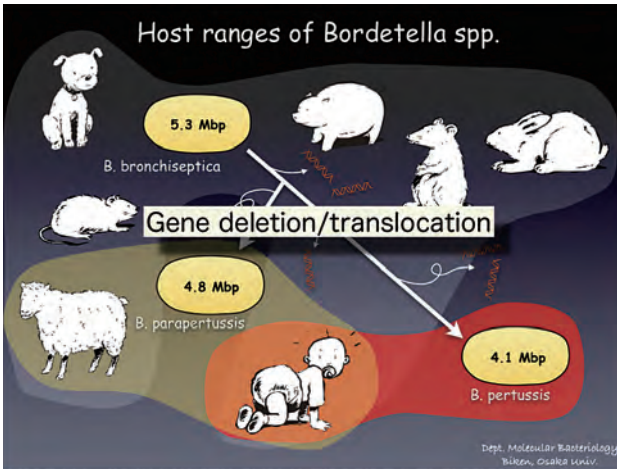


Fig. 3: *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica* are closely-related pathogenic bacteria.
B. bronchiseptica with the largest genome shows the broadest range of host while *B. pertussis* with the smallest genome shows the narrowest host range. It is believed that *B. pertussis* evolved from a lineage of *B. bronchiseptica* through deletion and/or translocation of a large number of genes.

Recent Publications

1. Substrate specificity of *Pasteurella multocida* toxin for α subunits of heterotrimeric G proteins. Orth JH, Fester I, Siegert P, Weise M, Lanner U, Kamitani S, Tachibana T, Wilson BA, Schlosser A, Horiguchi Y, Aktories K. ***FASEB J***. 2013 Feb;27(2):832-42.
2. Swine atrophic rhinitis caused by *Pasteurella multocida* toxin and *Bordetella* dermonecrotic toxin. Horiguchi Y. ***Curr Top Microbiol Immunol***. 2012;361:113-29.
3. Enzymatic actions of *Pasteurella multocida* toxin detected by monoclonal antibodies recognizing the deamidated α subunit of the heterotrimeric GTPase Gq. Kamitani S, Ao S, Toshima H, Tachibana T, Hashimoto M, Kitadokoro K, Fukui-Miyazaki A, Abe H, Horiguchi Y. ***FEBS J***. 2011 Aug;278(15):2702-12.
4. Crystal structure of *Clostridium perfringens* enterotoxin displays features of beta-pore-forming toxins. Kitadokoro K, Nishimura K, Kamitani S, Fukui-Miyazaki A, Toshima H, Abe H, Kamata Y, Sugita-Konishi Y, Yamamoto S, Karatani H, Horiguchi Y.***J Biol Chem***. 2011 Jun 3;286(22):19549-55.
5. Characterization of the membrane-targeting C1 domain in *Pasteurella multocida* toxin. Kamitani S, Kitadokoro K, Miyazawa M, Toshima H, Fukui A, Abe H, Miyake M, Horiguchi Y. ***J Biol Chem***. 2010 Aug 13;285(33):25467-75.

Department of Viral Infections

Research Group

Professor	Tatsuo Shioda, Ph.D.
Associate Professor	Emi E. Nakayama, M.D., Ph.D.
Assistant Professor	Jun-ichi Sakuragi, Ph.D.
Research Associate	Sayuri Sakuragi, Ph.D.
Research Associate	Eri Takeda, Ph.D.
Research Associate	Kahoru Taya, D.D.M., Ph.D.

The main focus of this department is to elucidate the molecular mechanisms of viral diseases, including human immunodeficiency virus (HIV). The following projects are currently underway.

(1) Antiretroviral factors

HIV does not establish a productive infection in any monkey other than the chimpanzee. This is thought to be due to the fact that inhibitors in simian lymphocytes act at the early stage (reverse transcription) of viral infection. To date, TRIM5α and TRIMCyp have been identified as such restriction factors. We have shown that differences in the amino acid sequences in the C-terminal domain of TRIM5α of different monkey species affect the species-specific restriction of retrovirus infection. We also found that sequence variations in the N-terminal half of the viral capsid protein determine viral sensitivity to TRIM5α-mediated restriction, which indicates that there is an interaction between TRIM5α and the virus capsid. In addition, we found that HIV-2 replication levels in infected individuals associate with capsid variations. Furthermore, a single nucleotide polymorphism in the human TRIM5α gene appears to affect the rate of HIV-1 infection. These results strongly suggest that TRIM5α acts as an anti-HIV factor in HIV-infected individuals, which raises the possibility of a novel anti-HIV strategy based on potentiating TRIM5α.

(2) Host factors that participate in the adverse effects of anti-retroviral drugs

Human genetic variations drive differences in human phenotypes. In collaboration with Thai groups, we are analyzing the relationship between human genomic variation and adverse effects of antiretroviral therapy, with the aim of establishing “tailor-made therapies” that will improve the quality of life of HIV-infected patients.

(3) Analysis of HIV-1 genome RNA dimerization

The genome of a retrovirus such as HIV-1 is a single-stranded, positive-sense RNA. The viral genome always exists as a dimer in virions. Genome dimerization plays important roles in various stages of the viral life cycle, including genome packaging and reverse transcription, and in the genome recombination processes that are involved in viral diversification. We have constructed HIV-1 Gag cleavage site mutants and are assessing the effect of these mutations on the virion maturation steps of Gag processing, RNA dimerization, virion morphology, and infectivity by steady-state observation. We found that the maturation of the viral RNA/protein plays a critical role in the acquisition of viral infectivity (Fig. 2A). We previously identified the region that is necessary and sufficient for HIV-1 genome dimerization in the virion (DLS: Dimer Linkage Structure). By further fine mapping of the DLS, we discovered the possibility of a long-range interaction, which has never been reported previously. We performed computer-assisted structural modeling and obtained new 3D models of the HIV-1 DLS that revealed a unique pseudoknot-like conformation (Fig. 2B). Since this conformation appears to be thermodynamically stable, forms a foundational skeleton for the DLS, and sterically restricts the spontaneous diversification of DLS conformations, its unique shape may potentially serve as a novel target for anti-HIV-1 therapies.

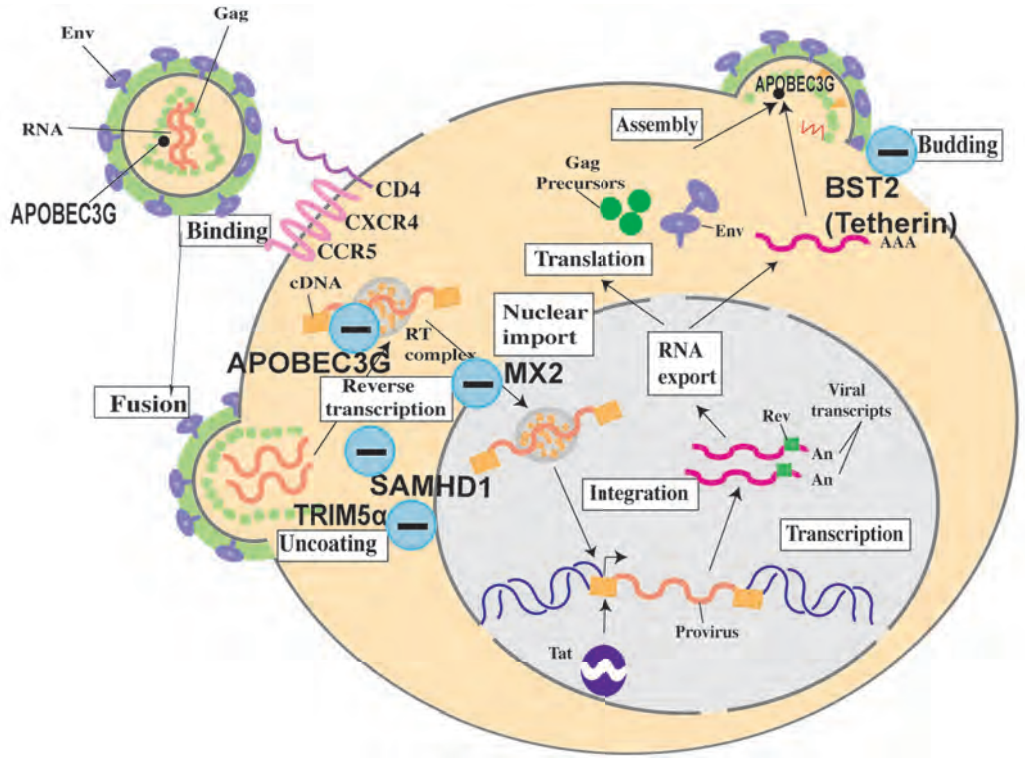


Fig. 1 Host factors that affect HIV replication. (-) indicates a negative regulator.

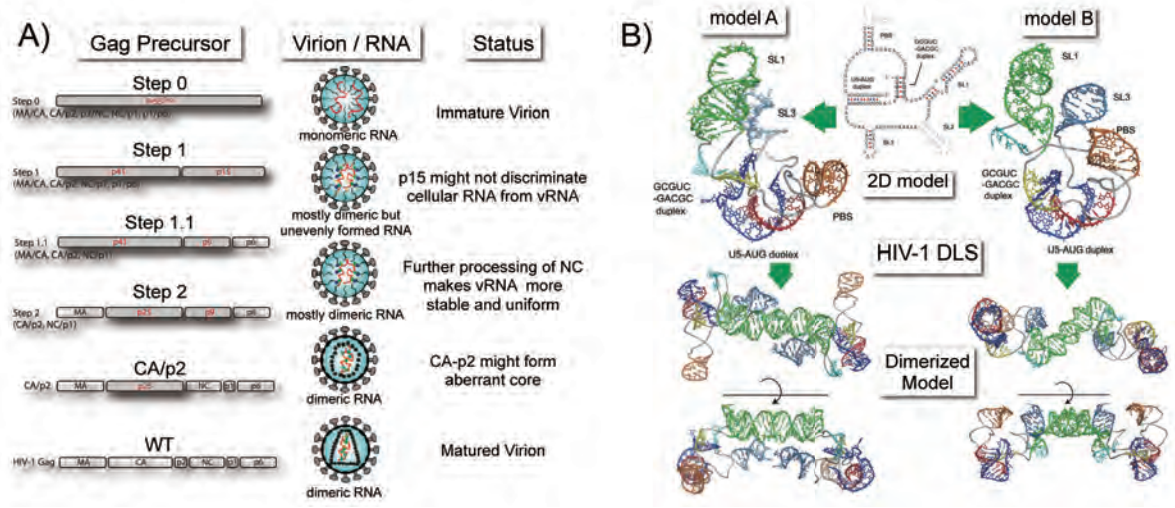


Fig. 2 HIV-1 genome RNA dimerization.

Recent Publications

1. Moderate restriction of macrophage-tropic human immunodeficiency virus type 1 by SAMHD1 in monocyte-derived macrophages. Taya K, Nakayama EE, Shioda T. ***PLoS ONE***. 2014 Mar5;9(3):e90969.
2. A naturally occurring single amino acid substitution in human TRIM5αlinker region affects its anti-HIV type 1 activity and susceptibility to HIV type 1 infection. Nakayama EE, Nakajima T, KaurG, Mimaya JI, Terunuma H, Mehra N, Kimura A, ShiodaT. ***AIDS Res Hum Retroviruses***. 2013 Jun;29(6):919-924.
3. Polymorphisms in Fas gene is associated with HIV-related lipoatrophy in Thai patients. Likanonsakul S, Rattanatham T, Feangvad S, Uttayamakul S, Prasithsirikul W, Srisopha S, Nitiyanontakij R, Tengtrakulcharoen P, Tarkowski M, Riva A, Nakayama EE, Shioda T. ***AIDS Res Hum Retroviruses***. 2013 Jan;29(1):142-50.
4. A proposal for a new HIV-1 DLS structural model. Sakuragi JI, Ode H, Sakuragi S, Shioda T, Sato H. ***Nucleic Aids Res***. 2012 Jun;40(11):5012-22.
5. TRIM5αand Species Tropism of HIV/SIV. Nakayama EE, Shioda T. ***Front Microbiol***. 2012 Jan 24;3:13.

Department of Molecular Virology

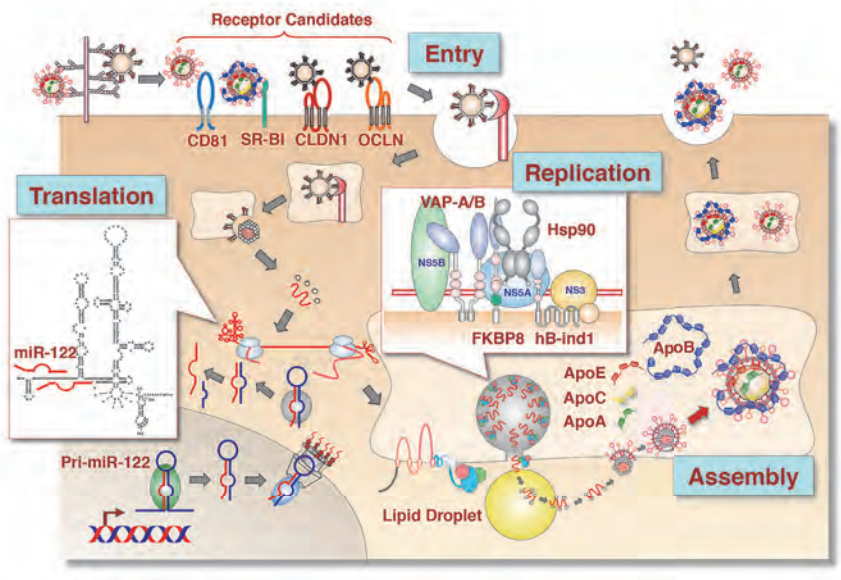
Research Group

Professor	Yoshiharu Matsuura	DVM, PhD
Assistant Professor	Toru Okamoto	PhD
Assistant Professor	Takasuke Fukuhara	MD, PhD
SA Researcher	Chikako Ono	PhD
SA Researcher	Sayaka Aizawa	PhD
SA Researcher	Masami Wada	PhD
SA Researcher	Puig-Basagoiti Francesc	PhD
Postdoctoral Fellow	Shinya Watanabe	DVM, PhD

We are working on understanding the molecular mechanisms of hepatitis C virus (HCV) entry, replication, assembly, and pathogenesis, and developing a novel virus vector for gene delivery.

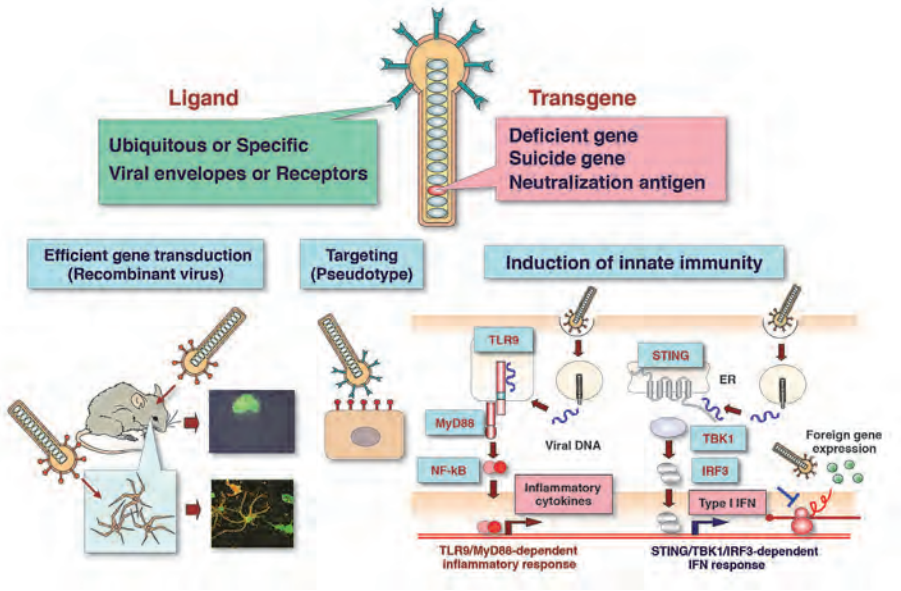
1. Studies on the molecular biology of HCV replication and pathogenesis

HCV infects over 130 million individuals worldwide and is one of the most common etiologic agents of chronic liver diseases, including liver cirrhosis and hepatocellular carcinoma (HCC). Although novel innovative anti-HCV agents achieve sustained virological responses in chronic hepatitis C patients, drug-resistant viruses emerge frequently. Therefore, host factors that are indispensable for HCV replication are the ideal targets for the development of new broad spectrum therapeutics against chronic hepatitis C that associate with a low possibility of emergence of breakthrough viruses against antiviral drugs. HCV internalizes into cells by endocytosis by interacting with several candidate receptors, including hCD81, SR-BI, Claudin1, and Occludin. After uncoating, the viral RNA is translated into a large precursor polyprotein composed of 3,000 amino acids, which is cleaved by signal peptidase, signal peptide peptidase, and virus-encoded proteases, resulting in at least 10 viral proteins. We have shown by using transgenic mice expressing the HCV core protein, which develop hepatic steatosis and HCC, that knocking out their PA28γ gene inhibits the development of liver steatosis and HCC. This suggests that PA28γ plays a crucial role in the development of the liver failure induced by HCV infection. Furthermore, we have shown that co-chaperones such as FKBP8 and hBind-1 recruit Hsp90 to the HCV replication complex by interacting with NS5A protein, thereby playing an important role in HCV replication. Liver-specific miR-122 enhances the translation of HCV RNA by interacting with its 5' UTR. Several non-hepatic cell lines permit HCV replication when miR-122 is expressed but infectious particles are not produced. This is probably because these cells have low expression levels of apolipoproteins such as ApoB and ApoE, unlike hepatic cell lines such as Huh7 and Hep3B. Indeed, Huh7 cells that are deficient in both ApoB and ApoE genes (DKO cells) exhibit severely impaired infectious particle formation, while exogenous expression of not only ApoE but also other apolipoproteins (including ApoA1, ApoA2, ApoC1, ApoC2, and ApoC3) rescues particle formation. This suggests that ApoA, ApoC and ApoE participate in HCV assembly in a redundant fashion. HCV belongs to the family of *Flaviviridae*, which includes flaviviruses such as Japanese encephalitis virus (JEV). Since there is a robust cell culture system and a small animal model for JEV, we are also investigating the replication and pathogenesis of JEV as a surrogate model for HCV.



2. Development of baculoviral vectors

Viral vectors are essential tools for studies on replication-deficient viral infectious diseases such as HCV. The development of novel viral vectors is also essential for future gene therapy. We are working on the baculovirus *Autographa californica* nucleopolyhedrovirus (AcNPV) to develop it into a versatile viral vector for gene delivery not only *in vitro* but also *in vivo*. AcNPV is an insect virus with a 134 kb double-stranded circular DNA genome. Due to its strong promoters, baculovirus is commonly used for the large-scale production of recombinant protein in insect cells. Baculovirus is also capable of entering a variety of mammalian cells and expressing foreign genes under the control of mammalian promoters without replicating its viral genome. Therefore, baculovirus is a useful viral vector, not only for the abundant expression of foreign genes in insect cells but also for efficient gene delivery to mammalian cells. AcNPV has a number of unique beneficial properties as a viral vector, including a large capacity for foreign gene incorporation, easy manipulation, and replication competence in insect cells combined with incompetence in mammalian cells. Therefore, it has a significantly lower likelihood of generating replication-competent revertants that express baculoviral gene products (which can lead to harmful immune responses against mammalian cells) than other viral vectors that are currently in use. Furthermore, intranasal inoculation with AcNPV induces a strong innate immune response that protects mice from lethal challenges of influenza viruses. We have shown that internalization of viral DNA by membrane fusion of the envelope glycoprotein is required to induce inflammatory cytokines and type I IFN *via* the TLR9-dependent and -independent pathway, respectively. Moreover, the induction of IFN-β through the STING/TBK1/IRF3 pathway attenuates the transgene expression in mammalian cells. Thus, innate immune responses induced by infection with AcNPV attenuate its transgene expression. This characteristic means that AcNPV may be useful for selective gene transduction into cells whose ability to evoke innate immunity is impaired by infection with various viruses.



Recent Publications

1. Novel permissive cell lines for a complete propagation of hepatitis C virus. Shiokawa M, Fukuhara T, Ono C, Yamamoto S, Okamoto T, Watanabe N, Wakita T, Matsuura Y. ***J Virol***. 2014 Mar 5. [Epub ahead of print]
2. Innate immune response induced by baculovirus attenuates transgene expression in Mammalian cells. Ono C, Ninomiya A, Yamamoto S, Abe T, Wen X, Fukuhara T, Sasai M, Yamamoto M, Saitoh T, Satoh T, Kawai T, Ishii KJ, Akira S, Okamoto T, Matsuura Y. ***J Virol***. 2014 Feb;88(4):2157-67.
3. Japanese encephalitis virus core protein inhibits stress granule formation through an interaction with Caprin-1 and facilitates viral propagation. Katoh H, Okamoto T, Fukuhara T, Kambara H, Morita E, Mori Y, Kamitani W, Matsuura Y. ***J Virol***. 2013 Jan;87(1):489-502.
4. Expression of microRNA miR-122 facilitates an efficient replication in nonhepatic cells upon infection with hepatitis C virus. Fukuhara T, Kambara H, Shiokawa M, Ono C, Katoh H, Morita E, Okuzaki D, Maehara Y, Koike K, Matsuura Y. ***J Virol***. 2012 Aug;86(15):7918-33.
5. CD44 participates in IP-10 induction in cells in which hepatitis C virus RNA is replicating, through an interaction with Toll-like receptor 2 and hyaluronan. Abe T, Fukuhara T, Wen X, Ninomiya A, Moriishi K, Maehara Y, Takeuchi O, Kawai T, Akira S, Matsuura Y. ***J Virol***. 2012 Jun;86(11):6159-70.

Department of Immunoparasitology

Research Group

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Assistant Professor（SUP）	Miwa Sasai, Ph.D.
Specially Appointed Assistant Professor	Hironori Bando, Ph.D.

In our immunoparasitology laboratory, the apicomplexan protozoan parasite called *Toxoplasma gondii* serves as a research model for analyzing host-to-pathogen interactions.

1) What is Toxoplasma?

T. gondii is a eukaryotic pathogen that causes life-threatening toxoplasmosis, a condition that includes encephalitis in immunocompromised individuals such as those who are being treated by chemotherapy or have AIDS. It also causes congenital diseases on primary infection during pregnancy in humans and animals. It is an obligate intracellular eukaryotic parasite that can proliferate exclusively inside a parasitophorous vacuole, which is formed during host–cell invasion. Taxonomically, *T. gondii* belongs to the phylum of Apicomplexa, which is characterized by the presence of an apical complex with secretory organelles such as conoids, micronemes, and rhoptries (Figure 1A). The rhoptries, which are large bulb-shaped organelles, contain a variety of proteins that are secreted into the host cytoplasm or in the forming parasitophorous vacuole during parasite entry. These proteins then co-opt the host cell for parasite growth and survival (Figure 1B and C).

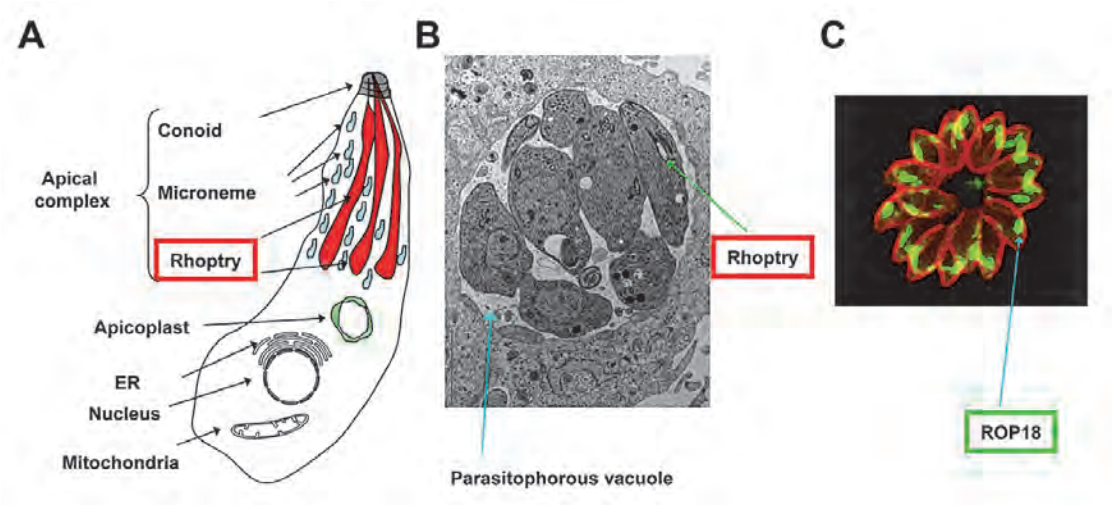

Figure 1 Toxoplasma gondii and the rhophry protein

（A） A schematic figure of *T. gondii*. The apical complex includes rhoptries (red)。
（B） An electron microscopic picture of *T. gondii* residing in a parasitophorous vacuole (blue) in an infected host cell.
（C） Immunofluorescent picture of *T. gondii* to detect a rhoptry protein ROP18.

2) Analysis of host cell-autonomous innate immunity against *T. gondii*.

Interferon-γ (IFN-γ) is essential for host defense against intracellular pathogens. When innate immune cells are stimulated by IFN-γ, they up-regulate ~2000 effector genes such as immunity-related GTPases, including the p65 guanylate-binding protein (GBP) family genes. We showed that a cluster of GBP genes is required for host cellular immunity against *T. gondii* by first generating mice that lack all six GBP genes on chromosome 3 (Gbp^{chr3}) by targeted chromosome engineering. The mice lacking Gbp^{chr3} were highly susceptible to *T. gondii* infection, resulting in increased parasite burden in immune organs. Furthermore, Gbp^{chr3}-deleted macrophages were not able to induce IFN-γ-mediated suppression of intracellular *T. gondii* growth. Although the IFN-γ-induced oxidative response and the localization of autophagy protein Atg4b were normal, deletion of Gbp^{chr3} impaired the recruitment of IFN-γ-inducible p47 GTPase Irgb6 to the parasitophorous vacuole. In addition, some members of Gbp^{chr3} restored the protective response against *T. gondii* in Gbp^{chr3}-deleted cells. Our results suggest that the Gbp^{chr3} proteins redundantly play a pivotal role in anti-*T. gondii* host defense by controlling IFN¬-γ-mediated Irgb6-dependent cellular innate immunity (Figure 2).

GBPs also participate in anti-bacterial responses via autophagy. An essential autophagy protein Atg5 was previously shown to play a critical role in cell-autonomous immunity against *T. gondii*. However, the involvement of other autophagy proteins remains unknown. Here, we show that some essential autophagy proteins, but not all, participate in anti-*T. gondii* cellular immunity by recruiting IFN-γ-inducible GTPases. In Atg7- or Atg16L1-deficient cells, IFN-γ-induced suppression of *T. gondii* proliferation and recruitment of the IRG Irgb6 and GBPs are profoundly impaired. However, cells lacking the other essential autophagy proteins Atg9a and Atg14 can mediate the anti-*T. gondii* response and recruit Irgb6 and GBPs to the parasites. Thus, mouse autophagy components Atg7 and Atg16L1, but not Atg9a and Atg14, participate in the IFN-γ-induced recruitment of immunity-related GTPases to the intracellular pathogen (Figure 2).

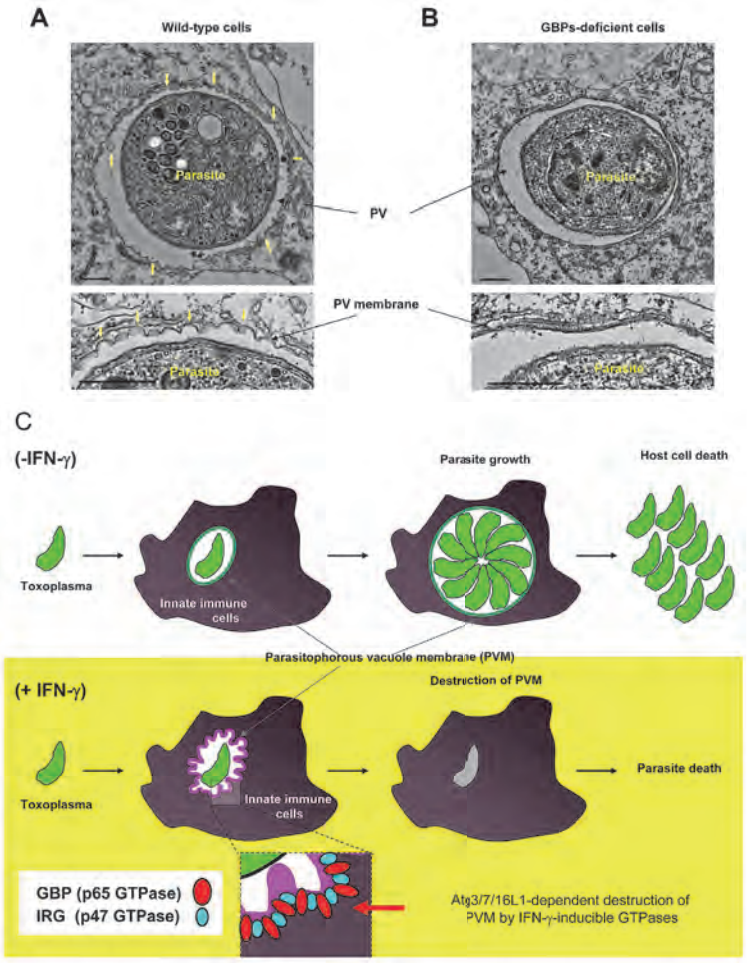

Figure 2 Role of IFN-γ-induced GBPs in anti-T. gondii cell-autonomous immunity

（A）Structure of PV membrane was destroyed in IFN-γ-treated wild-type cells, but not in （B） GBPs-deficient cells.

Recent Publications

1. Ohshima J, Lee Y, Sasai M, Saitoh T, Ma JS, Kamiyama N, Matsuura Y, Pann-Ghill S, Hayashi M, Ebisu S, Takeda K, Akira S, Yamamoto M. Role of the mouse and human autophagy proteins in IFN-γ-induced cell-autonomous responses against Toxoplasma gondii. ***J Immunol***. 2014, 192: 3328-3335.
2. Yamamoto M, Okuyama M, Ma JS, Kimura T, Kamiyama N, Saiga H, Ohshima J, Sasai M, Kayama H, Okamoto T, Huang DS, Soldati-Favre D, Horie K, Takeda J, Takeda K. A cluster of interferon-γ-inducible p65 GTPases plays a critical role in host defense against Toxoplasma gondii. ***Immunity***. 2012, 37:302-313.
3. Yamamoto M, Ma JS, Mueller C, Kamiyama N, Saiga H, Kubo E, Kimura T, Okamoto T, Okuyama M, Kayama H, Nagamune K, Takashima S, Matsuura Y, Soldati-Favre D, Takeda K. ATF6β is a host cellular target of the Toxoplasma gondii virulence factor ROP18. ***J Exp Med***. 2011, 208:1533-1546.
4. Yamamoto M, Standley DM, Takashima S, Saiga H, Okuyama M, Kayama H, Kubo E, Ito H, Takaura M, Matsuda T, Soldati-Favre D, Takeda K. A single polymorphic amino acid on Toxoplasma gondii kinase ROP16 determines the direct and strain-specific activation of Stat3. ***J Exp Med***. 2009, 206: 2747-2760.

Department of Molecular Immunology

Research Group

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Associate Professor	Teruhito Yasui, Ph.D
Assistant Professor	Shuhei Sakakibara, PhD
SA Researcher	Takeharu Minamitani, PhD
SA Researcher	Chiau-Yuang Tsai, PhD

1) Molecular mechanisms in B cell differentiation and survival

During effective humoral immune responses, the B cell receptor (BCR)- and CD40-mediated signaling pathways are activated and drive B cells to differentiate into antibody-secreting cells and memory B cells. Our goal is to understand the mechanisms that regulate humoral immune responses by molecularly characterizing the signal mediators.

During our search for novel signal molecules downstream of BCR, we found that Protein Kinase N1 (PKN1) is a negative regulator of BCR signals. PNK1 interacts with AKT kinase and inhibits its activity. In PKN1$^{-/-}$ mice, the selection of high affinity B cells is impaired, although germinal center (GC) formation is accelerated. Thus, the fine tuning of the BCR signal by PKN1 is indispensable for the quality control of humoral immune responses.

2) Molecular mechanisms in the immune pathology caused by host–pathogen interactions: Epstein-Barr virus (EBV)-encoded latent membrane proteins (LMP) 1 and 2a affect B cell survival and selection.

EBV infects human B cells, particularly the memory B cell population. This promotes its persistent infection. Of the various viral genes that are expressed in EBV-infected B cells, LMP1 and 2a constitutively activate and mimic the CD40 and BCR signals, respectively. However, it has been largely unknown what impact these virus-derived molecules have on the in vivo activation and differentiation of B cells. For this reason, we generated transgenic mice that conditionally express LMP1 or LMP2a in GC B cells.

Although the spleen of LMP2a Tg mice has normal GCs, the titers of antigen-specific antibodies are significantly low. Sequencing analysis of the immunoglobulin gene of the antigen-specific B cells revealed impaired affinity maturation after immunization. In addition, LMP2a Tg mice exhibit significantly accelerated plasma cell differentiation. Thus, EBV LMP2a reduces the threshold for high affinity B cell selection. This may promote the latent infection of EBV in memory B cells (Figure 1).

Unlike LMP2a, the expression of LMP1 in B cells strongly inhibits GC formation. Interestingly, GC formation and antibody responses are also impaired in the chimeric mice resulting from co-transfer of LMP1 Tg and wild-type-derived bone marrow cells. This suggests that LMP1^{+} B cells inhibit neighboring wild-type cells. Thus, LMP1 may contribute to EBV persistence by suppressing host humoral responses.

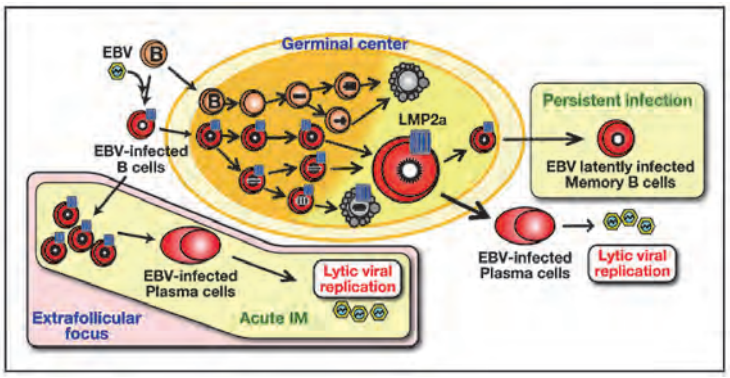

Figure 1. EBV LMP2a reduces the threshold for selection of high-affinity B cells and may contribute to EBV latent infection. Activation of the BCR signaling pathway by EBV LMP2a results in reduced threshold for selection of high-affinity B cells. (IM: infectious mononucleosis)

3) Understanding the mechanisms behind the emergence of auto- and polyreactive B cells and their physiological and/or pathological roles

A significant proportion of the circulating IgG^{+} memory B cells in healthy individuals are polyreactive clones that are reactive to multiple self-components. Since the immunoglobulin genes of these clones have multiple somatic hypermutations (SHM), it is expected that polyreactive IgG^{+} memory B cells are post-GC B cells. The inducers of polyreactive B cells have not been extensively investigated. However, the mouse infection model of MHV68 (murine γ-herpesvirus 68) provides strong evidence

that polyreactivity emerges and is selected from splenic IgG$^+$ GC B cells that harbor somatically mutated immunoglobulins. Therefore, MHV68 infection is an ideal experimental system for analyzing polyreactive B cell generation (Figure 2).

Is polyreactive antibody related to autoimmune diseases in humans? When we analyzed the polyreactivity of plasma cells from systemic erythematosus lupus (SLE) patients and healthy donors, both had similar frequencies of polyreactive clones. This indicates that at least the emergence of polyreactive clones does not correlate with SLE disease activity. By contrast, the patients with acute SLE had high frequencies (~5%) of anti-nuclear antibody (ANA)-producing cell clones. ANA production is generally used to diagnose SLE. These ANA-producing cell clones are not polyreactive and recognize each nuclear autoantigen in a specific manner. We are currently analyzing the ANA-encoding immunoglobulin genes by next generation sequencing (NGS) technologies to understand how autoreactive B cells in humans are generated and selected.

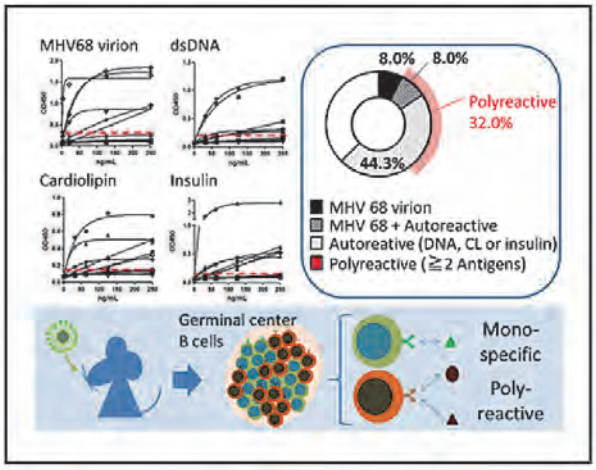

Figure 2. Polyreactive clones emerges in the splenic IgG$^+$ germinal center B cells during MHV68 infection. Single-cell-based cloning of immunoglobulins followed by ELISA revealed that ~30% of the splenic IgG$^+$ germinal center B cells showed polyreactivity. MHV68 infection will provide ideal experimental system for study of polyreactive B cell generation.

Recent Publications

1. Kumanogoh, A., and Kikutani H. Immunological functions of the neuropilin and plexin as receptors for semaphorins. ***Nat Rev Immunol***. 2013 Nov;13(11):802-14.
2. Tada S, Yasui T, Nakatsuji Y, Okuno T, Koda T, Mochizuki H, Sakoda S, Kikutani H. BAFF controls neural cell survival through BAFF receptor. ***PLoS One***. 2013 Jul 29;8(7):e70924.
3. Morihana T, Goya S, Mizui M, Yasui T, Prasad DV, Kumanogoh A, Tamura M, Shikina T, Maeda Y, Iwamoto Y, Inohara H, Kikutani H. An inhibitory role for Sema4A in antigen-specific allergic asthma. ***J Clin Immunol***. 2013 Jan;33(1):200-9.
4. Yasui T, Sakakibara-Yada K, Nishimura T, Morita K, Tada S, Mosialos G, Kieff E, Kikutani H. Protein kinase N1, a cell inhibitor of Akt kinase, has a central role in quality control of germinal center formation. ***Proc Natl Acad Sci U S A***. 2012 Dec 18;109(51):21022-7.
5. Tada, S., Okuno T., Yasui T., Nakatsuji N., Sugimoto T., Kikutani H., and Sakoda S. Deleterious effects of lymphocytes at the early stage of neurodegeneration in an animal model of amyotrophic lateral sclerosis. ***J Neuroinflammation***. 2011 Feb 23;8(1):1

Department of Immunoregulation

Research Group		
	Professor (SUP)	Taroh Kinoshita, D. Med. Sc.
	Associate Professor	Yusuke Maeda, M.D., D. Med. Sc.
	Associate Professor (SUP)	Yoshiko Murakami, M.D., D. Med. Sc.
	Assistant Professor	Yuko Tashima, D. Med. Sc.
	SA Assistant Professor	Noriyuki Kanzawa, D. Med. Sc.

Research Projects

Three research fields in diseases, immune defense, and homeostasis are our main focus. The first examines how glycosylphosphatidylinositol-anchored proteins are synthesized, processed, transported and secreted, and how defects in these processes lead to the onset and pathology of diseases. The second examines how the pH of intracellular organelles is regulated and how these pH values regulate many biological functions. The third aims to identify the components and their functions in membrane contact sites between closely apposing organelles where lipids and ions are transported.

1) Biogenesis, transport and remodeling of GPI-anchored proteins (GPI-APs).

Glycosylphosphatidylinositol (GPI) is a glycolipid that consists of phosphatidylinositol, glucosamine, mannoses and phosphoethanolamines. It acts as a lipid anchor for various plasma membrane proteins. GPI-APs play important roles in host self-defense, intercellular signal transduction, and other important processes. In addition, some GPI-APs function as receptors for certain viruses and toxins. The GPI-anchor is widely distributed and conserved in various eukaryotes, and is essential for the development of higher animals, as well as for the growth of yeasts and protozoan parasites. The modification of proteins due to the attachment of the GPI-anchor functions as a protein localization and sorting signal. Our current project is to identify and clarify the functions of all the genes involved in the biosynthesis of the GPI-anchor in the endoplasmic reticulum (ER) (PIG genes; PhosphatidylInositol Glycan), and in the sorting and localization of GPI-APs after their anchorage with GPI (PGAP genes; Post GPI-Attachment to Proteins). We expect that these studies will reveal why many proteins are modified by the GPI-anchor.

PIG genes are involved in the biosynthesis of the GPI-anchor in the ER. During this biosynthetic process, an alkyl-lipid generated in the peroxisome is utilized. Thereafter, GPI-APs are transported to the plasma membrane and are enriched in rafts. PGAP genes are involved in these latter processes. PGAP1 and PGAP5, which localize in the ER, and PGAP2 and PGAP3, which localize in the Golgi, are involved in the lipid or glycan remodeling of the GPI-anchor. Remodeling in the ER is necessary for the efficient transport of GPI-APs to the Golgi *via* their association with p24 family proteins. We found that this remodeling process affects the sorting of GPI-APs because it alters the physical characteristics of the GPI-anchor. The genes in yellow boxes are currently known to be responsible for IGD, and those in blue boxes are responsible for PNH (see below).

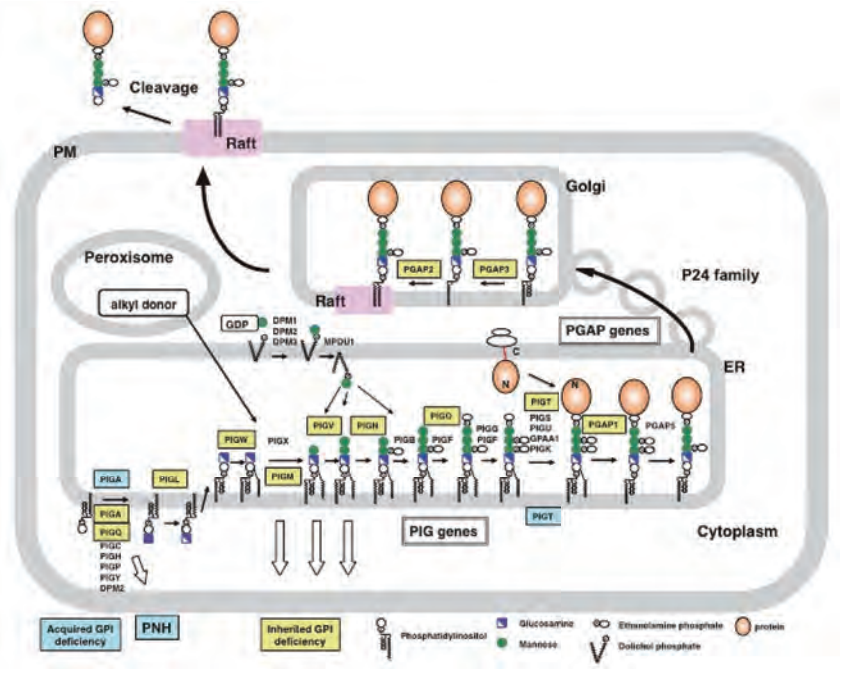

Fig. 1 GPI-anchor biosynthesis and the transport/remodeling of GPI-APs.
PIG genes are involved in the biosynthesis of the GPI-anchor in the ER. During the biosynthesis, an alkyl-lipid generated in the peroxisome is utilized. Thereafter, GPI-APs are transported to the plasma membrane and enriched in rafts. PGAP genes are involved in these later processes. PGAP1 and PGAP5, which localize in the ER, and PGAP2 and PGAP3, which localize in the Golgi, are involved in the lipid or glycan remodeling of the GPI-anchor. Remodeling in the ER is necessary for efficient transport of GPI-APs to the Golgi by association with p24 family proteins. We found that these remodeling affects the sorting of GPI-APs because it alters the physical characteristics of the GPI-anchor. The genes in yellow boxes are currently known to be responsible for IGD and those in blue boxes are responsible for PNH.

2) Molecular genetics of GPI deficiencies.

In collaboration with our colleagues in England, we identified a disease called inherited GPI deficiency (IGD) that is caused by the PIGM mutation. PIGM is a mannosyltransferase-encoding gene that plays an essential role in GPI biosynthesis. The recent analysis of the exomes of patients with IGD with the next generation sequencer has already revealed twelve kinds of IGD to

date. To further analyze IGDs, we are collaborating with many clinicians and scientists in Japan and abroad. As complete GPI deficiency is embryonically lethal, patients with IGD due to PIG gene mutations only have partial GPI deficiencies. The patients with IGD that is due to remodeling defects even have null mutations. The symptoms of IGD vary depending on the defective genes and the degree of defectiveness. The main symptoms are intellectual disability and seizures that are often accompanied by hyperphosphatasia, abnormal facial features, brachytelephalangy and sometimes other organ anomalies. IGD has been proven to be the main cause of Mabry syndrome/hyperphosphatasia mental retardation syndrome and CHIME syndrome. IGD has also been found in Early Onset Epileptic Encephalopathies such as Ohtahara syndrome and West syndrome. Good diagnostic markers for IGD are the presence of hyperphosphatasia and the decreased flow cytometric expression of granulocyte CD16, which is a GPI-AP . We will establish the diagnostic criteria by accumulating patient information and seek effective therapies.

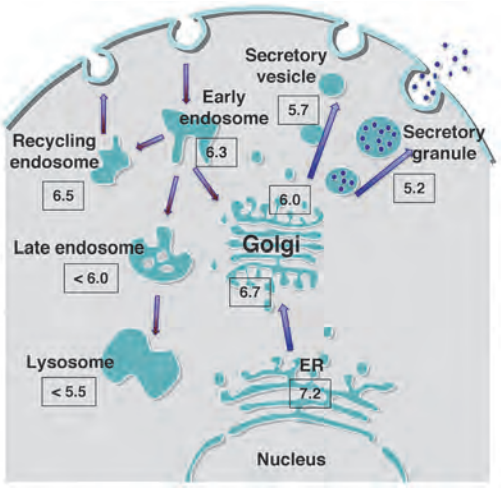

Fig. 3 pH regulation of intracellular organelles
The luminal pHs of organelles in secretory and endocytosis pathways are acidic as indicated in the boxes (the values are approximate).

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired GPI deficiency that is caused by somatic mutation of the X-linked PIGA gene in hematopoietic stem cells. However, it was recently reported that PNH can also be caused by somatic mutation of one allele of the PIGT gene in combination with a germline mutation in the other allele. This showed that PIGA is not always the responsible gene in PNH, although this situation is rarely seen. PNH is a hematopoietic disease that is characterized by the expansion of clonal cells that are defective in GPI biosynthesis. As a result, abnormal erythrocytes lacking the GPI-APs CD59 and DAF/CD55 (which inhibit the activation of complement) predominate, leading to massive hemolysis due to excessive complement attack during infections and other events. We are presently analyzing the pathogenesis of PNH by seeking the mechanism behind the clonal expansion of GPI-deficient hematopoietic stem cells.

3) pH regulation in acidic organelles and its biological significance, and analysis of membrane contact sites between organelles.
Each intracellular organelle is compartmentalized by lipid bilayers and possesses a characteristic environment, proteins and lipids that allow it to fully exercise its functions. A major environment factor is pH. The lumens of organelles in the secretory and endocytosis pathways are known to have acidic environments. This lumen acidity is believed to play critical roles in many biological processes in cells, including the transport, processing, and glycosylation of proteins and lipids. It is also known to influence the morphology of organelles and to participate in the onset and pathology of diseases that are characterized by pH regulation defects. However, the mechanisms behind the latter abnormal phenotypes are largely not known. Recently, we reported for the first time the establishment of mutant cells in which the Golgi pH is dysregulated. Analysis of these cells led to the identification of the gene that bore the mutations and was responsible for the deranged functions of the mutant cells. This project aims to clarify the mechanisms and biological significance of acidic pH regulation by establishing and analyzing additional mutant cells that are defective in organelle pH regulation and to improve our understanding about how such mutations can lead to pathology, as this will facilitate the identification of effective drugs. As an offshoot from the above study, we are also analyzing the components and functions of the membrane contact sites between closely apposed organelle membranes where lipids and ions are transported.

Recent Publications

1. Implication of lipid moiety in oligomerization and immunoreactivities of GPI-anchored proteins. Seong J, Wang Y, Kinoshita T, Maeda Y. ***J Lipid Res***. 2013 Apr;54(4):1077-91
2. Mechanism for release of alkaline phosphatase caused by glycosylphosphatidylinositol deficiency in patients with hyperphosphatasia mental retardation syndrome. Murakami Y, Kanzawa N, Saito K, Krawitz PM, Mundlos S, Robinson PN, Karadimitris A, Maeda Y, Kinoshita T. ***J Biol Chem***. 2012 Feb 24;287(9):6318-25.
3. Sorting of GPI-anchored proteins into ER exit sites by p24 proteins is dependent on remodeled GPI. Fujita M, Watanabe R, Jaensch N, Romanova-Michaelides M, Satoh T, Kato M, Riezman H, Yamaguchi Y, Maeda Y, Kinoshita T. ***J Cell Biol***. 2011 Jul 11;194(1):61-75.
4. Peroxisome dependency of alkyl-containing GPI-anchor biosynthesis in the endoplasmic reticulum. Kanzawa N, Maeda Y, Ogiso H, Murakami Y, Taguchi R, Kinoshita T. ***Proc Natl Acad Sci U S A***. 2009 Oct 20;106(42):17711-6.
5. GPI glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi. Fujita M, Maeda Y, Ra M, Yamaguchi Y, Taguchi R, Kinoshita T. ***Cell***. 2009 Oct 16;139(2):352-65.

Department of Host Defense

Research Group

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Associate Professor	Tatsuya Saitoh, Ph.D.
Assistant Professor	Takashi Satoh, Ph.D.
SA Assistant Professor (SUP)	Kenta Maruyama, M.D., Ph.D.

Main text

We study the innate immune system, which is an evolutionally conserved host defense mechanism against microbes. Pattern-recognition receptors (PRRs) play a critical role in the induction of innate immunity. PRRs detect various microorganisms ranging from bacteria to fungi, protozoa and viruses, and induce the production of inflammatory factors such as cytokines and interferons. However, PRRs also detect environmental irritants and host-derived stimulatory factors, and can cause the development of inflammatory diseases. Thus, the innate immune system can be a double-edged sword for the host. To gain a deeper understanding of the innate immune system, we have examined a mechanism that regulates PRR-mediated innate immune responses and a novel function of innate immune cells.

1. Microtubule-dependent activation of the NLRP3-inflammasome

NLRP3 forms an inflammasome with its adaptor ASC, and its excessive activation can cause inflammatory diseases. However, little was known about the mechanisms that control the assembly of the inflammasome complex. We found that microtubules mediate the assembly of the NLRP3 inflammasome (Misawa T et al., *Nat Immunol*, 2013). Thus, inducers of the NLRP3 inflammasome cause aberrant mitochondrial homeostasis, which in turn diminishes the concentration of the coenzyme NAD. This then inactivates the NAD-dependent protein α-tubulin deacetylase sirtuin 2, thereby causing acetylated α-tubulin to accumulate. The acetylated α-tubulin mediates the dynein-dependent transport of mitochondria and the subsequent apposition of ASC on mitochondria to NLRP3 on the endoplasmic reticulum. Therefore, the activation of the entire NLRP3 inflammasome requires both the direct activation of NLRP3 and the creation by microtubules of optimal sites for signal transduction (Figure 1).

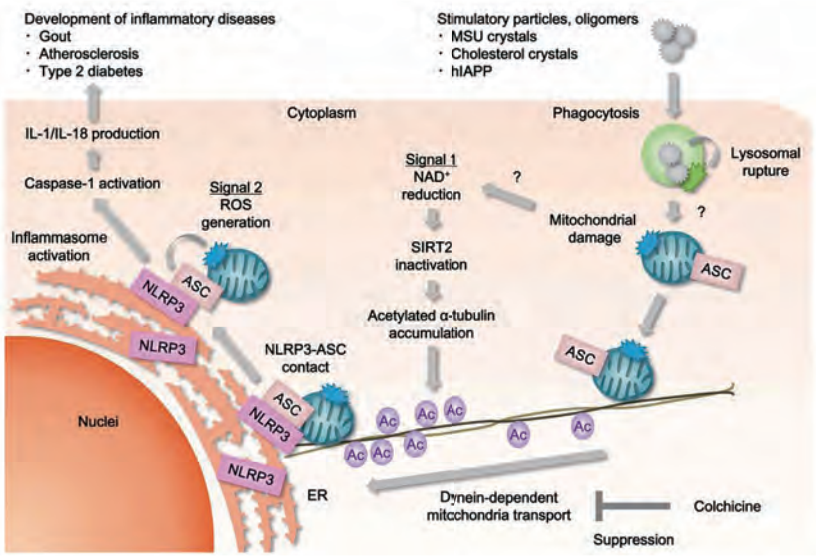


Figure 1. Activation of the NLRP3-inflammasome.

2. Involvement of cathepsin D in the immunomodulatory effects of synthetic double-stranded RNA

RIG-I-like receptors (RLRs) detect double-stranded (ds) RNA and induce antiviral immune responses. RLRs also mediate the adjuvant effect of polyinosinic-polycytidylic acid (poly IC), a synthetic dsRNA. We revealed the mechanisms of the poly IC-induced innate immune responses (Zou J et al., *Immunity*, 2013). Poly IC is taken up by dendritic cells (DCs) and induces lysosomal destabilization, which causes the release of cathepsin D into the cytoplasm from the lysosome. Cathepsin D then interacts with IPS-1, an adaptor molecule for RLRs. This interaction activates the RLR-dependent signaling pathway, as it facilitates the cathepsin D-mediated cleavage of caspase-8 and the activation of the transcription factor NF-κB, thus resulting

in enhanced cytokine production. Further recruitment of the kinase RIP-1 to the IPS-1-cathepsin D complex initiates the necroptosis of a small number of DCs. The HMGB1 released by the dying cells then enhances the IFN-β production caused by poly IC. Collectively, these findings indicate that cathepsin D-triggered necroptosis is a mechanism that propagates the adjuvant efficacy of poly IC (Figure 2).

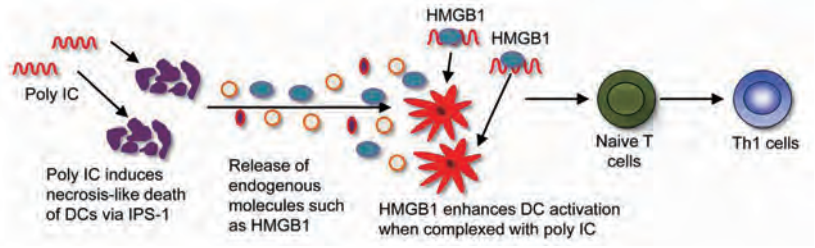


Figure 2. Activation of dendritic cells by poly IC.

3. A novel role of macrophages in the maintenance of adipose tissues

Macrophages consist of at least two subgroups, M1 and M2. Whereas M1 macrophages are pro-inflammatory and play a central role in host defense, M2 macrophages associate with anti-inflammatory reactions and tissue remodeling, among other processes. Genome-wide association studies in humans suggest that TRIB1 participates in lipid metabolism. We found that Trib1 is critical for the differentiation of tissue-resident macrophages, which we term M2-like macrophages (Satoh T et al., *Nature*, 2013). Trib1-deficiency results in severe reductions of M2-like macrophages in various organs, including adipose tissues. These defects in macrophage differentiation are caused by aberrant expression of C/EBPα in Trib1-deficient bone marrow cells. Mice lacking Trib1 in hematopoietic cells have a diminished adipose tissue mass that is accompanied by evidence of increased lipolysis. In response to a high-fat diet, mice lacking Trib1 in hematopoietic cells develop hypertriglyceridaemia and insulin resistance. Therefore, Trib1 is critical for adipose tissue maintenance and suppression of metabolic disorders by controlling the differentiation of tissue-resident M2-like macrophages (Figure 3).

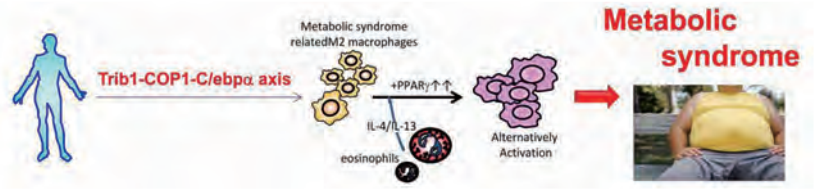


Figure 3. A role of M2-like macrophages.

Our future research will aim to generate a comprehensive understanding of the innate immune system and to develop an effective treatment for immune-related inflammatory diseases.

Recent Publications

1. Uehata T, Iwasaki H, Vandenbon A, Matsushita K, Hernandez-Cuellar E, Kuniyoshi K, Satoh T, Mino T, Suzuki Y, Standley DM, Tsujimura T, Rakugi H, Isaka Y, Takeuchi O, Akira S. Malt1-induced cleavage of regnase-1 in CD4 (+) helper T cells regulates immune activation. ***Cell***. 2013 May 23;153(5):1036-49.
2. Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, Akira S. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. ***Nat Immunol***. 2013 May;14(5):454-60.
3. Zou J, Kawai T, Tsuchida T, Kozaki T, Tanaka H, Shin KS, Kumar H, Akira S. Poly IC triggers a cathepsin D- and IPS-1-dependent pathway to enhance cytokine production and mediate dendritic cell necroptosis. ***Immunity***. 2013 Apr 18;38(4):717-28.
4. Satoh T, Kidoya H, Naito H, Yamamoto M, Takemura N, Nakagawa K, Yoshioka Y, Morii E, Takakura N, Takeuchi O, Akira S. Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. ***Nature***. 2013 Mar 28;495(7442):524-8.
5. Maruyama K, Fukasaka M, Vandenbon A, Saitoh T, Kawasaki T, Kondo T, Yokoyama KK, Kidoya H, Takakura N, Standley D, Takeuchi O, Akira S. The transcription factor Jdp2 controls bone homeostasis and antibacterial immunity by regulating osteoclast and neutrophil differentiation. ***Immunity***. 2012 Dec 14;37(6):1024-36.

Department of Cell Biology

Research Group

Professor	Eisuke Mekada, Ph. D.
Associate Professor	Ryo Iwamoto, Ph. D.
Assistant Professor	Hiroto Mizushima, Ph. D
Postdoctoral fellow	Takashi Nakamura, Ph. D.
Postdoctoral fellow	Tomoya Hikita, Ph. D.

We are studying the mechanisms of cell growth and differentiation that involve growth factors and adhesion molecules presented at cell–cell contact sites. In particular, we are focusing on the mode of action of HB-EGF, a membrane-anchored EGF family of growth factors, and the tetraspanin family, which are the molecules to which HB-EGF binds. These proteins function in morphogenesis and tissue maintenance and repair by regulating cell proliferation, migration and adhesion. They are also involved in the growth, invasion and metastasis of cancer cells.

1) Mode of action of HB-EGF

HB-EGF is a member of the EGF family of growth factors, and binds to and activates EGFR and ErbB4. It is synthesized as proHB-EGF, a membrane-anchored precursor protein, and is cleaved on the cell surface to yield the soluble growth factor (sHB-EGF). The conversion of proHB-EGF into the soluble form is critical for the activity of this growth factor, and therefore this process is tightly regulated. HB-EGF is secreted by various tissues and cells and functions in several physiological processes. For example, it maintains heart muscle function, suppresses cell proliferation in heart valve and lung alveolar development, promotes cell migration in wound healing and eyelid closure, supports blastocyst attachment to the uterus during implantation, and promotes cell proliferation in skin hyperplasia. ProHB-EGF is not only a precursor of the soluble form, it is also a biologically active molecule that regulates the growth of neighboring cells in a juxtacrine fashion. How is the conversion of the membrane-anchored form into the soluble form regulated? How does HB-EGF function in the manifold physiological processes that are dependent on this molecule? What roles do sHB-EGF and proHB-EGF play in such physiological processes? Do they participate in pathological processes? These questions are currently being analyzed at the molecular level.

In valve development, HB-EGF is expressed and secreted from endocardial cells. Secreted HB-EGF associates with heparan sulfate proteoglycans (HSPGs) in the valve mesenchyme (*upper right*). When EGFR on the surface of mesenchymal cells is activated by the HB-EGF-HSPG complex, this receptor transduces inhibitory signals for mesenchymal cell proliferation (*lower left*). Knock-in mice expressing a mutant HB-EGF that cannot bind to HSPGs (ΔHB), as well as KO mice, develop enlarged cardiac valves characterized by hyperproliferation of mesenchymal cells (*lower right*) (Iwamoto et al., 2010).

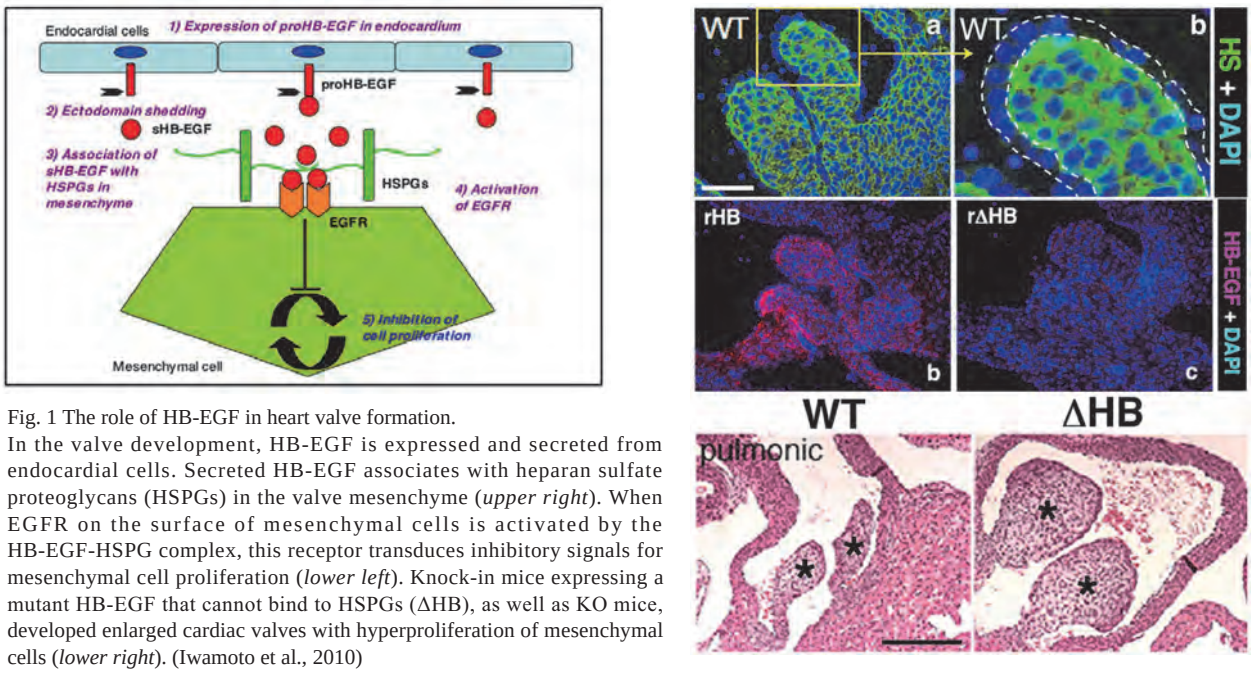


Fig. 1 The role of HB-EGF in heart valve formation.
In the valve development, HB-EGF is expressed and secreted from endocardial cells. Secreted HB-EGF associates with heparan sulfate proteoglycans (HSPGs) in the valve mesenchyme (*upper right*). When EGFR on the surface of mesenchymal cells is activated by the HB-EGF-HSPG complex, this receptor transduces inhibitory signals for mesenchymal cell proliferation (*lower left*). Knock-in mice expressing a mutant HB-EGF that cannot bind to HSPGs (ΔHB), as well as KO mice, developed enlarged cardiac valves with hyperproliferation of mesenchymal cells (*lower right*). (Iwamoto et al., 2010)

2) Role of HB-EGF in cancer malignancy

HB-EGF is expressed by cancer cells and/or cancer-derived stroma, and is involved in tumor growth, invasion and metastasis. To develop a novel strategy for cancer therapy, we are analyzing the role of HB-EGF in cancer malignancy.

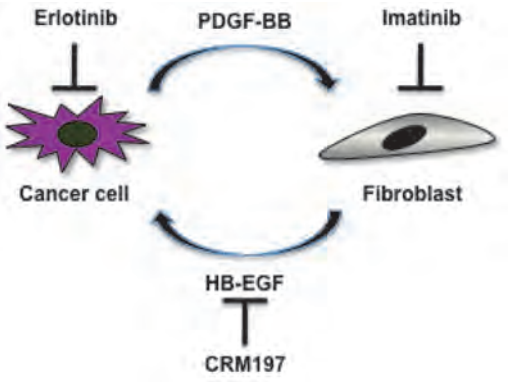


Fig. 2. Cancer cell-stromal fibroblast interactions in the cervix.

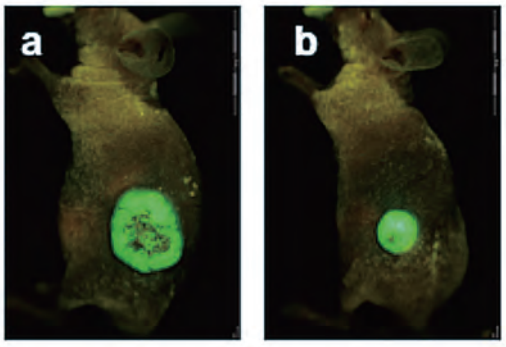


Fig.3. Cancer-derived fibroblasts enhance tumorigenesis of cervical cancer cells in nude mice. Fluorescence imaging of tumors injected cervical cancer cells with fibroblasts (a) or cervical cancer cells alone (b) (Murata et al. 2011).

3) Development of anti-cancer drugs targeting HB-EGF

We are developing new anti-cancer drugs that target HB-EGF. The pre-clinical and clinical studies of an anti-HB-EGF monoclonal antibody and a non-toxic mutant protein of diphtheria toxin CRM197 are currently in progress.

4) CD9 and the tetraspanin function

CD9 is a member of the tetraspanin superfamily. It is a membrane protein with four transmembrane domains. It associates with proHB-EGF and upregulates proHB-EGF function. CD9 is also involved in cell signaling, growth, motility, and adhesion, and in tumor cell metastasis and sperm–egg fusion. In addition, the *Caenorhabditis elegans* tetraspanin TSP-15 is essential for the epidermal integrity of the worm. We are analyzing the physiological activity of CD9 and other tetraspanins by using genetically engineered mice or worms (*C. elegans*) lacking CD9 or other tetraspanins.

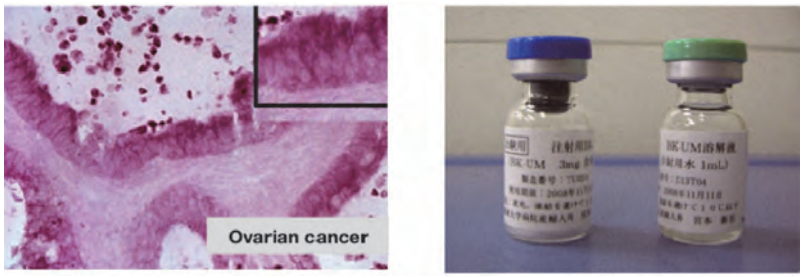


Fig. 4. Enhanced expression of HB-EGF in the ovarian cancer tissue (right) and BK-UM, therapeutics for ovarian cancer under development in our laboratory.

Recent Publications

1. Moribe H, Konakawa R, Koga D, Ushiki T, Nakamura K, Mekada E. Tetraspanin is required for generation of reactive oxygen species by the dual oxidase system in Caenorhabditis elegans. ***PLoS Genet***. 2012 Sep;8 (9):e1002957.
2. Miyamoto S, Iwamoto R, Furuya A, Takahashi K, Sasaki Y, Ando H, Yotsumoto F, Yoneda T, Hamaoka M, Yagi H, Murakami T, Hori S, Shitara K, Mekada E. A novel anti-human HB-EGF monoclonal antibody with multiple anti-tumor mechanisms against ovarian cancer cells. ***Clin Cancer Res***. 2011 Nov 1;17(21):6733-41.
3. Murata T, Mizushima H, Chinen I, Moribe H, Yagi S, Hoffman RM, Kimura T, Yoshino K, Ueda Y, Enomoto T, Mekada E. HB-EGF and PDGF mediate reciprocal interactions of carcinoma cells with cancer-associated fibroblasts to support progression of uterine cervical cancers. ***Cancer Res***. 2011 Nov 1;71(21):6633-42.
4. Iwamoto R, Mine N, Kawaguchi T, Minami S, Saeki K, Mekada E. HB-EGF function in cardiac valve development requires interaction with heparan sulfate proteoglycans. ***Development***. 2010 Jul;137(13):2205-14.
5. Ichise T, Adachi S, Ohishi M, Ikawa M, Okabe M, Iwamoto R, Mekada E. Humanized gene replacement in mice reveals the contribution of cancer stroma-derived HB-EGF to tumor growth. ***Cell Struct Funct***. 2010;35(1):3-13.

Department of Immunochemistry

Research Group

Professor（SUP）	Hisashi Arase, M.D., Ph.D.
Assistant Professor	Tadahiro Suenaga, M.D., Ph.D.
Assistant Professor	Masako Kohyama, Ph.D.
SA Researcher	Fumiji Saito, Ph.D.

Research Projects

Our department analyzes various immunological aspects in host–pathogen interactions and autoimmune diseases. In particular, we are analyzing receptor pairs that consist of an activating and an inhibitory receptor, and that are expressed on immune cells to test the hypothesis that these paired receptors may have evolved with pathogens. Moreover, since infections are often followed by autoimmune diseases, we are also analyzing how infection abrogates the homeostasis of the immune system.

(1) Immune regulation by paired receptors

Immune cells express cell surface receptor pairs that consist of activating and inhibitory receptors. The inhibitory receptors recognize self-antigens such as MHC class I molecules. We have found that these paired receptors also recognize viral proteins and play an important role in determining host resistance to pathogens (Figure 1). We are presently analyzing the functions of these receptors in immune regulation and host–pathogen interactions by using various pathogens, including viruses, parasites and bacteria.

(2) Entry mechanism of virus into cells

Several viruses that exhibit persistent infection downregulate their host' s immune response by stimulating the host's inhibitory receptors. We have found that some viruses further exploit the host' s inhibitory receptors to enter the cells. For example, interactions between immune receptors and viral proteins play an important role in membrane fusion during the infection of herpes simplex virus (HSV) and varicella-zoster virus (VZV) (Figure 2). Since other viruses may also use similar receptors to enter cells, we intend to further investigate the molecular mechanisms involved in viral entry of cells.

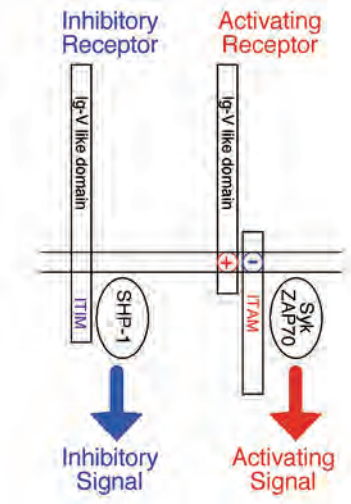


Figure 1. Paired receptor
Inhibitory receptors play an important role in immune regulation (Wang et al. ***Nat. Immunol***. 2013), whereas pathogens exploit inhibitory receptors for immune evasion. On the other hand, activating receptors might have evolved to protect infections.

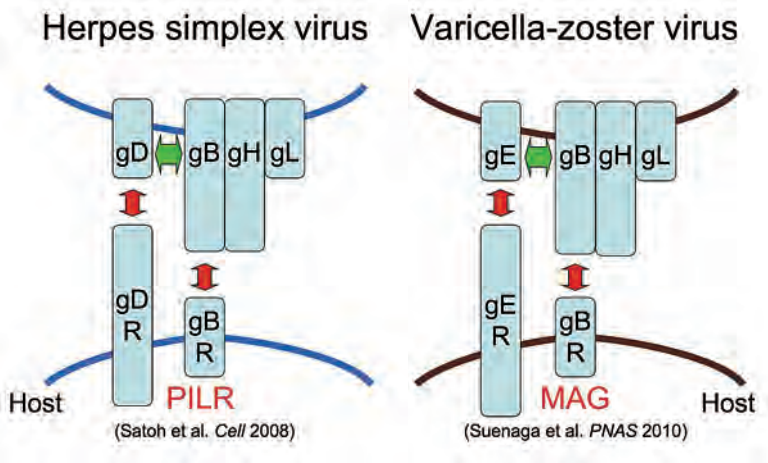


Figure 2. Paired receptor mediated viral infection.
Paired receptors receptors such as PILRα and Siglec (MAG) are involved in membrane fusion during viral infection by associating with envelope proteins of herpes simplex virus and varicella-zoster virus (Satoh et al. ***Cell*** 2008; Suenaga et al. ***Proc. Natl. Acad. Sci. USA***. 2010)

(3) Mechanism of autoimmune pathogenesis

MHC class II allelic polymorphisms associate with susceptibility to many autoimmune diseases. However, it has been unclear how MHC class II molecules regulate autoimmune disease susceptibility. We found that cellular misfolded autoantigens that are rescued from protein degradation and complex with MHC class II molecules can become targets for autoantibodies in patients with autoimmune diseases. Moreover, autoantibodies recognize misfolded proteins that are complexed with MHC class II molecules of disease-susceptible alleles but not disease-resistant MHC class II alleles. In addition, autoantibody binding to misfolded proteins that are transported by MHC class II molecules correlates strongly with susceptibility to autoimmune disease. This suggests that misfolded proteins complexed with MHC class II molecules are natural autoantigens for autoantibodies. Therefore, misfolded proteins, which normally would not be exposed to the immune system, can become targets for autoantibodies when they avoid protein degradation (Figure 3). Notably, MHC class II molecules are both induced by inflammation during infections and aberrantly expressed in many autoimmune diseased tissues. This suggests that the rescue of misfolded proteins in the context of infection may promote the development of autoimmune diseases. This notion is supported by the fact that autoimmune disease onset is often preceded by an infection. We are further analyzing how aberrant misfolded protein/MHC class II molecule complexes are generated and how they induce autoimmune diseases.

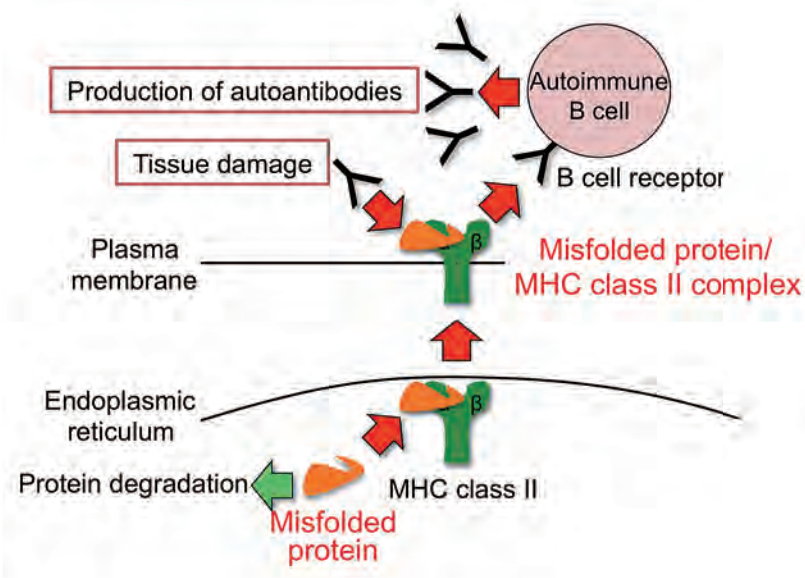


Figure 3. Misfolded proteins complexed with MHC class II molecules are targets for autoantibodies.
Cellular misfolded proteins are transported to the cell surface without processing to peptides by associating with MHC class II molecules at ER (Jiang et al. ***Int. Immunol***. 2013). Furthermore, misfolded proteins complexed with MHC class II molecules of disease-susceptible alleles are specifically recognized by autoantibodies. This suggested that misfolded proteins complexed with MHC class II molecules are natural autoantigens for autoantibodies, which affects autoimmune disease susceptibility (Jin et al. ***Proc. Natl. Acad. Sci. USA***. 2014).

Recent Publications

1. Autoantibodies to IgG/HLA-DR complexes are associated with rheumatoid arthritis susceptibility.
Jin H, Arase N, Hirayasu K, Kohyama M, Suenaga T, Saito F, Tanimura K, Matsuoka S, Ebina K, Shi K, Toyama-Sorimachi N, Yasuda S, Horita T, Hiwa R, Takasugi K, Ohmura K, Yoshikawa H, Saito T, Atsumi T, Sasazuki T, Katayama I, Lanier LL, Arase H. ***Proc Natl Acad Sci USA***. 2014 Mar 111(10): 3787-92.
2. Herpesvirus 6 Glycoproteins B (gB), gH, gL and gQ are Necessary and Sufficient for Cell-to-Cell Fusion.
Tanaka Y, Suenaga T, Matsumoto M, Seya T, Arase H. ***J Virol***. 2013 Oct; 87 (19): 10900-3.
3. Transport of misfolded endoplasmic reticulum proteins to the cell surface by MHC class II molecules.
Jiang Y, Arase N, Kohyama M, Hirayasu K, Suenaga T, Jin H, Matsumoto M, Shida K, Lanier LL, Saito T, Arase H. ***Int Immunol***. 2013 Apr; 25(4): 235-46.
4. Neutrophil infiltration during inflammation is regulated by PILRα via modulation of integrin activation.
Wang J, Shiratori I, Uehori J, Ikawa M, Arase H. ***Nat Immunol***. 2013 Jan; 14(1): 34-40.
5. Myelin-associated glycoprotein mediates membrane fusion and entry of neurotropic herpesviruses. Suenaga T, Satoh T, Somboonthum P, Kawaguchi Y, Mori Y, and Arase H. ***Proc Natl Acad Sci USA***. 2010 Jan 12;107(2):866-71.

Department of Molecular Genetics

Research Group

Professor	Hiroshi Nojima, Ph.D.
Associate Professor	Norikazu Yabuta, Ph.D.
Assistant Professor (SUP)	Daisuke Okuzaki, Ph.D.
SA Researcher	Satomi Mukai, Ph.D.

Research Projects:

We are studying the eukaryotic cell cycle to understand the mechanisms underlying the observed chromosomal instability in cancer cells. Many human cancer cells exhibit mitotic defects (such as centrosome aberrations, abnormal spindle formation, and chromosome missegregation). The resulting chromosomal instability is a major cause of malignant tumor progression. Our research focuses on functional analyses of the Ser/Thr kinases Lats (large tumor suppressor) and GAK (cyclin G-associated kinase). These kinases localize to the centrosome, regulate mitotic progression in response to DNA damage, and cause chromosome instability when their functions are disrupted. Both Lats (Lats1 and Lats2) and GAK form complexes with Mdm2. In turn, Mdm2 controls the stability of p53, which is a transcriptional regulator of the Lats2, cyclin G1, and Mdm2 genes. Thus, the Lats and GAK complexes have intimate correlations in their regulation of cell cycle (Fig. 1).

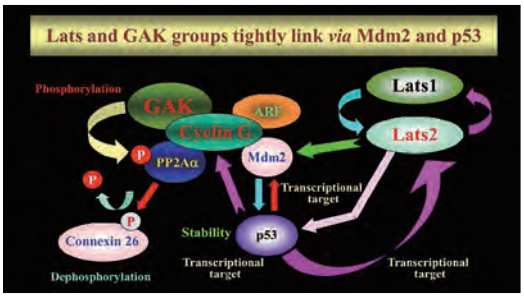


Fig. 1: The functions of the Lats and GAK complexes correlate closely.

Our research subjects are as follows:

(1) Lats Group

Lats1 and Lats2, which belong to the Lats kinase family, are highly conserved across species and localize to the centrosome during the cell cycle. Lats1/2 plays an important role in the cell cycle checkpoint and the Hippo pathway that mainly regulates organ size and cell growth. In particular, Lats2 binds to Mdm2, thereby inhibiting its E3 ligase activity and activating p53. Thereafter, p53 rapidly and selectively upregulates Lats2 expression in G2/M cells. This positive feedback loop constitutes a novel checkpoint that plays a critical role in the maintenance of proper chromosome numbers (Aylon *et al.*, Gene Dev., 2006).

We have discovered the following: (A) *LATS2* knockout mice are embryonic lethal, which indicates the essential role of Lats2 in the development and differentiation of mammalian germ cells. (B) *Lats2*$^{-/-}$ mouse embryonic fibroblasts (MEF) display mitotic defects such as centrosome fragmentation, abnormal chromosome segregation, and aberrant cytokinesis (Yabuta *et al.*, J. Biol. Chem., 2007). (C) MEFs from mice in which Lats1 is knocked out by disrupting its N-terminal region (*Lats1*$^{\Delta N/\Delta N}$) display downregulation of Lats2 mRNA expression and protein accumulation of Yap, a downstream factor of Lats2 in the Hippo pathway (Ref. 4). Moreover, the MEFs also show mitotic defects such as centrosome amplification, chromosome missegregation (Fig. 2), cytokinesis failure, and anchorage-independent growth (Fig. 3). These results indicate the essential role that Lats2 and Lats1 play in proper M phase progression and chromosome stability. (D) Lats2 mediates two novel signaling pathways: CLP (Chk1/2—Lats2—P-body) and ALB (Aurora-A—Lats1/2—Aurora-B). In the CLP pathway, Lats2 phosphorylates 14-3-3 for processing body (P-body) formation downstream of Chk1 in response to DNA damage (Okada *et al.*, J. Cell Sci., 2011). Moreover, under severe DNA damage, Lats2 is phosphorylated both by Chk1 and itself and fully activated, thereby inducing caspase-dependent apoptosis through Lats2-mediated phosphorylation of p21/CDKN1A and subsequent protein degradation of p21 (Ref. 2, Fig. 4). In the ALB pathway, Lats2 is phosphorylated by Aurora-A. Phosphorylated Lats2 localizes to the chromosomes and the

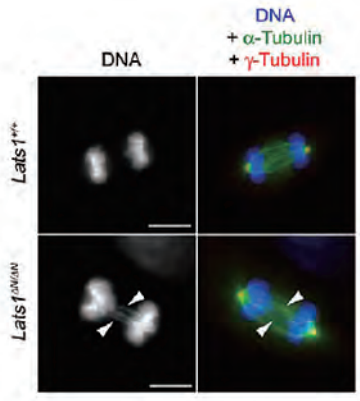


Fig. 2: *Lats1*$^{\Delta N/\Delta N}$MEFs exhibit chromosomal missegregation during anaphase.

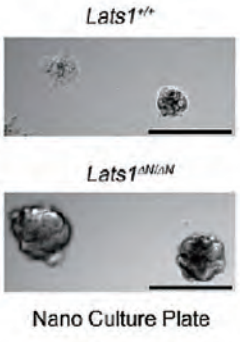


Fig. 3: *Lats1*$^{\Delta N/\Delta N}$MEFs exhibit anchorage-independent growth.

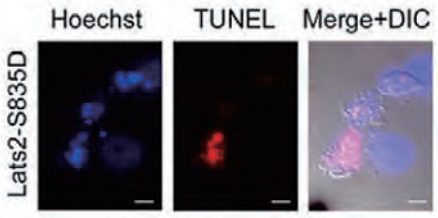


Fig. 4: Activation of Lats2 by Chk1-mediated and auto-phosphorylation induces apoptosis (red).

central spindle, and regulates Lats1 and Aurora-B, thereby executing proper chromosomal segregation during mitosis (Yabuta *et al*., Cell Cycle, 2011). (D) Lats2 phosphorylates and regulates ASPP1/p53 complexes, thereby inducing apoptosis in malignant tumor cells with aneuploidy and polyploidy (Aylon *et al*., Genes Dev., 2010). Moreover, Lats2 phosphorylates the transcription factor Snail, thereby regulating the epithelial-mesenchymal transition (EMT) (Zhang *et al*., EMBO J., 2012).

(2) GAK Group

GAK is an association partner of clathrin heavy chain (CHC) and is essential for clathrin-mediated membrane trafficking. Unlike neuron-specific auxilin, which plays a similar role in neural cells, GAK has a kinase domain whose function was unclear. We have discovered the following: (a) GAK forms a complex with PP2A B'γ and cyclin G (cyclin G1 and cyclin G2), which regulates the dephosphorylation activity of PP2A (Ref5, Fig. 5); (b) GAK localizes to the cytoplasm and the nucleus, where it has two additional functions (*i.e*., maintenance of proper centrosome maturation and mitotic chromosome congression; Shimizu *et al*., J. Cell Sci., 2009); and (c) *GAK-kd*$^{-/-}$ mice exhibit neonatal lethality with pulmonary dysfunction (Fig. 6). Since the EGFR inhibitor Gefitinib (Iressa) also efficiently inhibits the kinase activity of GAK, the side effects of Gefitinib, including interstitial pneumonia, may be due to the inhibition of GAK (Tabara *et al*., PLoS One, 2011). (d) Cyclin G2 knockout (*Ccng2*$^{-/-}$) mice are born and develop normally. However, when *Ccng2*$^{-/-}$ MEFs are irradiated with ionizing radiation (IR), their dephosphorylation of γH2AX after DNA damage (*i.e*., the cancellation of the DNA damage response) is severely retarded (Ref. 3, Fig. 7). These results suggest that Cyclin G2 regulates the dephosphorylation of γH2AX by recruiting PP2A after the completion of DNA repair, thereby causing the cells to recover from DNA damage checkpoint-dependent cell cycle arrest.

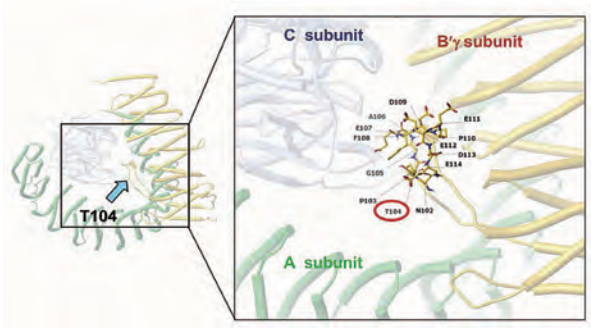


Fig. 5: T104 of PP2A B'γ by GAK is an important phosphorylation site for the activity of PP2A complex.

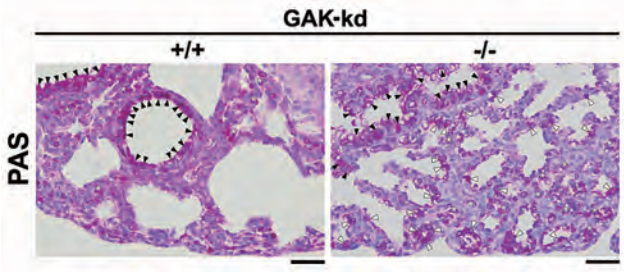


Fig. 6: Severe defects of fetal lung maturity in *GAK-kd*$^{-/-}$mice.

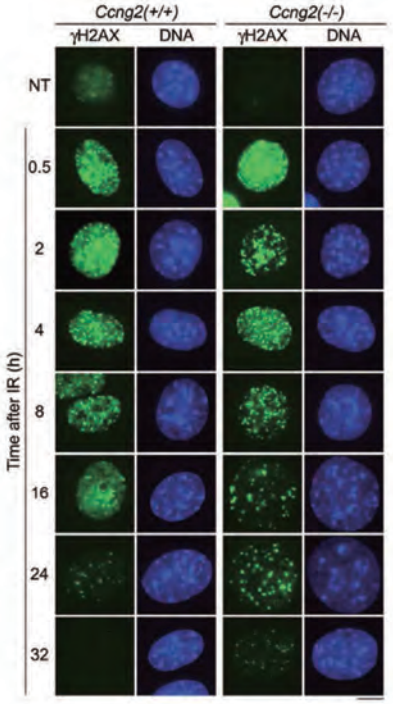


Fig. 7；Cyclin G2 knockout cells show that the dephosphorylation of γH2AX is retarded after IR irradiation (24 h and 32 h, green).

Recent Publications

1. Shao D, Zhai P, Del Re DP, Sciarretta S, Yabuta N, Nojima H, Lim DS, Pan D, Sadoshima J. A functional interaction between Hippo-YAP signalling and FoxO1 mediates the oxidative stress response. ***Nat Commun***. 2014 Feb 14;5:3315.
2. Suzuki H, Yabuta N, Okada N, Torigata K, Aylon Y, Oren M, Nojima H. Lats2 phosphorylates p21/CDKN1A after UV irradiation and regulates apoptosis. ***J Cell Sci***. 2013 Oct 1;126(Pt 19):4358-68.
3. Naito Y, Yabuta N, Sato J, Ohno S, Sakata M, Kasama T, Ikawa M, Nojima H. Recruitment of cyclin G2 to promyelocytic leukemia nuclear bodies promotes dephosphorylation of γH2AX following treatment with ionizing radiation. ***Cell Cycle***. 2013 Jun 1;12(11):1773-84.
4. Yabuta N, Mukai S, Okamoto A, Okuzaki D, Suzuki H, Torigata K, Yoshida K, Okada N, Miura D, Ito A, Ikawa M, Okabe M, Nojima H. N-terminal truncation of Lats1 causes abnormal cell growth control and chromosomal instability. ***J Cell Sci***. 2013 Jan 15;126(Pt 2):508-20.
5. Naito Y, Shimizu H, Kasama T, Sato J, Tabara H, Okamoto A, Yabuta N, Nojima H. Cyclin G-associated kinase regulates protein phosphatase 2A by phosphorylation of its B'γ subunit. ***Cell Cycle***. 2012 Feb 1;11(3):604-16.

Department of Oncogene Research

Research Group

Professor	Masato Okada, D.Sc.
Associate Professor	Shigeyuki Nada, D.Sc.
Associate Professor	Chitose Oneyama, D.Sc.
SA Researcher	Kentaro Kajiwara, D.Sc.

Research Projects

Cancers arise, evolve and develop progressively due to the accumulations of mutations and/or modifications in the genomic DNA. Loss-of-function mutations in “tumor suppressor genes” induce cell immortalization, while gain-of-function mutations in “proto-oncogenes” induce cell transformation. Cell immortalization prevents the induction of apoptosis and/or senescence, which is a defense mechanism against cancer development. Cell transformation involves the gain of autonomous cell growth, the loss of cell communication, morphological changes, and the elevated production of matrix proteases and growth factors that participate in invasion, metastasis and angiogenesis. These cellular events thus lead to the malignant conversion of cancer cells. The primary focus of this department is to understand the molecular basis of the cell transformation that is induced by the gain-of-function mutations of proto-oncogenes. As a representative proto-oncogene, we have focused on the c-Src proto-oncogene, which encodes a non-receptor tyrosine kinase. To date, we have analyzed its physiological roles in development and the mechanisms by which its specific regulators, such as Csk and Cbp, regulate it. To obtain a full picture of the cell signaling pathways that lead to c-Src–mediated cell transformation and to search for new therapeutic targets that will block c-Src–mediated cancer progression, the following projects are currently in progress:

I. Function and regulation of c-Src.

In normal cells, c-Src is present as an inactive form that is phosphorylated by its negative regulatory kinase Csk. Extracellular stimuli transiently activate it, after which it in turn activates downstream components such as the MAPK pathway and Rho family GTPases, thereby inducing gene expression and cytoskeletal rearrangements, respectively. This promotes the cell growth and phenotypic changes that are involved in cell transformation (Fig. 1). While the *c-SRC* gene is rarely mutated in human cancers, its protein is frequently hyperactivated and overexpressed. This aberrant activation of c-Src is suggested to contribute to cancer malignancy. Recently, we showed that the oncogenic potential of c-Src is regulated by the membrane adaptor protein Cbp, which is exclusively localized to the membrane microdomain. We are currently analyzing the relationship between the disruption of this regulatory system and cancer progression.

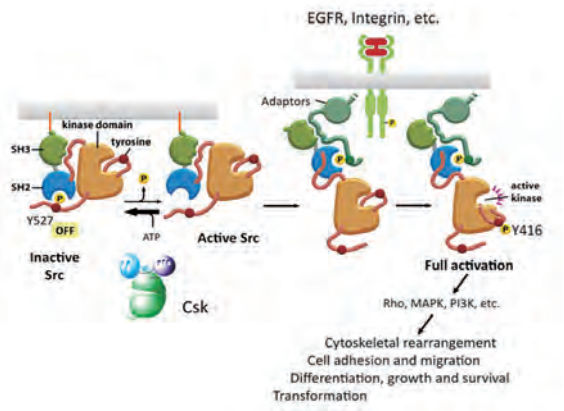


Fig. 1. Function and regulation of c-Src.

II. Mechanism of cell transformation induced by c-Src activation.

To define the signaling pathway that leads to c-Src-mediated cell transformation, we performed a comprehensive study of target molecules by using a newly developed c-Src inducible system. To date, proteomic analyses have found that Arhgef5 is a critical c-Src target. Arhgef5 is a Dbl family of GEF for Rho GTPase. A miRNA array analysis also revealed that c-Src activation alters the gene expression of a limited number of miRNAs. We identified several target genes for these miRNAs and analyzed their functions. Based on the finding that many of the target molecules (*e.g.*, FGFR3, mTOR and ILK) are components of the c-Src signaling pathway, we proposed a new tuning system of c-Src-mediated cell transformation *via* miRNA. Analysis of the regulatory mechanism for c-Src-mediated miRNA expression is currently in progress.

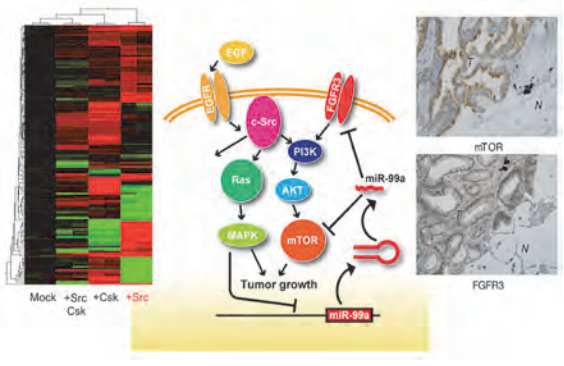


Fig. 2. A mechanism of c-Src-induced cell transformation regulated by miRNA.

III. Regulation of cell growth signaling via the late endosome/lysosome.

During the search for c-Src targets, we recently found a new regulator of cell growth signaling, namely, p18, which is a membrane adaptor protein that localizes exclusively to late endosomes/lysosomes. p18 forms a ternary complex with the scaffolding p14/MP1 proteins and plays an essential role in the activation of mTORC1 on late endosomes/lysosomes. p18 KO mice are embryonic lethal and exhibit severe defects in lysosome biogenesis (Fig. 3). To elucidate the function of p18 in lysosome biogenesis, we are currently searching for target molecules for the p18-mTORC1 pathway in late endosomes/lysosomes. We also found that the p18-dependent pathway is crucial for controlling cell growth and oncogene-mediated cell transformation. The elucidation of the underlying mechanism is an important objective of our project as well.

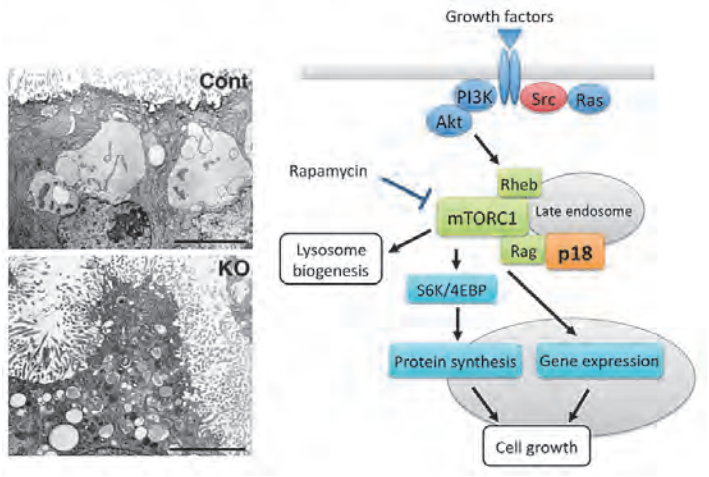


Fig. 3. Roles played by the late endosome/lysosome-anchored p18-mTORC1 pathway.

Recent Publications

1 : Nada S, Mori S, Takahashi Y, Okada M. p18/LAMTOR1: a late endosome/lysosome-specific anchor protein for the mTORC1/MAPK signaling pathway. ***Methods Enzymol***. 2014;535:249-63

2 : Soma-Nagae T, Nada S, Kitagawa M, Takahashi Y, Mori S, Oneyama C, Okada M. The lysosomal signaling anchor p18/LAMTOR1 controls epidermal development by regulating lysosome-mediated catabolic processes. ***J Cell Sci***. 2013 Aug 15;126(Pt 16):3575-84.

3 : Kajiwara K, Yamada T, Bamba T, Fukusaki E, Imamoto F, Okada M, Oneyama C. c-Src-induced activation of ceramide metabolism impairs membrane microdomains and promotes malignant progression by facilitating the translocation of c-Src to focal adhesions. ***Biochem J***. 2014 Feb 15;458(1):81-93

4 : Oneyama C, Ikeda J, Okuzaki D, Suzuki K, Kanou T, Shintani Y, Morii E, Okumura M, Aozasa K, Okada M. MicroRNA-mediated downregulation of mTOR/FGFR3 controls tumor growth induced by Src-related oncogenic pathways. ***Oncogene***. 2011 Aug 11;30(32):3489-501.

5 : Oneyama C, Hikita T, Enya K, Dobenecker MW, Saito K, Nada S, Tarakhovsky A, Okada M. The lipid raft-anchored adaptor protein Cbp controls the oncogenic potential of c-Src. ***Mol Cell***. 2008 May 23;30(4):426-36.

Department of Signal Transduction

Research Group

Professor	Nobuyuki Takakura, M.D., Ph.D.
Assistant Professor	Hiroyasu Kidoya, Ph.D.
Assistant Professor	Hisamichi Naito, M.D., Ph.D.
SA Researcher	Yinglu Han, Ph.D.
SA Researcher	Daishi Yamakawa, Ph.D.
SA Researcher	Weizhen Jia, Ph.D.
SA Researcher	Taku Wakabayashi, M.D., Ph.D.

Research Projects

It is well known that the development of normal tissues and organs requires the generation of tissue-specific cells from stem cells. The maintenance of this stem cell system also requires the generation of an appropriate microenvironment. Blood vessels are the most essential structures in tissues and organs, as without blood vessel formation, almost all tissues cannot develop (there are some exceptions). In our research group, we are analyzing the molecular mechanisms by which blood vessels form in physiological and pathological conditions, including in cancers and inflammation. We are also elucidating the mechanisms that cause stem cells to associate closely with blood vessels. Ultimately, we wish to employ our results to establish strategies that will inhibit the malignant progression of various diseases. Our specific research projects are as follows:

I. Analysis of the molecular mechanism of blood vessel formation

1) Molecular analysis of angiogenesis, with a particular focus on the Tie1 and Tie2 receptors.
2) Physiologic and pathologic function of endothelial stem cells (ESCs).
3) Molecular characterization of arterio-venous patterning, with a particular focus on the apelin/APJ system.

II. Molecular analysis of self-renewal in normal and cancer stem cells

1) Analysis of cell cycle regulation in stem cells, with a special focus on Galectin-3 and the GINS complex.
2) Establishment of a strategy that can inhibit the formation of the vascular niche inhabited by cancer stem cells.

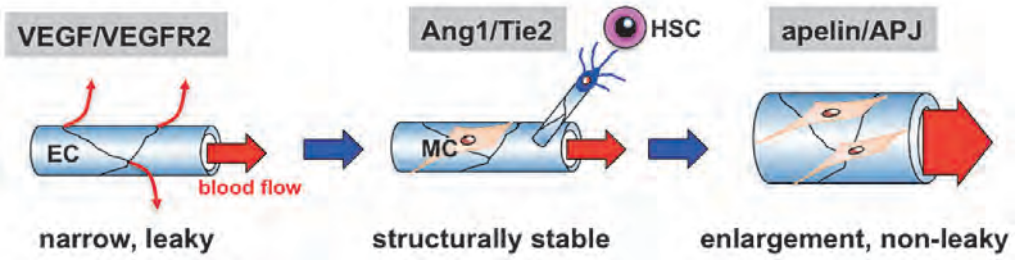


Figure 1. Maturation of blood vessels.

We elicited the concept that Angiopoietin-1 (Ang1) from mural cells (MCs) generates structurally stable blood vessels by inducing cell adhesion between endothelial cells (ECs) and MCs (Takakura, *Immunity* 1998). In addition, we found that Ang1 from hematopoietic stem cells (HSCs) induces non-leaky new blood vessels during angiogenesis (Takakura, *Cell* 2000, Yamada, *J Exp Med* 2006). Apelin produced from ECs on stimulation with Ang1 induces non-leaky and enlarged blood vessels (Kidoya, *EMBO* J 2008, *Blood* 2000). Apelin overexpression induces larger blood vessels in tumors and promotes effective drug delivery into tumors (Kidoya, *Oncogene* 2012), thereby suppressing tumor growth.

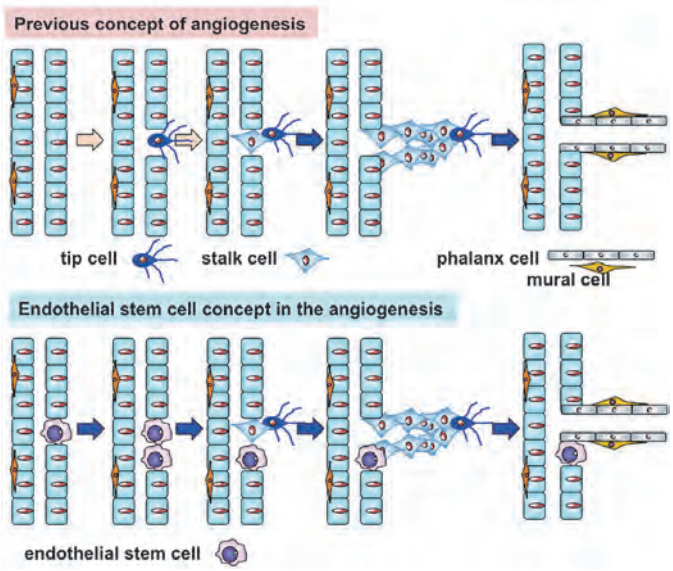
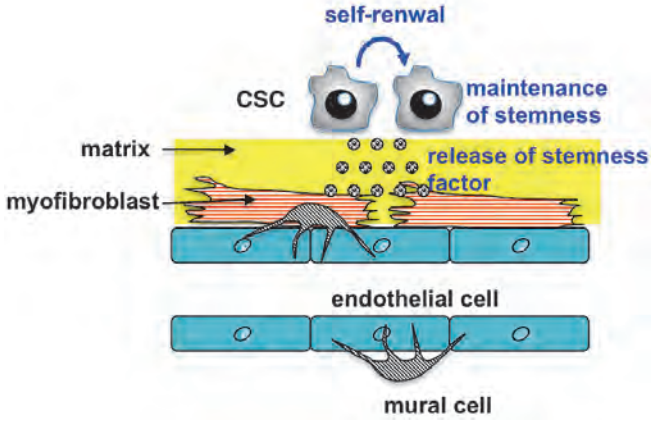


Figure 2. Endothelial stem cells (ESCs) and angiogenesis.

During angiogenesis, the tip cells in front of new vessels determine the migration direction of the new vessels, stalk cells produce a higher number of endothelial cells (ECs) for elongation, and finally phalanx cells induce the maturation of new blood vessels. We recently identified ESCs in the preexisting blood vessels that may generate heterogeneous ECs as described above, and that play a critical regulatory role in pathological and physiological angiogenesis (Naito, *EMBO J* 2012).

Figure 3. Vascular niche of cancer stem cells.

By using PSF1, a component of the DNA replication factor GINS, we succeeded in identifying cancer stem cells (CSCs) in the vascular niche (Nagahama, *Cancer Res* 2010, Matsui, *Am J pathol* 2013). Moreover, we found that stromal CD44 acts as a niche factor that supports the stemness of CSCs (Kinugasa, *Stem Cells* 2014).



Recent Publications

1. CD44 expressed on cancer-associated fibroblasts is a functional molecule supporting the stemness and drug resistance of malignant cancer cells in the tumor microenvironment. Kinugasa Y, Matsui T, Takakura N. ***Stem Cells***. 2014 Jan;32(1):145-56.
2. microRNA-125b inhibits tube formation of blood vessels through translational suppression of VE-cadherin. Muramatsu F, Kidoya H, Naito H, Sakimoto S, Takakura N. ***Oncogene***. 2013 Jan 24;32(4):414-21.
3. A role for endothelial cells in promoting the maturation of astrocytes through the apelin/APJ system in mice. Sakimoto S, Kidoya H, Naito H, Kamei M, Sakaguchi H, Goda N, Fukamizu A, Nishida K, Takakura N. ***Development***. 2012 Apr;139(7):1327-35.
4. Identification and characterization of a resident vascular stem/progenitor cell population in preexisting blood vessels. Naito H, Kidoya H, Sakimoto S, Wakabayashi T, Takakura N. ***EMBO J***. 2012 Feb 15;31(4):842-55.
5. The apelin/APJ system induces maturation of the tumor vasculature and improves the efficiency of immune therapy. Kidoya H, Kunii N, Naito H, Muramatsu F, Okamoto Y, Nakayama T, Takakura N. ***Oncogene***. 2012 Jul 5;31 (27):3254-64.

Department of Cellular Regulation

Research Group

Professor	Hiroaki Miki, Ph.D.
Assistant Professor	Daisuke Yamazaki, Ph.D.
Assistant Professor	Yosuke Funato, Ph.D.
SA Researcher	Yusuke Hirata, Ph.D.

Cells are equipped with the signal transduction system, which enables them to respond appropriately to the surrounding environment, such as stimulation with various hormones/growth factors and physical interactions with other cells or the extracellular matrix. Malfunction in the signaling system is responsible for various human diseases. A typical pathogenic consequence of such malfunction is the abnormal proliferation of cancer cells. In our laboratory, we are investigating the intracellular signaling that regulates the proliferation, differentiation, and motility of cells at various levels, ranging from molecules to organisms (nematodes and mice). The present main research interests are (1) oxidative stress signaling by reversible oxidation of proteins, and (2) regulation of intracellular magnesium levels and signal transduction.

(1) Oxidative stress signaling by reversible oxidation of proteins

We discovered that thioredoxin-related protein nucleoredoxin (NRX) is a novel regulator of Wnt signaling, which plays important roles in early development and oncogenesis. NRX binds directly to Wnt signal transducer Dishevelled (Dvl) and inhibits its function. Interestingly, the NRX-Dvl interaction is negatively regulated by the formation of intramolecular S–S bonds in NRX. Thus, NRX regulates Wnt signaling in a redox-dependent manner. We also developed a novel method to search for proteins forming S–S bonds in cells by using thioredoxin mutants. With this method, we identified several proteins such as CRMP2, which functions in Semaphorin signaling. Semaphorin treatment stimulates H_2O_2 generation and CRMP2 oxidation, resulting in the formation of homodimers linked with S–S bonds. This oxidation of CRMP2 mediates the repulsive axon guidance induced by Semaphorin stimulation (Fig. 1).

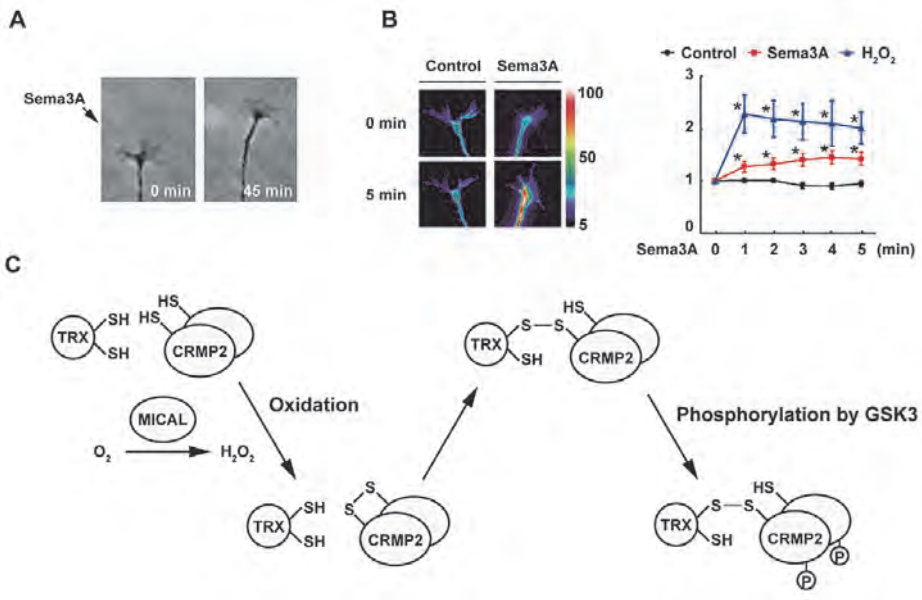


Fig. 1: CRMP2 oxidation in Semaphorin signaling

(A) Repulsive guidance of axons by Semaphorin 3A (Sema3A). (B) Increase of H2O2 in growth cones of neurons by Sema3A. (C) Schematic illustration of the mechanism of Semaphorin signaling via CRMP2 oxidation. Sema3A stimulation generates H2O2 and oxidizes CRMP2. Oxidized CRMP2 transiently forms protein complexes with TRX, which induces CRMP2 phosphorylation by GSK3.

(2) Regulation of intracellular magnesium levels and signal transduction

We found that PRL, a tyrosine phosphatase with unknown function, is a novel S–S bond-containing protein. It has been reported that PRL is consistently overexpressed in various human metastatic cancers and can promote experimental metastasis in mice. We searched for novel PRL-binding proteins and identified Magnesium-Exporting protein (MagEx). MagEx regulates the intracellular Mg^{2+} levels by exporting Mg^{2+}. This function of MagEx is inhibited by the interaction with PRL. Since this PRL-MagEx interaction is dependent on the redox state of PRL, PRL functions as a redox switch that regulates intracellular Mg^{2+} levels. Endogenous MagEx is highly expressed in the intestinal epithelia and specifically localizes in their basolateral membrane and causes hypomagnesemia. Gene disruption of *MagEx* in mice severely impairs the ability of their intestines to absorb magnesium. In addition, MagEx is also highly expressed in the epithelial cells that form enamel (ameloblasts). Indeed, *MagEx*-knockout mice show symptoms of amelogenesis imperfecta (Fig. 2). These results demonstrate the importance of MagEx in the control of systemic and local magnesium levels, the dysfunction of which results in the development of various diseases.

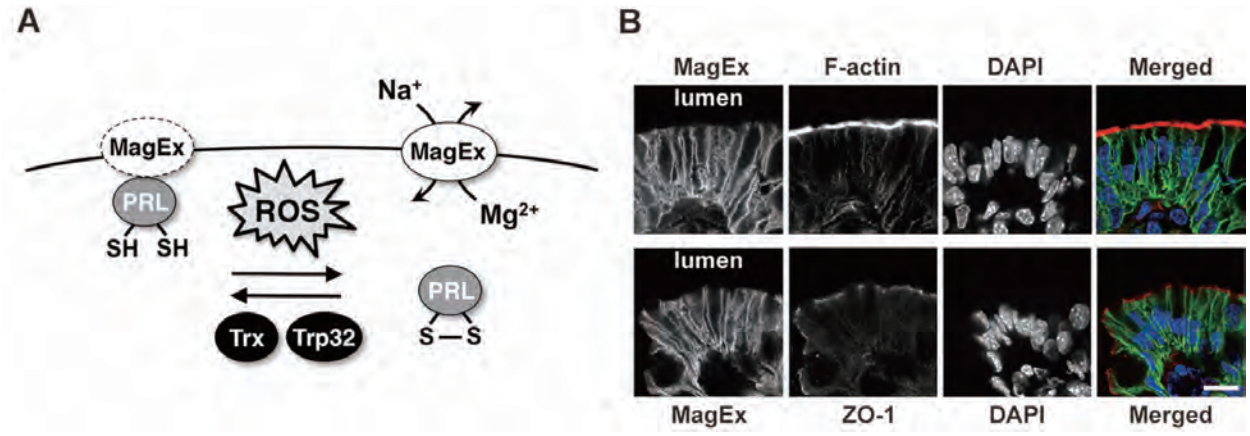


Fig. 2: Redox regulation of the intracellular Mg^{2+} level by MagEx
(A) MagEx stimulates Na^+/Mg^{2+}-exchange and decreases the intracellular Mg^{2+} level. PRL directly binds to MagEx and inhibits its Mg^{2+}-transporting function in a manner dependent on its redox state. Oxidized PRL is reversibly reduced by S–S bond-reducing enzymes, such as Trx and Trp32. (B) Localization of endogenous MagEx at the basolateral membrane of the epithelial cells in the intestine.

Recent Publications

1. Basolateral Mg^{2+} extrusion via CNNM4 mediates transcellular Mg^{2+} transport across epithelia: a mouse model. Yamazaki D, Funato Y, Miura J, Sato S, Toyosawa S, Furutani K, Kurachi Y, Omori Y, Furukawa T, Tsuda T, Kuwabata S, Mizukami S, Kikuchi K, Miki H. ***PLoS Genet***. 2013 Dec;9(12):e1003983.
2. Oligomeric peroxiredoxin-I is an essential intermediate for p53 to activate MST1 kinase and apoptosis. Morinaka A, Funato Y, Uesugi K, Miki H. ***Oncogene***. 2011 Oct 6;30(40):4208-18.
3. Thioredoxin mediates oxidation-dependent phosphorylation of CRMP2 and growth cone collapse. Morinaka A, Yamada M, Itofusa R, Funato Y, Yoshimura Y, Nakamura F, Yoshimura T, Kaibuchi K, Goshima Y, Hoshino M, Kamiguchi H, Miki H. ***Sci Signal***. 2011 Apr 26;4(170):ra26.
4. Nucleoredoxin sustains Wnt/β-catenin signaling by retaining a pool of inactive dishevelled protein. Funato Y, Terabayashi T, Sakamoto R, Okuzaki D, Ichise H, Nojima H, Yoshida N, Miki H. ***Curr Biol***. 2010 Nov 9;20 (21):1945-52.
5. Par1b/MARK2 phosphorylates kinesin-like motor protein GAKIN/KIF13B to regulate axon formation. Yoshimura Y, Terabayashi T, Miki H. ***Mol Cell Biol***. 2010 May;30(9):2206-19.

Department of Molecular Protozoology

Research Group		
	Professor	Toshihiro Horii, Ph. D.
	SA Professor	Eisaku Kimura, Ph. D.
	SA Associate Professor	Nirianne M. Q. Palacpac, Ph. D.
	Assistant Professor	Nobuko Arisue, Ph. D.
	Assistant Professor	Takahiro Tougan, Ph. D.
	SA Researcher	Masanori Yagi, Ph. D
	SA Researcher	Jyotheeswara Reddy Edula, Ph. D

Research Projects

Malaria is a serious threat to global human health. More than 40% of the world's population lives in malaria-endemic areas, and two million people die from the disease annually. Controlling malaria has become more challenging since the emergence of drug-resistant malaria parasites. This stresses the need for novel drug-targeting strategies and an effective malaria vaccine. Our department focuses on both malaria vaccine and anti-malarial drug development. We also seek to understand the mechanisms used by the malaria parasite to survive in the host.

(1) Development of a recombinant vaccine based on the malaria protein SERA

We are developing a malaria vaccine based on SE36, a recombinant protein that corresponds to the N-terminal amino acid sequence present in the serine repeat antigen (SERA) 5 of malaria parasites (Ref. 1, 2). An epidemiological study of children living in a malaria-endemic area in Uganda revealed that the children with high antibody titers against the N-terminal domain of SERA5 did not have fever. There was a clear negative correlation between blood parasitemia and anti-SERA IgG antibody titers. In collaboration with The Research Foundation for Microbial Diseases of Osaka University, we produced the GMP-grade malaria vaccine candidate BK-SE36, which is composed of SE36 and aluminum hydroxide gel. We conducted a Phase Ib clinical trial of BK-SE36 with 140 volunteers in Uganda between 2010 and 2011. The safety of BK-SE36 was demonstrated in Stage 1 of the trial with Ugandan male and female adults and in the following Stage 2 with Ugandan children aged 6–20 years. We followed up the volunteers for 1 year after vaccination and observed their malaria infection. An efficacy rate of 72% (p=0.003) against symptomatic malaria was obtained when BK-SE36 vaccinees were compared to the control cohort (Ref. 2). Although the polymorphism of malaria antigens often complicates malaria vaccine development, SERA genes do not show antigenic variation (Aoki; *JBC*, 2002) and the polymorphism of the antigen is also limited (Ref. 4). This suggests that this vaccine may be effective against a wide variety of malaria parasite strains. We will conduct further clinical trials and proceed to the development of the vaccine for use in malaria-endemic areas.

(2) Approaches to understanding the evolution of malaria parasites

Malaria parasites have many unique features, such as a highly restricted host range, a complex life cycle, and host immune

Fig. 1. Patients at Apac hospital (Uganda). Of the patients waiting at the Out-patient Department of Apac Hospital in Northern Uganda, majority are children under 5 years old.

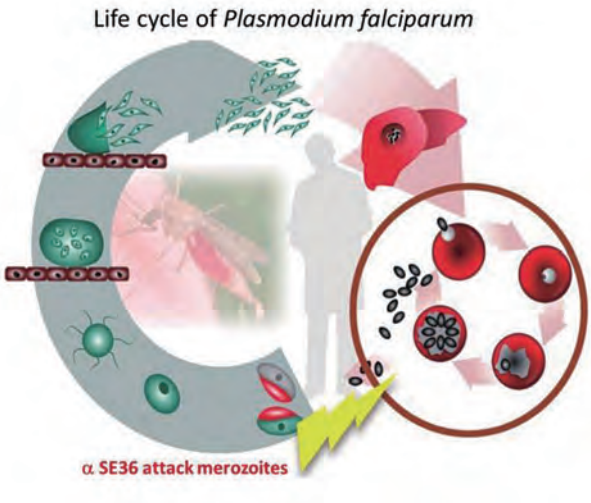


Fig. 2. Life cycle of *Plasmodium falciparum* and the action point of anti-SE36 antibody induced by SE36 malaria vaccine.

evasion systems. To understand the molecular basis of those features, we utilized several genetic approaches, including nuclear and organelle genome analyses, as well as population genetic analyses of the antigen-coding genes (Ref. 4, 5). The findings from these studies will be useful for vaccine development.

Fig. 3. The SE36 malaria vaccine for clinical trials is produced under Good Manufacturing Practices (GMP) conditions at the Kanonji Institute of The Research Foundation for Microbial Diseases of Osaka University.

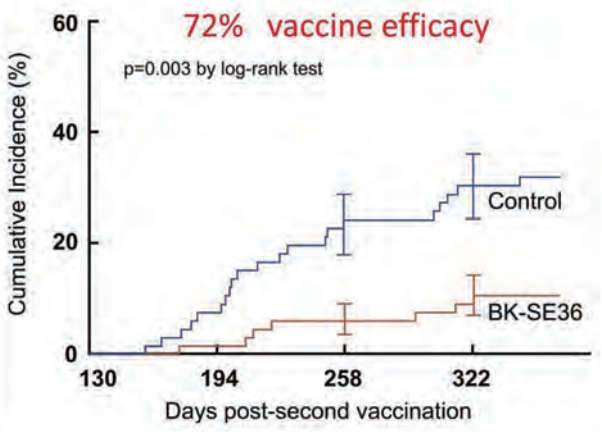


Fig. 4. Kaplan-Meier plot of *P. falciparum* malaria episodes after vaccination.

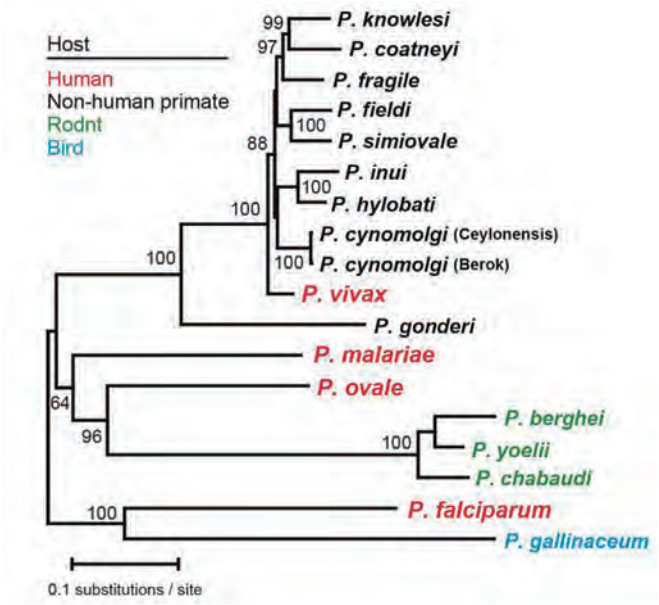


Fig. 5. Evolutionary tree of malaria parasites inferred from 30 protein-encoding genes from the apicoplast genome. Four human parasites do not show a monophyletic relationship revealing host-switching during parasite evolution.

Recent Publications

1. Protective epitopes of the *Plasmodium falciparum* SERA5 malaria vaccine reside in intrinsically unstructured N-terminal repetitive sequence. Yagi M, Bang G, Tougan T, Palacpac NMQ, Arisue N, Aoshi T, Matsumoto Y, Ishii KJ, Egwang TG, Druilhe P, Horii T. ***PLoS One***. 2014 Jun 2;9(6):e98460.
2. Phase 1b randomized trial and follow-up study in Uganda of the blood-stage malaria vaccine candidate BK-SE36. Palacpac NM, Ntege E, Yeka A, Balikagala B, Suzuki N, Shirai H, Yagi M, Ito K, Fukushima W, Hirota Y, Nsereko C, Okada T, Kanoi BN, Tetsutani K, Arisue N, Itagaki S, Tougan T, Ishii KJ, Ueda S, Egwang TG, Horii T. ***PLoS One***. 2013 May 28;8(5):e64073.
3. TLR9 adjuvants enhance immunogenicity and protective efficacy of the SE36/AHG malaria vaccine in nonhuman primate models. Tougan T, Aoshi T, Coban C, Katakai Y, Kai C, Yasutomi Y, Ishii KJ, Horii T. ***Hum Vaccin Immunother***. 2013 Jan 4;9(2):283-90.
4. Geographic differentiation of polymorphism in the *Plasmodium falciparum* malaria vaccine candidate gene SERA5. Tanabe K, Arisue N, Palacpac NM, Yagi M, Tougan T, Honma H, Ferreira MU, Färnert A, Björkman A, Kaneko A, Nakamura M, Hirayama K, Mita T, Horii T. ***Vaccine***. 2012 Feb 21;30(9):1583-93.
5. The *Plasmodium* apicoplast genome: conserved structure and close relationship of *P. ovale* to rodent malaria parasites. Arisue N, Hashimoto T, Mitsui H, Palacpac NM, Kaneko A, Kawai S, Hasegawa M, Tanabe K, Horii T. ***Mol Biol Evol***. 2012 Sep;29(9):2095-9.

Department of Experimental Genome Research

Research Group

Professor	Masahito Ikawa, Ph.D.
SA Associate Professor	Ayako Isotani, Ph.D.
Assistant Professor	Yoshitaka Fujihara, Ph.D.
Assistant Professor	Haruhiko Miyata, Ph.D.
Assistant Professor	Yuhkoh Satouh, Ph.D.

In the past, naturally mutated animals were used to elucidate the mechanisms of various diseases. In the "post-genome project era", however, genetically manipulated animals play a key role in such investigations as they are highly useful for providing disease models. Our laboratory, in collaboration with the Animal Resource Center for Infectious Diseases, is researching the mammalian reproductive system by genetically manipulating mice (http://www.egr.biken.osaka-u.ac.jp/).

Research Projects

We were the first group in the world to produce a genetically altered “green mouse” that glows in the dark. These GFP-expressing mice are highly useful for many fields of research, such as stem cell transplantation and regeneration. By utilizing these animals, we showed that it was possible to label sperm with fluorescent proteins and to visualize the fertilization process (Fig. 1–3). By utilizing gene knockout (KO) technology, we also showed that testis-specific chaperones (CLGN, CALR3, and PDILT) are required for the quality control of the sperm membrane protein ADAM3 in the endoplasmic reticulum. The *Pdilt* KO mice were found to be male infertile because their spermatozoa lack ADAM3 and cannot migrate through the utero-tubal junction (Fig. 2, #5). The testicular germ cell-specific GPI-anchored protein TEX101 was also found to be required for the presence of ADAM3 (#3). Finally, we were successful in visualizing the moment of sperm-egg fusion by using transgenic mouse lines in which IZUMO1 was tagged with a fluorescent protein (Fig. 3, #4).

We are also seeking to invent new technologies for novel investigations in the field of reproduction. By transducing blastocysts with lentiviral vectors, we developed the placenta-specific gene manipulation method (*Nat Biotechnol*, 2007). Expression of soluble FLT1 in the placenta generates preeclampsia model mice (*PNAS*, 2011). We established rat embryonic stem (ES) cells and used them to generate Mouse↔Rat chimeric animals (Fig. 4, *Genes Cells*, 2011). These chimeric animals may be useful for generating various organs from ES or iPS cells. To examine the role(s) of miRNA, we have analyzed miRNA KO mice (#2). We also successfully generated genetically modified animals (mice and rats) by using the CRISPR/Cas system (#1). Our recent interest is to use the CRISPR/Cas system to generate gene-modified animals for studying fertilization, implantation, and placentation.

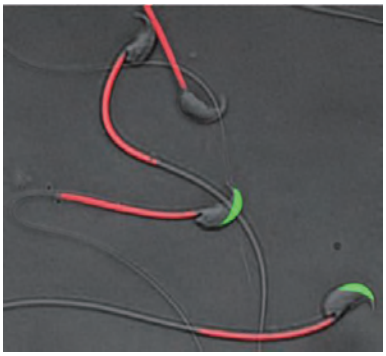


Fig. 1) A transgenic mouse spermatozoa labeled with green fluorescent protein (GFP) in their acrosome and red fluorescent protein (RFP) in their mitochondria. Spermatozoa lose green fluorescence after acrosome reaction.

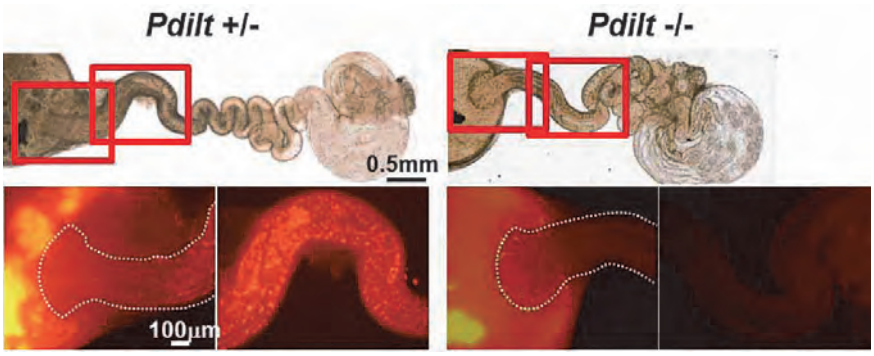


Fig. 2) *Pdilt* $^{-/-}$ mouse spermatozoa have impaired ability to migrate from uterus into the oviduct.

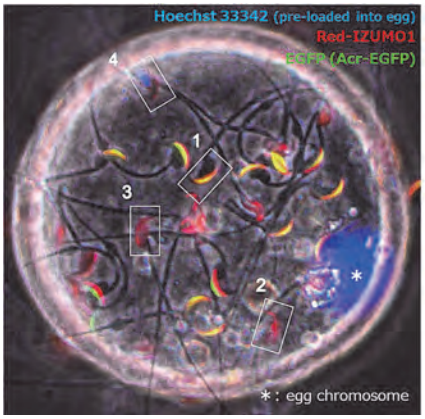


Fig. 3) Red-IZUMO1 transgenic spermatozoa bind onto oocyte plasma membrane. Acrosome reacted spermatozoa indicate altered distribution of IZUMO1 by red fluorescence (2 or 3, GFP negative). Fused sperm (4) shows specific pattern of fluorescent lost.

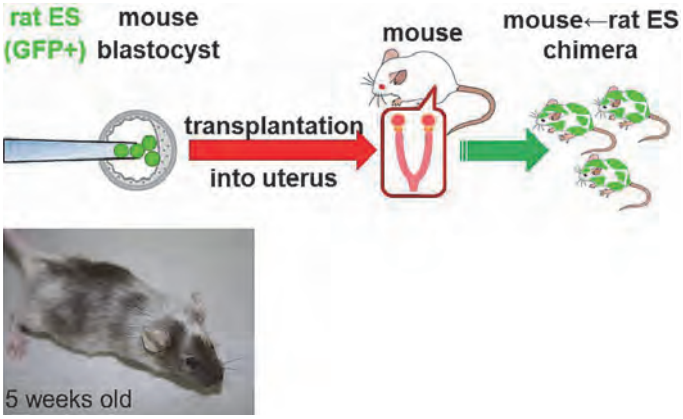


Fig. 4) Mouse↔Rat chimeric animals are generated by injecting rat ES cells into mouse blastocysts (top). Contribution of mouse (white) and rat (black) cells are determined by coat color (bottom).

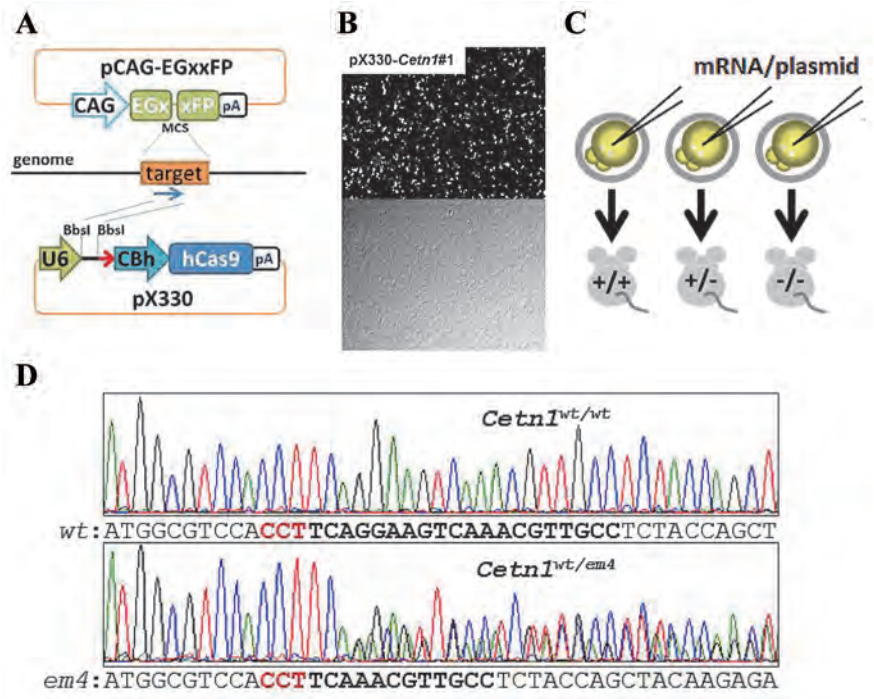


図 5)
A. pCAG-EGxxFP target plasmid contains overlapping 5' and 3' EGFP fragments under the CAG promoter. pX330 plasmid expresses both guide RNA (gRNA) and hCas9 endonuclease.
B. The efficiency of DSB mediated homology dependent repair was validated by observing EGFP fluorescence 48 hrs after the transfection (top; fluorescent field , bottom; bright field).
C. To generate gene modified mice, fertilized eggs were injected with mRNAs coding hCas9 and gRNA into cytoplasm or pX330 plasmid into pronuclei.
D. CRISPR/Cas9 mediated *Cetn1* mutations observed in founder mice. Representative *Cetn1* genomic sequences from founder mice (top; wild-type (WT), bottom; heterozygous 8 bp deletion (em4)).

Recent Publications

1. Generation of mutant mice by pronuclear injection of circular plasmid expressing Cas9 and single guided RNA. Mashiko D, Fujihara Y, Satouh Y, Miyata H, Isotani A, Ikawa M. ***Sci Rep***. 2013 Nov 27;3:3355.
2. MiR-200b and miR-429 Function in Mouse Ovulation and Are Essential for Female Fertility. Hasuwa H, Ueda J, Ikawa M, Okabe M. ***Science***. 2013 Jul 5;341(6141):71-3.
3. Expression of TEX101, regulated by ACE, is essential for the production of fertile mouse spermatozoa. Fujihara Y, Tokuhiro K, Muro Y, Kondoh G, Araki Y, Ikawa M, Okabe M. ***Proc Natl Acad Sci U S A***. 2013 May 14;110 (20):8111-6.
4. Visualization of the moment of mouse sperm-egg fusion and dynamic localization of IZUMO1. Satouh Y, Inoue N, Ikawa M, Okabe M. ***J Cell Sci***. 2012 Nov 1;125(Pt 21):4985-90.
5. Testis specific PDILT is required for quality control of sperm membrane protein ADAM3 and male fertility. Tokuhiro K, Ikawa M, Benham AM, Okabe M. ***Proc Natl Acad Sci U S A***. 2012 March 6;109 (10):3850-3855.

Laboratory of Genome Research

Research Group Associate Professor Takeshi Miwa, Ph.D.

We are investigating the molecular biological mechanisms involved in human diseases, especially cardiovascular diseases, by using animal models.

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 subsequently activated Na^+/Ca^{2+} exchanger entry mode may play important roles in the development of hypertensive HFpEF and the effect of carnitine when it is administered to the HFpEF model (3). In addition, serum interleukin-16 (IL-16) levels are elevated in patients with HFpEF and the rat model (1). Enhanced cardiac expression of IL-16 in transgenic mice induces cardiac fibrosis and LV myocardial stiffening that is accompanied by increased macrophage infiltration (Figure 1).
2) To understand the cellular and molecular aspects of vascular smooth muscle (SM) cell growth in atherosclerotic plaques, we characterized the transcriptional mechanisms of SM-specific genes, especially, the human SM alpha-actin (SmαA) gene (Figure 2). Several cis-acting DNA elements and transcriptional nuclear factors that are essential for SmαA expression have been identified. Since SmαA is also expressed in many tissues during acute inflammation, we are analyzing its gene expression and its functions.

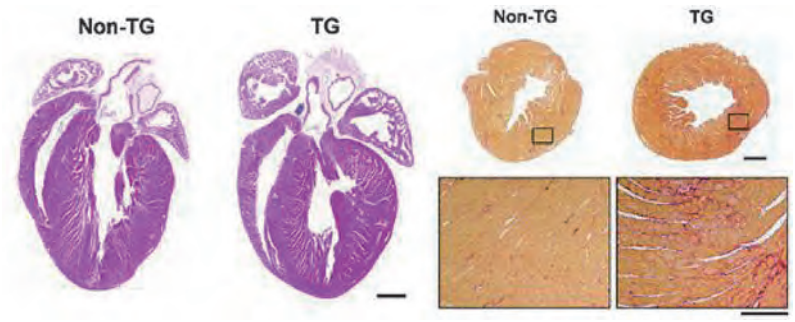


Figure 1. Enhanced cardiac expression of IL-16 under the α-MHC promoter causes increased myocardial fibrosis and stiffness in mice. (left) Four-chamber view of the hearts from non-transgenic and transgenic mice. (right) Sirius Red-stained heart LV sections of fibrosis areas.

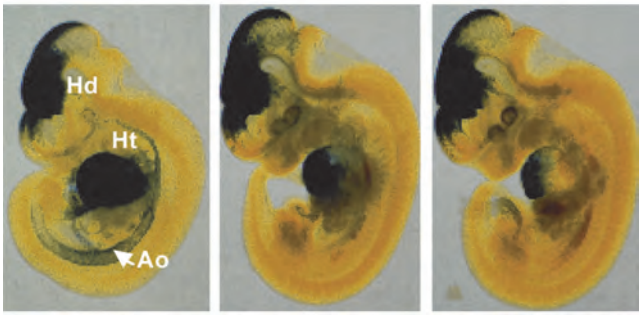


Figure 2. The human vascular smooth muscle α-actin promoter (left) expressed in embryonic aorta (Ao), but those including -1M (center) and 4M (right) point mutants in the transcriptional nuclear factor binding regions specifically did not.

Recent Publications

1. Tamaki S, Mano T, Sakata Y, Miwa T, et.al. Interleukin-16 promotes cardiac fibrosis and myocardial stiffening in heart failure with preserved ejection fraction. **PLoS One**. 2013 Jul 19;8(7):e68893.
2. Tsukamoto Y, Mano T, Sakata Y, Miwa T, et.al. A novel heart failure mice model of hypertensive heart disease by angiotensin II infusion, nephrectomy, and salt loading. **Am J Physiol Heart Circ Physiol**. 2013 Dec 1;305 (11):H1658-67.
3. Kamimura D, Ohtani T, Miwa T, et al.Ca2+ Entry mode of Na+/Ca2+ exchanger as a new therapeutic target for heart failure with preserved ejection fraction. ***European Heart J***. 2012, 11, 1408-1416.

Department of Genome Informatics

Research Group

Professor	Teruo Yasunaga, Ph.D.
Professor (SUP)	Tatsuya Takagi, Ph.D.
Assistant Professor	Naohisa Goto, Ph.D.
Assistant Professor	Shota Nakamura, Ph.D.
Assistant Professor (SUP)	Norihito Kawashita, Ph.D.

Research Projects

Our group studies the genome information of various organisms by using high-performance computers to identify new biological phenomena and to understand how organisms evolve. In addition, we develop software tools for bioinformatics and molecular biology. Our laboratory operates a computer system for genome sequence data analyses that is made available to researchers in our university. We also hold training courses for genome analysis at least once every year.

(1) Large-scale genome analysis

The complete genome sequences of more than 1,000 organisms are currently available. By using bioinformatics and molecular evolution techniques, we intend to analyze this enormous body of genome data. We are developing software and algorithms for large-scale genome analysis. In particular, we developed CONSERV, a conserved sequence finder. By analyzing the complete genome sequences of 266 organisms, we identified invariant sequences that may have been present in the last common ancestor of all extant life forms (Fig. 1). By comprehensively analyzing the genome of the influenza virus, we also intend to elucidate the evolutionary pathways this virus has taken (Fig. 2).

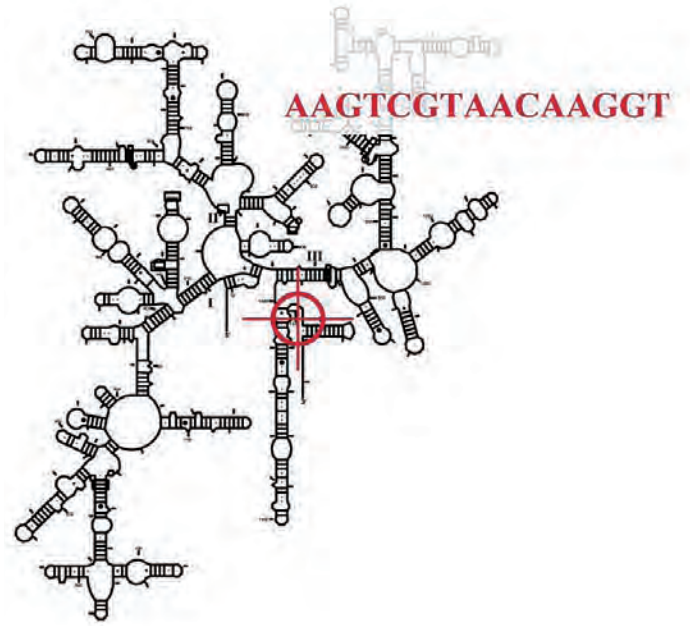


Figure 1. Large-scale genome analysis of 266 organisms. Identification of sequences conserved in almost all known genomes.

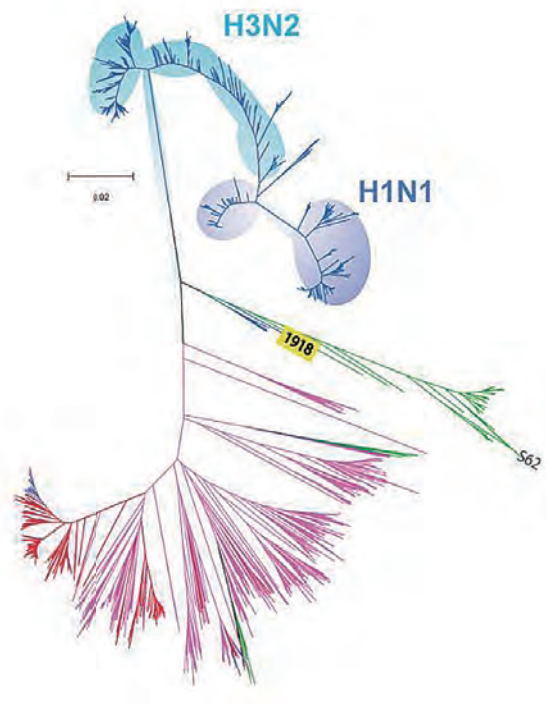


Figure 2. Comprehensive analysis of the influenza virus genome.

(2) Next generation sequencer data analysis

The recently developed “next generation sequencing” technology has enabled sequencing of the whole genome of any microorganism in one sequencer run, thus producing massive amounts of nucleotide sequence data in each run. We have been developing software to handle these data and have set up an analysis system for collaborative sequencing projects of microorganisms with other laboratories (Fig. 3).

(3) Operation of a computer system for genome information analysis at Osaka University

We provide computer resources for researchers in our university. We also provide mirrored access to major nucleotide, protein, and genome databases through our servers (Fig. 4), which are fully synchronized with the mother servers and are kept up to date at all times.

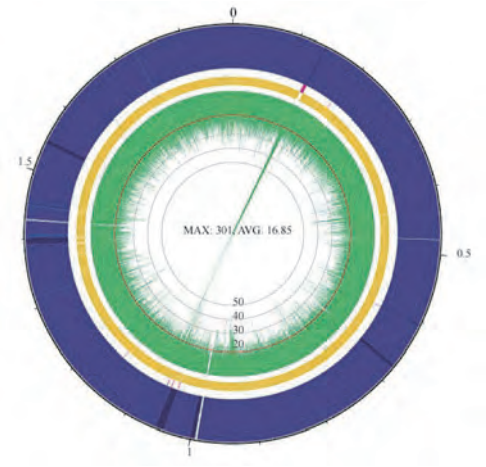


Figure 3. Next generation sequencing enables sequencing of a whole genome in one run.

Figure 4. Genome Information Research Center Computer System: Analysis servers (left) and 1.8PB storage system (right).

Recent Publications

1. Tachibana S, Sullivan SA, Kawai S, Nakamura S, Kim HR, Goto N, Arisue N, Palacpac NM, Honma H, Yagi M, Tougan T, Katakai Y, Kaneko O, Mita T, Kita K, Yasutomi Y, Sutton PL, Shakhbatyan R, Horii T, Yasunaga T, Barnwell JW, Escalante AA, Carlton JM, Tanabe K. Plasmodium cynomolgi genome sequences provide insight into Plasmodium vivax and the monkey malaria clade. ***Nat Genet***. 2012 Sep;44(9):1051-5.
2. de Silva UC, Tanaka H, Nakamura S, Goto N, Yasunaga T. A comprehensive analysis of reassortment in influenza A virus. ***Biol Open***. 2012 Feb 23;1(4):385-90.
3. Yamashita A, Kawashita N, Kubota-Koketsu R, Inoue Y, Watanabe Y, Ibrahim MS, Ideno S, Yunoki M, Okuno Y, Takagi T, Yasunaga T, Ikuta K. Highly conserved sequences for human neutralization epitope on hemagglutinin of influenza A viruses H3N2, H1N1 and H5N1: Implication for human monoclonal antibody recognition. Biochem ***Biophys Res Commun***. 2010 Mar 19;393(4):614-8.
4. Nakamura S, Yang CS, Sakon N, Ueda M, Tougan T, Yamashita A, Goto N, Takahashi K, Yasunaga T, Ikuta K, Mizutani T, Okamoto Y, Tagami M, Morita R, Maeda N, Kawai J, Hayashizaki Y, Nagai Y, Horii T, Iida T, Nakaya T. Direct metagenomic detection of viral pathogens in nasal and fecal specimens using an unbiased high-throughput sequencing approach. ***PLoS One***. 2009;4(1):e4219.
5. Yamashita A, Goto N, Nishiguchi S, Shimada K, Yamanishi H, Yasunaga T. Computational search for over-represented 8-mers within the 5'-regulatory regions of 634 mouse testis-specific genes. ***Gene***. 2008 Dec 31;427 (1-2):93-8.

Department of Infection Metagenomics

Research Group

Professor (SUP)	Toshihiro Horii, Ph.D.
Professor (SUP)	Teruo Yasunaga, Ph.D.
SA Professor (SUP)	Tetsuya Iida, Ph.D.
Assistant Professor (SUP)	Naohisa Goto, Ph.D.
Assistant Professor (SUP)	Shota Nakamura, Ph.D.

1. RAPID (Robotics Assisted Pathogen IDentification)

Under the aegis of the Program of Research Centers for Emerging and Re-emerging Infectious Diseases of MEXT, Japan, and in collaboration with the Omics Science Center, RIKEN, we are constructing a framework called “RAPID” that will facilitate the emergency diagnosis of infectious diseases. We are also cooperating with the research centers of eight countries in Asia and Africa in an effort to identify the causative agents in naturally occurring outbreaks.

2. Metagenomic Diagnosis of Infectious Diseases

Metagenomic analysis allows us to diagnose many of the major human infectious diseases (including respiratory tract infections, enteric infections, and blood-borne infections) by using a single common protocol. In addition, to pre-empt zoonotic disease outbreaks, we are seeking to identify new pathogenic microorganisms in animal-derived samples that may have zoonotic potential.

3. Metagenomic Analysis of the Intestinal Microbiome

The intestinal microbiome plays an important role in protecting the host from pathogen invasion. We are currently analyzing the intestinal microbiome of patients with diarrheal diseases to elucidate how the human host, the intestinal microbiome, and pathogenic microorganisms interact. This analysis will help us to understand the changes that occur in the intestinal microbiome during the course of infection.

4. Development of Novel Methods for Pathogen Detection

To develop more efficient and comprehensive methods of identifying pathogens, we are studying the efficacy of different methods for amplifying the genome of pathogenic microorganisms and subtracting the host genome.

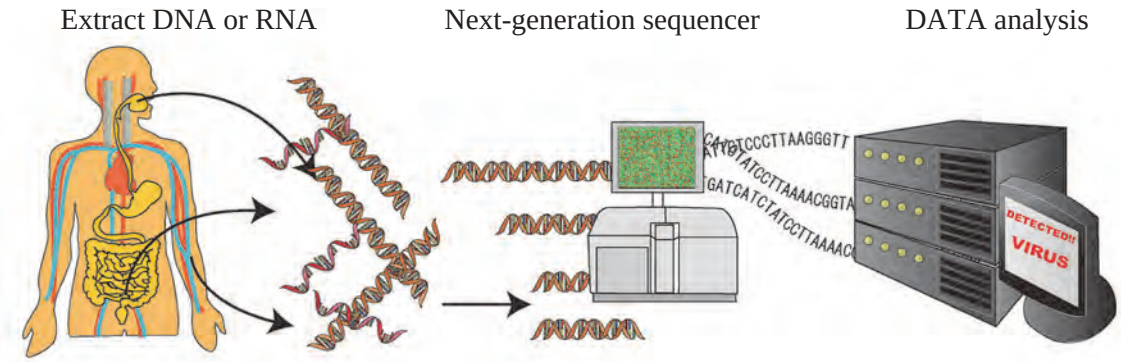


Fig. 1. Metagenomic diagnosis of infectious diseases using a next-generation sequencer.

Fig. 2. The next-generation sequencers installed in our department, Roche 454 GS Junior Bench Top System, illumina MiSeq Personal Sequencer, Pacific Biosciences RS System.

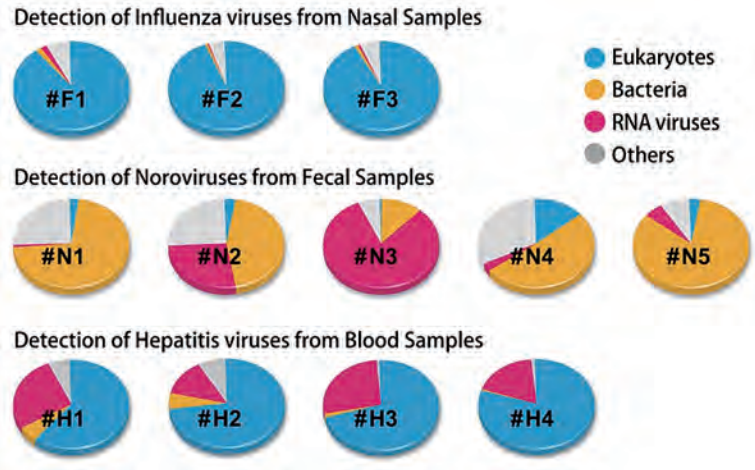


Fig. 3. Distribution of detected organisms in viral infection cases

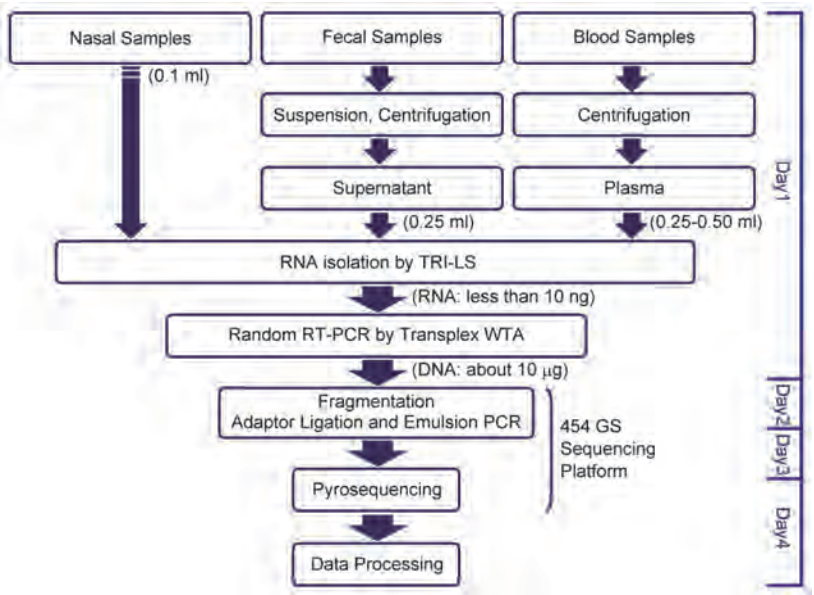


Fig. 4. Standard operating protocol for RAPID

Recent Publications

1. Masahata K, Umemoto E, Kayama H, Kotani M, Nakamura S, Kurakawa T, Kikuta J,Gotoh K, Motooka D, Sato S, Higuchi T, Baba Y, Kurosaki T, Kinoshita M, ShimadaY, Kimura T, Okumura R, Takeda A, Tajima M, Yoshie O, Fukuzawa M, Kiyono H,Fagarasan S, Iida T, Ishii M, Takeda K. Generation of colonic IgA-secreting cellsin the caecal patch. ***Nat Commun***. 2014 Apr 10;5:3704.
2. Yonogi S, Matsuda S, Kawai T, Yoda T, Harada T, Kumeda Y, Gotoh K, Hiyoshi H, Nakamura S, Kodama T, Iida T. BEC, a novel enterotoxin of Clostridium perfringensfound in human clinical isolates from acute gastroenteritis outbreaks. ***InfectImmun***. 2014 Mar 24.
3. Imai A, Gotoh K, Asano Y, Yamada N, Motooka D, Fukushima M, Kanzaki M, Ohtani T, Sakata Y, Nishi H, Toda K, Sawa Y, Komuro I, Horii T, Iida T, Nakamura S, Takashima S. Comprehensive metagenomic approach for detecting causativemicroorganisms in culture-negative infective endocarditis. ***Int J Cardiol***. 2014 Mar 15;172 (2):e288-9.
4. Seki M, Gotoh K, Nakamura S, Akeda Y, Yoshii T, Miyaguchi S, Inohara H, Horii T, Oishi K, Iida T, Tomono K. Fatal sepsis caused by an unusual Klebsiellaspecies that was misidentified by an automated identification system. ***J Med Microbiol***. 2013 May;62(Pt 5):801-3
5. Monira S, Nakamura S, Gotoh K, Izutsu K, Watanabe H, Alam NH, Nakaya T, Horii T, Ali SI, Iida T, Alam M. Metagenomic profile of gut microbiota in childrenduring cholera and recovery. ***Gut Pathog***. 2013 Feb 1;5(1):1.

Laboratory of Clinical Research on Infectious Diseases 1

Research Group

SA Associate Professor Wataru Kamitani, PhD.
SA Researcher Yusuke Sakai, DVM, PhD.

Research projects: Our group studies the molecular biology and host cell–virus interactions of Severe Acute Respiratory syndrome (SARS) coronavirus (SARS-CoV). 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. Coronavirus is an enveloped virus that carries a long, single-stranded, positive-sense genomic RNA. On infection, the genomic RNA is translated to produce two large polyproteins that are then proteolytically processed by two viral proteinases into 16 mature viral proteins called nsp1 to nsp16. Currently, our group is studying the effect of SARS-CoV nsp1 protein on host cells. SARS-CoV nsp1 protein inhibits host gene expression by first binding to the 40S ribosome and then inactivating the translational machinery. In addition, the 40S ribosome-bound nsp1 induces RNA cleavage of host mRNA. The nsp1 protein also binds to the 5' untranslated region (UTR) of SARS-CoV RNA, which confers resistance to the nsp1-mediated shutoff of translation. Our group is also studying various SARS-CoV–host cell interactions to understand SARS-CoV pathogenesis at the molecular level. More recently, another coronavirus called the Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in the Middle East in April of 2012. It too causes acute respiratory distress syndrome. Our group is presently studying the molecular biology and host cell–virus interactions of MERS-CoV.

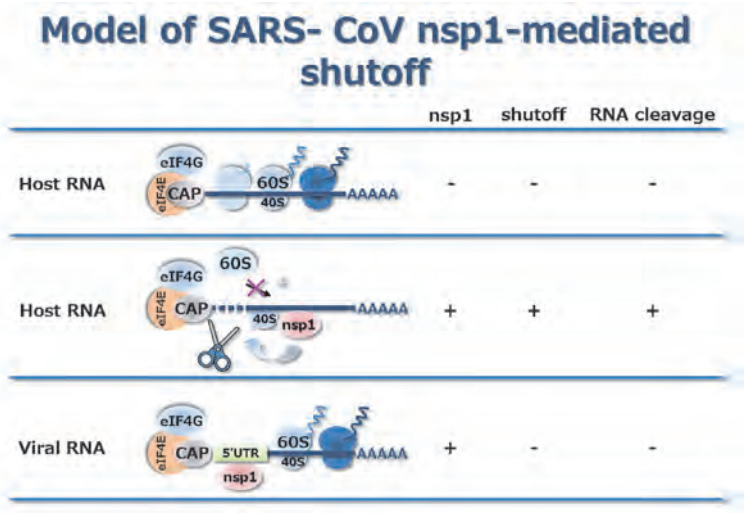


Figure: Model of SARS-CoV nsp1-mediated shutoff. SARS-CoV nsp1 causes the translational shutoff by binding to the 40S ribosome. The nsp1-40S ribosome complex also induces the cleavage of host mRNA. SARS-CoV nsp1 also binds to the 5' UTR of its own RNA. This latter interaction is crucial for the evasion by the coronavirus of its nsp1-mediated shutoff.

Recent Publications

1. Severe acute respiratory syndrome coronavirus nsp1 facilitates efficient propagation in cells through a specific translational shutoff of host mRNA. Tanaka T, Kamitani W, DeDiego ML, Enjuanes L, Matsuura Y. ***J Virol***. 2012 Oct;86(20):11128-37.
2. A two-pronged strategy to suppress host protein synthesis by SARS coronavirus Nsp1 protein. Kamitani W, Huang C, Narayanan K, Lokugamage KG, Makino S. ***Nat Struct Mol Biol***. 2009 Nov;16(11):1134-40.
3. Suppression of host gene expression by nsp1 proteins of group 2 bat coronaviruses. Tohya Y, Narayanan K, Kamitani W, Huang C, Lokugamage K, Makino S. ***J Virol***. 2009 May;83(10):5282-8.
4. Rift Valley fever virus NSs protein promotes post-transcriptional downregulation of protein kinase PKR and inhibits eIF2alpha phosphorylation. Ikegami T, Narayanan K, Won S, Kamitani W, Peters CJ, Makino S. ***PLoS Pathog***. 2009 Feb;5(2):e1000287.

Laboratory of Clinical Research on Infectious Diseases 2

Research Group

Invited Professor — Kazunori Oishi M.D., Ph.D.
SA Associate Professor — Yukihiro Akeda Ph.D.
SA Researcher — Dan Takeuchi M.D., Ph.D.

The research activities in our group include 1) studies on the diagnosis, pathogenesis, and prevention of invasive bacterial diseases such as pneumococcal and *Haemophilus influenzae* infection; 2) studies on the epidemiology and pathogenesis of the zoonotic pathogen *Streptococcus suis*; 3) the development of novel vaccine antigens; 4) the development of diagnostic test kits for infectious diseases, including drug-resistant bacteria, and for clinical research using these test kits; and 5) studies on the protein secretion systems of pathogenic bacteria. Our research has been conducted with foreign collaborators to clarify the pathogenic mechanisms of infectious diseases, with the hope that the research outcomes will eventually be translated to successful clinical interventions. Our most recent achievements include the proposal of a surrogate marker for the immunogenicity of current pneumococcal vaccines and breakthrough research on the mechanism by which *Streptococcus suis* causes meningitis (Figure).

In addition, our research group is conducting a training course in tropical infectious diseases for young medical doctors under the Research Institute for Microbial Diseases and the Graduate School of Medicine, Osaka University. This successful training course develops the skills of many young doctors working in the field of infectious diseases.

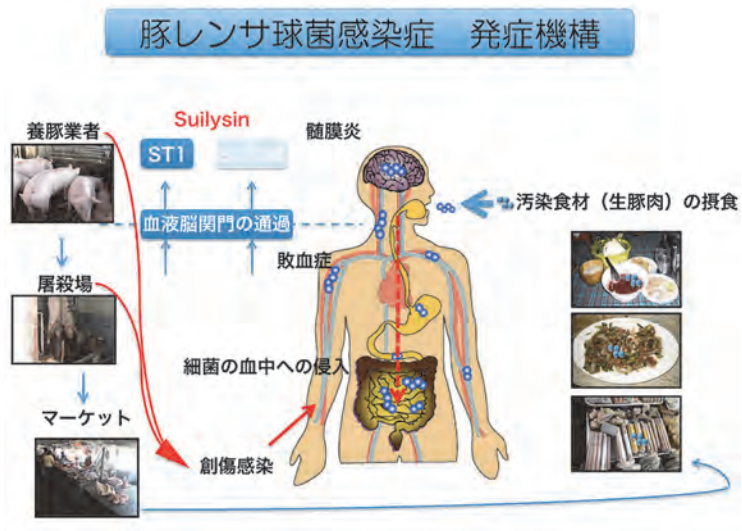


Figure. Mechanism of *Streptococcus suis* infection in Thailand (*Emerg Infect Dis* 2011, *J Infect Dis* 2014)

Recent Publications

1. Hamaguchi S, Hirose T, Matsumoto N, Akeda Y, Irisawa T, Seki M, Hosotsubo H, Yamamoto K, Tasaki O, Oishi K, Shimazu T, Tomono K. Neutrophil extracellular traps in bronchial aspirates: a quantitative analysis. ***Eur Respir J***. 2014 (In press)
2. Tamura K, Matsubara K, Ishiwada N, Nishi J, Ohnishi H, Suga S, Ihara T, Chang B, Akeda Y, Oishi K; Japanese IPD Study Group. Hyporesponsiveness to the infecting serotype after vaccination of children with seven-valent pneumococcal conjugate vaccine following invasive pneumococcal disease. ***Vaccine***. 2014 (In press).
3. Takeuchi D, Akeda Y, Nakayama T, Kerdsin A, Sano Y, Kanda T, Hamada S, Dejsirilert S, Oishi K. The Contribution of Suilysin to the Pathogenesis of *Streptococcus suis* Meningitis. ***J Infect Dis***. 2014 (In press).
4. Kerdsin A, Dejsirilert S, Sawanpanyalert P, Boonnark A, Noithachang W, Sriyakum D, Simkum S, Chokngam S, Gottschalk M, Akeda Y, Oishi K. Sepsis and spontaneous bacterial peritonitis in Thailand. ***Lancet***. 2011 Sep 3;378 (9794):960.
5. Kerdsin A, Dejsirilert S, Puangpatra P, Sripakdee S, Chumla K, Boonkerd N, Polwichai P, Tanimura S, Takeuchi D, Nakayama T, Nakamura S, Akeda Y, Gottschalk M, Sawanpanyalert P, Oishi K. Genotypic profile of *Streptococcus suis* serotype 2 and clinical features of infection in humans, Thailand. ***Emerg Infect Dis***. 2011 May;17(5):835-42.

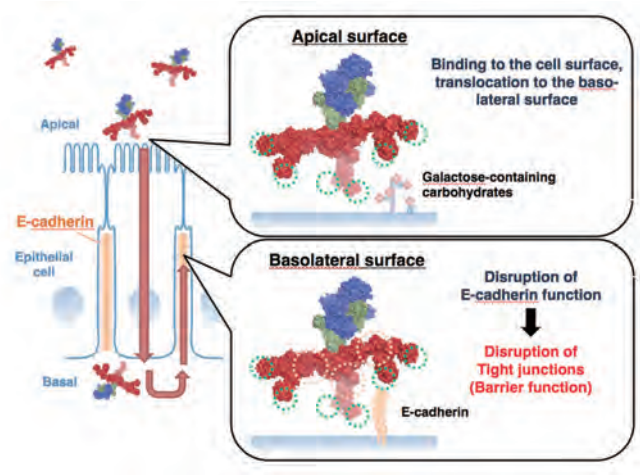
Laboratory for Infection Cell Biology

Research Group

SA Professor	Yukako Fujinaga, Ph.D.
SA Assistant Professor	Yo Sugawara, Ph.D.
SA Assistant Professor	Takuhiro Matsumura, Ph.D.
SA Researcher	Masahiro Yutani, Ph.D.

Research Projects:

Many bacterial toxins can severely damage the host, even at very low concentrations. Most bacterial toxins are enzymes that act catalytically and with high specificity on functional host cell molecules, thereby markedly modulating host homeostasis. The toxins are also often highly efficient in accessing their target molecule in the host. The ingenious transport systems involved often exploit the fundamental host machinery of membrane trafficking and the functions of intracellular organelles. Therefore, studies seeking to elucidate toxin trafficking could provide us with valuable information about basic cellular function, as well as aiding our understanding of the pathology induced by these toxins and helping us to develop effective therapeutic strategies against them. We are currently engaged in studying the structure and function of the botulinum neurotoxin complex, which must pass down the digestive tract and cross the epithelial barrier lining the intestine to cause food-borne botulism.



Hypothetical model for intestinal absorption of botuliunum neurotoxin complex (type B 16S toxin). We propose a three-step mechanism by which the botulinum neurotoxin complex breaches the intestinal epithelial barrier. First, apically located 16S toxin binds to intestinal epithelial cells via the interaction of HA (hemagglutinin) component and galactose containing-glycoconjugates, and is transcytosed. Then, once located in the basolateral compartment, HA component binds to E-cadherin, and thereby disrupts the paracellular barrier. Finally, a large amount of toxin accumulates in the basolateral area via paracellular movement.

Recent Publications

1. Amatsu S[1], Sugawara Y[1], Matsumura T, Yutani M, Kitadokoro K, and Fujinaga Y. Crystal structure of botulinum whole hemagglutinin reveals a huge triskelion-shaped molecular complex. ***J Biol Chem***. 2013; 288; 49: 35617-25. [1]These authors contributed equally.
2. Fujinaga Y, Yo Sugawara, Takuhiro Matsumura. Uptake of Boulinum Neurotoxin in the Intestine. ***Curr Top Microbiol Immunol***. 2013; 364: 45-59 [Review]
3. Sugawara Y, Fujinaga Y. The botulinum toxin complex meets E-cadherin on the way to its destination. ***Cell Adh Migr.*** 2011; 5(1): 34-36. [Review]
4. Sugawara Y, Matsumura T, Takegahara Y, Jin Y, Tsukasaki Y, Takeichi M, Fujinaga Y. Botulinum HA disrupts the intercellular epithelial barrier by directly binding E-cadherin. ***J Cell Biol***. 2010; 189 (4), 691-700
5. Jin Y[1], Takegahara Y[1], Sugawara Y, Matsumura T, Fujinaga Y. Disruption of the epithelial barrier by botulinum hemagglutinin (HA) proteins - Differences in cell tropism and the mechanism of action between HA proteins of types A or B, and HA proteins of type C. ***Microbiology***. 2009; 155(Pt 1): 35-45. [1]These authors are contributed equally.

Laboratory of Viral Infection

Research Group SA Associate Professor Eiji Morita, Ph. D.

Positive-strand RNA viruses can dramatically rearrange the intracellular membranes of the host cell and produce unusual organelle-like structures called 'replication complexes' or 'membranous webs' . These membrane structures appear in close proximity to the endoplasmic reticulum, and probably serve as a scaffold for the assembly of virus replication machinery by providing an organization and an environment that facilitate viral propagation. These structures are also known to serve as shells that protect the viruses against various cellular stress responses and allow persistent viral replication in the cytoplasm. Our group has been focusing on the molecular mechanisms involved in the formation of these replication complexes to determine the dynamic state of viral and/or host factors during the viral propagation cycle. Recently, we succeeded in purifying replication complexes from cells infected with hepatitis C virus or flavivirus. Quantitative mass spectrometry analyses led to the identification of several cellular factors that are specifically recruited to these viral replication complexes. We are currently focusing on the molecular functions of these newly identified cellular factors on the presumption that they are involved in the biosynthesis of the viral replication complexes. We are also studying the molecular mechanisms of viral particle formation and the mechanisms of autophagy induction seen in virus-infected cells. These studies may contribute to the development of novel antiviral therapies.

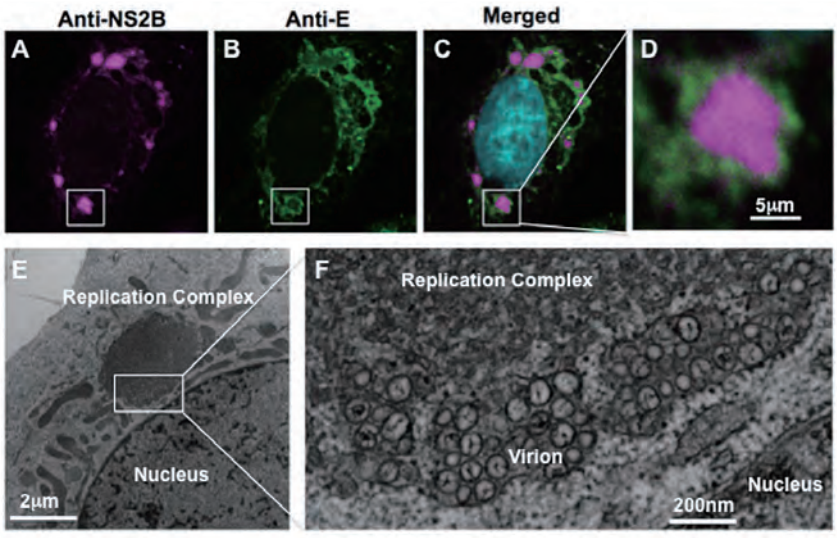


Figure: Fluorescent (A-D) and electron (E and F) microscope images of viral replication complexes in flavivirus-infected Vero cells. Twenty-four hours after infection, the cells were fixed and stained with antibodies against viral non-structural (anti-NS2B, magenta), structural (anti-E, green), and nuclear (DAPI, cyan) proteins. Unusual membrane structures, 5 to 10 nm in diameter and positive for viral antigens, are detected adjacent to the nucleus in the virus-infected cells. The endoplasmic reticulum is observed at the periphery of this structure, and many virus-like particles are detected inside of the lumen.

Recent Publications

1. Fujita, N.†, **Morita, E.** †, Itoh, T., Tanaka, A., Nakaoka, M., Osada, Y., Umemoto, T., Saitoh, T., Nakatogawa, H., Kobayashi, S., Haraguchi, T., Guan, J.L., Iwai, K., Tokunaga, F., Saito, K., Ishibashi, K., Akira, S., Fukuda, M., Noda, T., Yoshimori, T. Recruitment of the autophagic machinery to endosomes during infection is mediated by ubiquitin. ***J Cell Biol***. 2013 †These authors contributed equally
2. **Morita, E.**, Arii J, Christensen D, Votteler J, Sundquist WI. Attenuated protein expression vectors for use in siRNA rescue experiments. ***Biotechniques***. 2012 Aug;0(0):1-5.
3. **Morita, E.**, Sandrin, V., McCullough, J., Katsuyama, A., Hamilton, IB., Sundquist, WI. ESCRT-III protein requirements for HIV-1 budding. ***Cell Host Microbe***. 2011, 3:235-42.
4. **Morita, E.**, Colf, LA., Karren, MA., Sandrin, V., Rodesch, CK., Sundquist, WI. Human 5. ESCRT-III and VPS4 proteins are required for centrosome and spindle maintenance. ***Proc Natl Acad Sci U S A***. 2010 107(29):12889-94.
5. Bajorek, M. †, **Morita, E.** †, Skalicky, JJ, Morham, SG., Babst, M., Sundquist, WI. Biochemical analyses of human IST1 and its function in cytokinesis. ***Mol Biol Cell***. 2009, 20(5):1360-73. †These authors contributed equally.

Laboratory of Genomic Research on Pathogenic Bacteria

Research Group

SA Professor	Tetsuya Iida, Ph.D.
SA Assistant Professor	Shigeaki Matsuda, Ph.D.
SA Researcher	Chonchanok Theethakaew, Ph.D.

This research group is studying pathogenic bacteria from the genomic point of view.

Our main research targets are as follows:

1. Characterization of the mechanism(s) used by bacterial pathogens to infect host organisms by identifying infection-related changes in pathogen genome expression: To understand the molecular mechanisms by which bacterial pathogens infect host organisms, we are using DNA microarrays and other molecular methods to investigate the changes in the genome expression pattern of various bacterial pathogens that occur during their interaction with their target host.

2. Analysis of the mechanism(s) that lead to the emergence of new infectious diseases: The unique features of various newly emerged bacterial pathogens are being studied by analyzing their genomes and comparing them with those of other bacterial strains.

3. Investigation of the life cycles of bacterial pathogens in their natural environments: Based on what is currently understood about various bacterial pathogens, we are seeking to characterize their life cycles in their natural habitats.

4. Development of new methods for the rapid identification of bacterial pathogens based on genomic information: To rapidly diagnose bacterial infections, a novel system for identifying bacterial pathogens by high-throughput DNA sequencing is being developed.

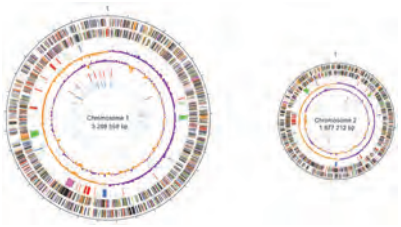


Figure 1. Whole genome sequence of *Vibrio parahaemolyticus*

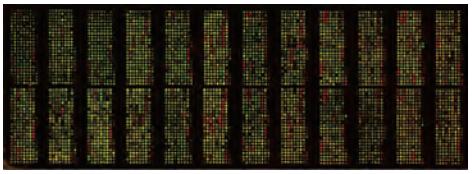


Figure 2. Characterization and comparison of the genomes of pathogenic bacteria by using DNA microarrays

Recent Publications

1. Okada R, Zhou X, Hiyoshi H, Matsuda S, Chen X, Akeda Y, Kashimoto T, Davis BM, Iida T, Waldor MK, Kodama T. The *Vibrio parahaemolyticus* effector VopC mediates Cdc42-dependent invasion of cultured cells but is not required for pathogenicity in an animal model of infection. ***Cell Microbiol***. 2014 Jun;16(6):938-47.
2. Matsuda S, Okada N, Kodama T, Honda T, Iida T. A cytotoxic type III secretion effector of *Vibrio parahaemolyticus* targets vacuolar H^+-ATPase subunit c and ruptures host cell lysosomes. ***PLoS Pathog***. 2012 Jul 19;8(7):e1002803.
3. Hiyoshi H, Kodama T, Saito K, Gotoh K, Matsuda S, Akeda Y, Honda T, Iida T. VopV, an F-actin-binding type III secretion effector, is required for *Vibrio parahaemolyticus*-induced enterotoxicity. ***Cell Host Microbe***. 2011 Oct 20;10(4):401-9.
4. Monira S, Nakamura S, Gotoh K, Izutsu K, Watanabe H, Alam NH, Endtz HP, Cravioto A, Ali SI, Nakaya T, Horii T, Iida T, Alam M. Gut microbiota of healthy and malnourished children in Bangladesh. ***Front Microbiol***. 2011 Nov 21;2:228.
5. Gotoh K, Kodama T, Hiyoshi H, Izutsu K, Park KS, Dryselius R, Akeda Y, Honda T, Iida T. Bile acid-induced virulence gene expression of *Vibrio parahaemolyticus* reveals a novel therapeutic potential for bile acid sequestrants. ***PLoS One***. 2010 Oct 13;5(10):e13365.

Laboratory of Viral Replication

Research Group

SA Associate Professor Takeshi Kobayashi, D.V.M., Ph.D.
SA Associate Professor Yuta Kanai, D.V.M., Ph.D.

Research projects

1) Oncolytic viral therapy using reovirus

Mammalian orthoreoviruses (reoviruses) are members of the family *Reoviridae* and contain a genome consisting of 10 segments of double-stranded (ds) RNA. Reoviruses are highly tractable experimental models for studies of dsRNA virus replication and pathogenesis. In the last decade, the potential of reoviruses as oncolytic agents against various tumors, including head and neck, colon, breast, and pancreatic cancers, has been investigated in animal models and humans. The putative oncolytic potential of reoviruses is based on the observation that reoviruses induce cell death and apoptosis in tumor cells with an activated Ras signaling pathway. However, while wild-type reovirus-based oncolytic therapies have been safe, the efficacy so far is limited. We are thus using genetic modification to develop safer and more effective reovirus-based cancer therapeutics.

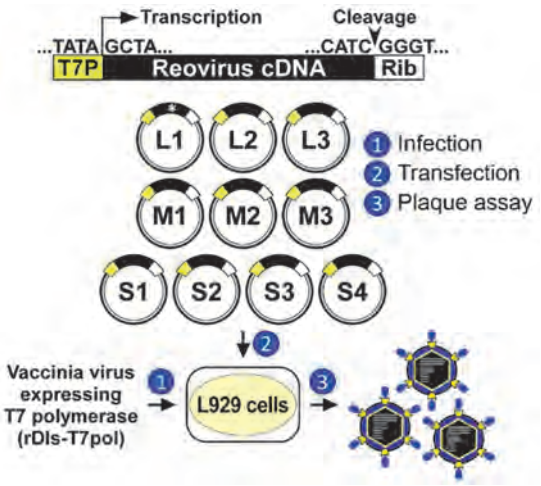


Fig1 Strategy to generate reovirus from cloned cDNA

2) Highly pathogenic bat reovirus

Bats are a natural reservoir for many important zoonotic viruses, including Hendra virus, Nipah virus, and potentially SARS coronavirus and Ebola virus. In 1968, the *Pteropine orthoreovirus* (PRV) was isolated from flying fox. While it was not associated with any disease, Melaka virus, which is genetically similar to PRV, was recently isolated from a human patient in Malaysia with acute respiratory tract infection (RTI). Subsequently, other related strains of bat-associated orthoreoviruses have been isolated in Malaysia, Indonesia, and China. We also isolated and characterized another new PRV, Miyazaki virus, from a patient with acute RTI after returning to Japan from Indonesia in 2007. These isolates have given rise to increasing concern about bat-transmitted orthoreovirus infections in humans. We are investigating how PRV replicates and causes disease by using a combination of genetic, biochemical and biophysical approaches. Our aim is ultimately to develop vaccines, diagnostics, and therapeutics for bat reovirus-related diseases.

Recent Publications

1. A plasmid-based reverse genetics system for mammalian orthoreoviruses driven by a plasmid-encoded T7 RNA polymerase. Komoto S, Kawagishi T, Kobayashi T, Ikizler M, Iskarpatyoti J, Dermody TS, Taniguchi K. ***J Virol Methods***. 2014 Feb;196:36-9.
2. Nonstructural protein σ1s mediates reovirus-induced cell cycle arrest and apoptosis.Boehme KW, Hammer K, Tollefson WC, Konopka-Anstadt JL, Kobayashi T, Dermody TS. ***J Virol***. 2013 Dec;87(23):12967-79.
3. The reovirus sigma1s protein is a determinant of hematogenous but not neural virus dissemination in mice. Boehme KW, Frierson JM, Konopka JL, Kobayashi T, Dermody TS. ***J Virol***. 2011 Nov;85(22):11781-90.
4. An improved reverse genetics system for mammalian orthoreoviruses. Kobayashi T, Ooms LS, Ikizler M, Chappell JD, Dermody TS. ***Virology***. 2010 Mar 15;398(2):194-200.
5. Identification of functional domains in reovirus replication proteins muNS and mu2. Kobayashi T, Ooms LS, Chappell JD, Dermody TS. ***J Virol***. 2009 Apr;83(7):2892-906

Laboratory of Combined Research on Microbiology and Immunology

Research Group

Associate Professor	Hiroki Nagai, Ph.D.
SA Associate Professor	Tomoko Kubori, Ph.D.
SA Researcher	Andree Marie Hubber, Ph.D.
SA Researcher	Xuan Thanh Bui, Ph.D
SA Researcher	Akiko Okura
SA Researcher	Masayo Yamada

Research Projects

Protein secretion is a process of fundamental importance for bacterial pathogenesis. Whether they deliver toxins or directly inject effector proteins into the cytoplasm of host cells, bacterial protein secretion systems play a central role in modulating eukaryotic cell functions. *Legionella pneumophila* are Gram-negative bacteria that are found ubiquitously in soil and freshwater environments. Once inhaled by humans, *Legionella* infections can result in a severe form of pneumonia known as Legionnaires' disease. *Legionella* use a type IV secretion system (T4SS) to deliver effector proteins, which mediates the establishment of a replicative niche in host cells.

The goal of our research is to understand, at the molecular level, how *Legionella* subverts host cellular functions to accomplish successful intracellular replication. To this end, the following research projects are currently in progress.

(1) Analysis of the structure and function of the type IV secretion apparatus.

Essentially nothing is known about the substrate transfer across eukaryotic and bacterial membranes that occurs *via* type IV secretion systems. Furthermore, the macromolecular structure of the type IV secretion apparatus is largely unknown. To address these questions, we are analyzing the structure and function of the type IV secretion apparatus of *Legionella*.

(2) Analysis of effector proteins that translocate from Legionella to host cells.

We previously demonstrated that RalF is translocated by the type IV secretion system of *Legionella* into host cells and is required for the recruitment of host ARF proteins to *Legionella*-containing vacuoles. We also recently demonstrated that the effector LubX acts as an E3 ligase and targets another effector for proteasomal degradation within host cells. LubX is the first effector protein that has been shown to target and regulate another effector within host cells.

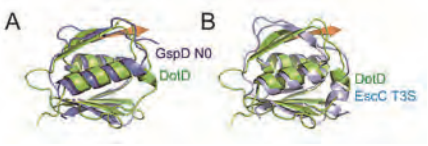


Fig. 1. T4SS core component DotD represents a conserved structural motif of the periplasmic ring.

(3) Interaction of Legionella and its natural hosts.

The ability of *Legionella* to survive and replicate within eukaryotic cells has been acquired during its history of interactions with natural hosts such as free-living amoebae. Taking advantage of the recent development of the NGS technique, we are analyzing the molecular interactions between amoebae and amoeba symbionts/pathogens, including *Legionella*.

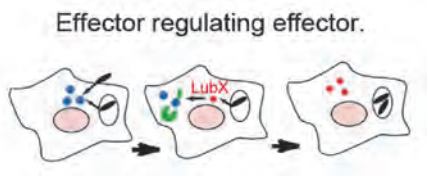


Fig. 2 Discovery of metaeffector.

Recent Publications

1. Hubber A, Kubori T, *Nagai H. Modulation of the ubiquitination machinery by *Legionella*. ***Curr Top Microbiol Immunol***. 2014:**376**, 227-247
2. Hori JI, Pereira MS, Roy CR, *Nagai H, Zamboni DS. Identification and functional characterization of K(+) transporters encoded by *Legionella pneumophila kup* genes. ***Cell Microbiol***. 2013: **15**, 2006-2019
3. Nagai H and Kubori T. Dot/Icm type IVB secretion systems of *Legionella* and other gram-negative bacteria. ***Front Microbiol***.,2011: **2**,136.
4. Kubori T, Shinzawa N, Kanuka H, Nagai H. *Legionella* metaeffector exploits host proteasome to temporally regulate cognate effector. ***PLoS Pathog***. 2010;**6**(12),e1001216.
5. Nakano N, Kubori T, Kinoshita M, Imada K, Nagai H. Crystal structure of *Legionella* DotD: insights into the relationship between type IVB and type II/III secretion systems. ***PLoS Pathog***. 2010;**6**(10),e1001129.

Pathogenic Microbes Repository Unit

Head
SA Professor (SUP) Tetsuya Iida, Ph.D.
Associate Professor Toshio Kodama, Ph.D.

In this facility, as a part of the activity of the National BioResource Project (NBRP) directed by the Ministry of Education, Culture, Sports, Science and Technology, Japan, and as a member of the Japan Society for Culture Collections (JSCC), pathogenic bacterial strains are being collected and preserved. Those strains will be distributed upon request to investigators in and outside this country. The list of the collection is available at the websites of this facility and NBRP.

Office of Combined Program on Microbiology and Immunology

Research promotion group Associate Professor Yoshiko Murakami, M.D., Ph.D
Education promotion group Associate Professor Hodaka Fujii, M.D., Ph.D.

Office activities

Our institute and the Immunology Frontier Research Center are world-class institutes for microbiological and immunological research, respectively. To take maximal advantage of this situation and to promote the cross-fertilization of research between these institutes, we have developed the combined microbiology and immunology program.

Research promotion

To promote combined microbiological and immunological research, we have the following plans.

1. Organization of the Awaji international forum on infection and immunology, which is held in September annually.
2. Organization of the research progress report, which is held every 2 months within the institute.
3. Organization of the large research presentation program and the competition in research presentation, which are held annually.

The aim of these activities is to facilitate microbiological and immunological research by promoting research collaboration, information exchange, and personal exchange between laboratories, and by generating a conducive research environment.

Education promotion

To facilitate seamless research on microbiology and immunology, we direct a multidisciplinary graduate program on microbiology and immunology, including designing the curriculum and its contents. We also organize Open House sessions of the institute and give guidance to new students. In addition, we organize the combined program on clinical microbiology and immunology in close cooperation with other research centers, in particular, the Thailand–Japan Research Collaboration Center on Emerging and Re-emerging Infections.

Research group Associate Professor Yoshiko Murakami, MD. PhD

Research projects

I have an additional appointment in the Department of Immunoregulation as the leader of the PNH group.
(See details on the Department page).

1. Investigation of the pathogenesis of acquired glycosylphosphatidylinositol (GPI) deficiency, paroxysmal nocturnal hemoglobinuria (PNH).
2. Investigation of the pathogenesis of inherited GPI deficiency.

Recent Publications

1. *PIGA* mutations cause early-onset epileptic enceohalopathies and distinctive features. M. Kato* H. Saitsu* Y. Murakami* K. Kikuchi, Watanabe, M. Iai, K. Miya, R. Matsuura, R. Takayama, C. Ohba, M. Nakashima,Y. Tsurusaki, N. Miyake, S. Hamano, H. Osaka, K. Hayasaka, T. Kinoshita, N. Matsumoto. ***Neurology***. 2014 *in press* (*equally contribution).
2. Mutations in PGAP3 impair GPI-anchor maturation and lead to intellectual disability with hyperphosphatasia and additional phenotypic features. Howard MF* Murakami Y* Pagnamenta AT, Haas CD, Fischer B, Hecht J, Keays DA, Knight SJL, Kölsch U, Krüger U, Leiz S, Maeda Y, Mitchell D, Mundlos S, Philipps JA, Robinson PN, Kini U, Taylor JC, Horn D, Kinoshita T, Krawitz PM. ***Am J Hum Genet***.2014;94(2)278-87(*equally contribution).
3. Glycosylphosphatidylinositol (GPI) anchor deficiency caused by mutations in PIGW is associated with West syndrome and hyperphosphatasia with mental retardation syndrome. Chiyonobu T, Inoue N, Morimoto M, Kinoshita T, Murakami Y. ***J Med Genet***. 2014; 51(3): 203-7.
4. Case report with vitamin B6 responsive epilepsy due to inherited GPI deficiency. Kuki, I., Y. Takahashi, Okazaki, Ebara, N. Inoue, T. Kinoshita, Y. Murakami. ***Neurology***. 2013;81(16):1467-9.
5. Deregulated expression of HMGA2 is implicated in clonal expansion of PIGA deficient cells in paroxysmal nocturnal haemoglobinuria. Murakami Y, Inoue N, Shichishima T, Ohta R, Noji H, Maeda Y, Nishimura J, Kanakura Y, Kinoshita T. ***Br J Haematol***. 2012 Feb;156(3):383-7.

Chromatin Biochemistry Research Group

Research Group

Associate Professor Hodaka Fujii, M.D., Ph.D.
Assistant Professor Toshitsugu Fujita, Ph.D.

Locus-specific biochemical analysis of genome functions

A comprehensive understanding of the mechanisms behind genome functions such as transcription and epigenetic regulation requires the identification of the molecules that bind to the genomic regions of interest *in vivo*. However, there are few non-biased methods for identifying such molecules. To facilitate the biochemical and molecular biological analysis of specific genomic regions, we developed the locus-specific chromatin immunoprecipitation (ChIP) technologies to purify the genomic regions of interest. The ChIP technologies consist of insertional ChIP (iChIP) and engineered DNA-binding molecule-mediated ChIP (enChIP).

The scheme of iChIP is as follows (left panel): (i) The recognition sequence(s) of an exogenous DNA-binding protein such as LexA are inserted into the genomic region of interest in the cell that is to be analyzed. (ii) The DNA-binding domain (DNA DB) of the exogenous DNA-binding protein is fused with a tag(s) and a nuclear localization signal(s), and then expressed in the cell that is to be analyzed. (iii) The resulting cell is stimulated and crosslinked with formaldehyde or other crosslinkers, if necessary. (iv) The cell is lysed and the crosslinked DNA is fragmented by sonication. (v) The complexes, including the complexes bearing exogenous DNA DB, are immunoprecipitated with an antibody against the tag.

The scheme of enChIP is as follows (right panel): (i) A DNA-binding molecule/complex (DB) that recognizes the DNA sequence in a genomic region of interest is generated. This can be achieved by utilizing technologies such as engineered zinc-finger proteins, transcription activator-like (TAL) proteins, and the CRISPR system consisting of dCas9 together with gRNA. The engineered DB is fused with a tag(s) and an NLS(s), and then expressed in the cell that is to be analyzed. (ii) The resulting cell is stimulated and crosslinked with formaldehyde or other crosslinkers, if necessary. (iii) The cell is lysed, and the DNA is fragmented by sonication or digested with nucleases such as restriction enzymes. (iv) The complexes, including those containing the engineered DB, are subjected to affinity purification methods such as immunoprecipitation.

Thus, the complexes isolated by iChIP and enChIP retain molecules interacting with the genomic region of interest. Reverse crosslinking and subsequent purification of DNA, RNA, proteins, or other molecules allow these molecules to be identified and characterized.

We will apply locus-specific ChIP to elucidate the molecular mechanisms behind important epigenetic phenomena, including: (a) the lineage commitment of lymphocytes, (b) the regulation of odorant receptor expression, (c) the insulator function, (d) the detection and repair of DNA double-strand break, and (e) the epigenetic suppression of the expression of tumor suppressor genes.

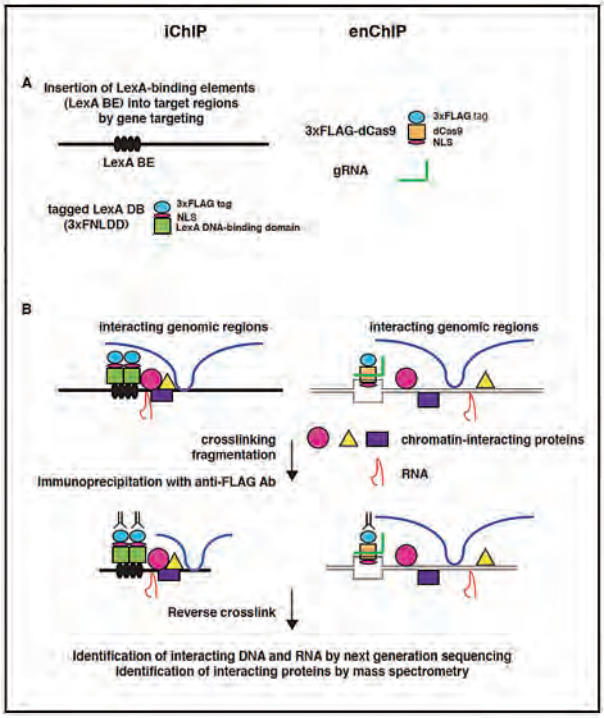


Figure. A scheme of locus-specific ChIP consisting of iChIP and enChIP

Recent Publications

1. Identification of telomere-associated molecules by engineered DNA-binding molecule-mediated chromatin immunoprecipitation (enChIP). Fujita T, Asano Y, Ohtsuka J, Takada Y, Saito K, Ohki R, Fujii H. ***Sci Rep***. 2013 Nov 8;3:3171.
2. Efficient isolation of specific genomic regions and identification of associated proteins by engineered DNA-binding molecule-mediated chromatin immunoprecipitation (enChIP) using CRISPR. Fujita T, Fujii H. ***Biochem Biophys Res Commun***. 2013 Sep 13;439(1):132-6.
3. Direct identification of insulator components by insertional chromatin immunoprecipitation. Fujita T, Fujii H. ***PLoS One***. 2011;6(10):e26109
4. Insertional chromatin immunoprecipitation: a method for isolating specific genomic regions. Hoshino A, Fujii H. ***J Biosci Bioeng***. 2009 Nov;108(5):446-9.
5. Regulation of Fas-mediated immune homeostasis by an activation-induced protein, Cyclon. Saint Fleur S, Hoshino A, Kondo K, Egawa T, Fujii H. ***Blood***. 2009 Aug 13;114(7):1355-65.

Germ Cell Group

Research Group　Associate Professor　Masami Nozaki, Ph. D.

Research Projects

(1) Epigenetics in germ cells

Many testicular germ cell-specific genes are retroposons, most of which contain a CpG-rich region within their ORFs. We discovered that in somatic cells methylation of CpG dinucleotides within the ORF represses its promoter and that demethylation is necessary for gene expression in spermatogenic cells. We are currently examining the molecular basis of epigenetic modifications, including DNA methylation and histone methylation, that occur within a distinct genomic region in germ cells.

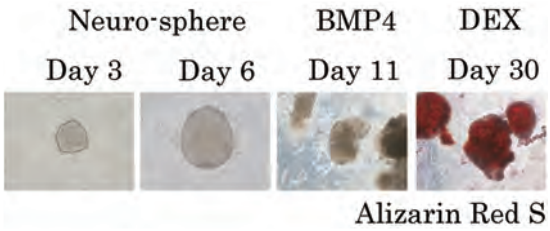


Figure legend. Neural crest cells derived from ES cells differentiated into osteoblasts.

(2) Unique structure of sperm chromatin

The haploid genome in the mammalian sperm nucleus is packaged into a highly compact structure containing protamines and some remaining histones. We are currently analyzing the physiological importance of the somatic-like, histone-containing regions of sperm chromatin.

(3) Establishment of neural crest stem cell lines from ES cells and application of these cell lines in regenerative medicine

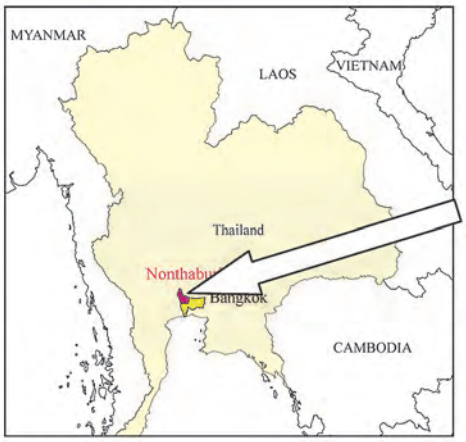
Recent Publications

Inoue H, Ohnishi Y, Nakajima M, Kakudo M, Nozaki M. A novel function of EpCAM in oral squamous cell carcinoma cells under anchorage-independent conditions. ***Int J Oncol***. 2011 Dec; 39 (6): 1401-5.

Thailand – Japan Research Collaboration Center on Emerging and Re-emerging Infections

Director SA Professor Naokazu Takeda, Ph.D.

It was believed until recently that infectious diseases could be conquered through the development of chemotherapies and vaccines; however, the recent worldwide emergence of new infectious diseases and reemergence of infectious diseases that were once considered to be controlled has seriously challenged this notion. Under these circumstances, intensive research that closely monitors and rapidly analyzes emerging and re-emerging infections is urgently required. Since a variety of infectious diseases can spread rapidly across national borders, it is obvious that these diseases cannot be controlled by the independent efforts of individual nations.



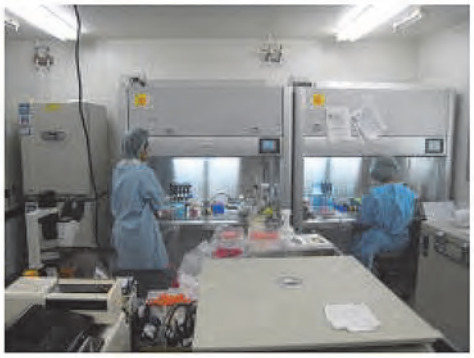
Campus of Ministry of Public Health

To this end, Osaka University founded the Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI) in the Thai National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health of Thailand in 2005. The second phase of the program (2010-2014), which is now named the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), is on-going.

The facility consists of P2 and P3 biohazard containment laboratories and various other equipment and facilities in 600 m^2 floor space. Previously, most research projects conducted abroad were short-term, with the researchers only staying for at most a few months to complete their experiments. Due to the installation of the RCC-ERI, researchers are now able to stay for longer periods of time. The RCC-ERI aims to carry out research projects on both emerging and re-emerging infections in close collaboration with the researchers at the NIH, while at the same time developing the talents of young scientists from Japan and Southeast Asian countries in the field of infection.

To conduct basic and applied research and to develop human resources, the RCC-ERI consists of two sections that are devoted to bacterial and viral infection research. In addition, we aim to establish an effective system that would (i) provide information that would help to prevent the emergence of emerging and re-emerging infections, and (ii) promptly activate a variety of countermeasures should such a disease emerge, including developing therapeutics or vaccines. Finally, we wish to begin collaborations with laboratories from the nations that neighbor Thailand so that we can be at the frontline with the capacity to quickly respond to any globally spreading infectious disease.

BSL-2 Laboratory



BSL-3 Laboratory

Section of Bacterial Infections

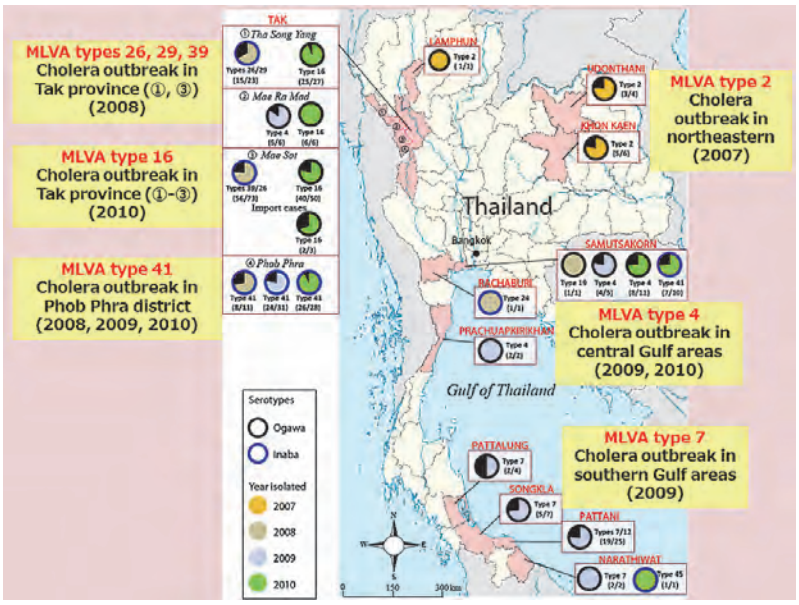
Research Group

SA Professor	Shigeyuki Hamada, D.D.S. Ph.D.
SA Assistant Professor	Kazuhisa Okada, Ph.D.
SA Researcher	Kentaro Inoue, Ph.D.
Postdoctoral Fellow	Mathukorn Na-Ubol, Ph.D.
Postdoctoral Fellow	Warawan Wongboot, Ph.D.

In collaboration with the National Institute of Health, Department of Medical Sciences, Ministry of Public Health of Thailand, the Section of Bacterial Infections pays special attention to emerging and reemerging bacterial diseases that are prevalent or break out in Asian countries. We conduct molecular epidemiology studies on enteric or systemic bacterial infections. Moreover, we also develop detection and identification techniques for the diagnosis of bacterial diseases described below.

It is reported that pneumonia, tuberculosis, and acute diarrheal diseases are associated with high morbidity and mortality rates in Thailand. Consequently, in the Program of Promotion of Research Network for Emerging and Reemerging Infectious Diseases during the 2010–2014 fiscal years, we prioritized research on cholera in Thailand and its causative agent, *Vibrio cholerae*.

In addition, *Streptopcoccus suis*, which is occasionally pathogenic and frequently isolated from diseased pigs, has been found to cause several systemic (zoonotic) infectious diseases in humans, namely meningitis, infective endocarditis, and toxic shock-like syndrome. This has mainly been observed in Asian countries, including Northern Thailand. We will examine this emerging zoonotic infection closely, and elucidate the molecular pathogenesis of S. *suis* infections.



Geographical spread and temporal changes of *Vibrio cholerae* O1 during the 2007–2010 cholera outbreaks in Thailand.
Yellow squares indicate the major MLVA type(s) in each outbreak site shown in the map.

Recent Publications

1. Okada K, Roobthaisong A, Swaddiwudhipong W, Hamada S, Chantaroj S. *Vibrio cholerae* O1 Isolate with Novel Genetic Background, Thailand-Myanmar. ***Emerg Infect Dis***. 2013 19(6):1015-17.
2. Takeuchi D, Akeda Y, Nakayama T, Kerdsin A, Sano Y, Kanda T, Hamada S, Dejsirilert S, Oishi K. The Contribution of Suilysin to the Pathogenesis of *Streptococcus suis* Meningitis. ***J Infect Dis***. 2013 first published online.
3. Okada K, Roobthaisong A, Nakagawa I, Hamada S, Chantaroj S. Genotypic and PFGE/MLVA analyses of *Vibrio cholerae* O1: geographical spread and temporal changes of isolates during the 2007-2010 cholera outbreaks in Thailand. ***PLoS One***. 2012 7(1):e30863.
4. Takeuchi D, Kerdsin A, Pienpringam A, Loetthong P, Samerchea S, Luangsuk P, Khamisara K, Wongwan N, Areeratana P, Chiranairadul P, Lertchayanti S, Petcharat S, Yowang A, Chaiwongsaen P, Nakayama T, Akeda Y, Hamada S, Sawanpanyalert P, Dejsirilert S, Oishi K. Population-Based Study of *Streptococcus suis* Infection in Humans in Phayao Province in Northern Thailand. ***PLoS One***. 2012 7(2):e31265.
5. Okada K, Chantaroj S, Taniguchi T, Suzuki Y, Roobthaisong A, Puiprom O, Honda T, Sawanpanyalert P. A rapid, simple, and sensitive loop-mediated isothermal amplification method to detect toxigenic **Vibrio cholerae** in rectal swab samples. ***Diagn Microbiol Infect Dis***. 2010 66(2):135-9.

Section of Viral Infections

Research Group

SA Professor	Naokazu Takeda, Ph.D.
SA Associate Professor	Kazushi Motomura, M.D., Ph.D.
SA Associate Professor	Atsushi Tanaka, V.M.D., Ph.D.
Postdoctoral Fellow	Nitchakarn Noranate, Ph. D.
Research Fellow	Chris Verathamjamras, M. Sc.
Research Fellow	Uranan Tumkosit , M. Sc.
Research Fellow	Michittra Boonchan, M.Sc.

The Section of viral Infections focuses on three emerging and reemerging viral diseases that are prevalent in Asian countries including Thailand in collaboration with the National Institute of Health, Department of Medical Sciences, Ministry of Public Health of Thailand. The first one is mosquito-borne infectious diseases, and we study chikungunya fever from epidemiological, molecular biological and immunological aspects. The second one is blood-borne infectious diseases (HIV diseases/AIDS), and we perform virological and immunological characterization of the HIV-1 CRF01_AE strains prevalent in Southeast Asian countries including Thailand. In addition, the studies to elucidate the mechanism of the anti-retroviral drug resistance are in progress. The last one is enteric viral diseases including norovirus and hepatitis A virus infections, in which we perform molecular epidemiological studies using specimens from patients with diarrhea as well as environmental water. We also perform genetic analysis of the viruses and develop control measures of the diseases.

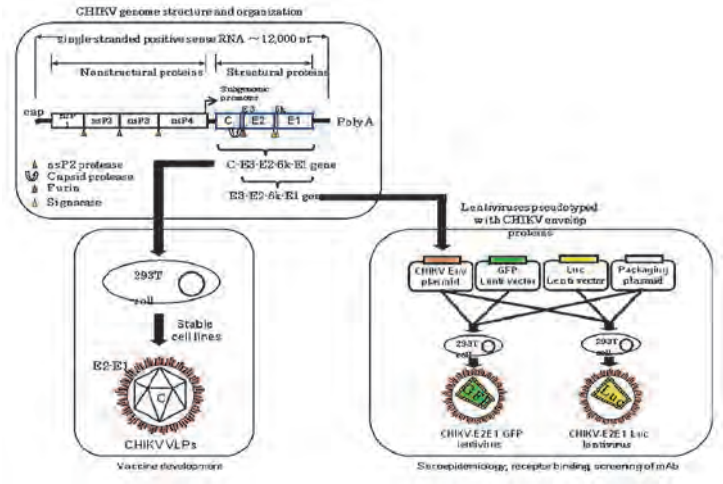


Figure 1. Genome organization of chikungunya virus (CHIKV) and research projects on chikungunya fever. CHIKV (BSL3) is a positive-sense single stranded RNA virus with 60-70 nm in diameter. When the subgenomic region encoding entire structural proteins was expressed, they self-assembled into virus-like particles, which were morphologically and antigenically similar to those of native CHIKV. Expression vectors encoding envelop proteins, E3, E2, 6k and E1, lentivirus packaging vector, and lentivirus vector encoding luciferase were used to transfect 293T cells. The resultant lentivirus pseudotyped with CHIKV envelop proteins (BSL2) was applicable to the CHIKV neutralization test (Ref.3).

Recent Publications

1. Characterization of human immunodeficiency virus type 1 CRF01_AE env genes derived from recently infected Thai individuals. Chaitaveep N, Utachee P, Nakamura S, Chuenchitra T, Ekpo P, Takeda N, Pattanapanyasat K, Kameoka M. ***Microbes Infect***. 2014 Feb;16(2):142-52.
2. CRF01_AE-specific neutralizing activity observed in plasma derived from HIV-1-infected Thai patients residing in northern Thailand: comparison of neutralizing breadth and potency between plasma derived from rapid and slow progressors. Sapsutthipas S, Tsuchiya N, Pathipavanich P, Ariyoshi K, Sawanpanyalert P, Takeda N, Isarangkura-na-ayuthaya P, Kameoka M. ***PLoS One*** 2013; 8: e53920.
3. Development of a Pseudotyped Lentiviral Vector--Based Neutralization Assay for Chikungunya Virus Infection. Kishishita N, Takeda N, Anuegoonpipat A, Anantapreecha S. ***J Clin Microbiol*** 2013; 51: 1389-95.
4. Poly (I:C), an agonist of toll-like receptor-3, inhibits replication of the Chikungunya virus in BEAS-2B cells. Li YG, Siripanyaphinyo U, Tumkosit U, Noranate N, A AN, Pan Y, Kameoka M, Kurosu T, Ikuta K, Takeda N, Anantapreecha S, ***Virol J*** 2012; 9: 114.
5. Two N-linked glycosylation sites in the V2 and C2 regions of human immunodeficiency virus type 1 CRF01_AE envelope glycoprotein gp120 regulate viral neutralization susceptibility to the human monoclonal antibody specific for the CD4 binding domain. Utachee P, Nakamura S, Isarangkura-Na-Ayuthaya P, Tokunaga K, Sawanpanyalert P, Ikuta K, Auwanit W, Kameoka M. ***J Virol*** 2010; 84: 4311-20.

Mahidol-Osaka Center for Infectious Diseases (MOCID)

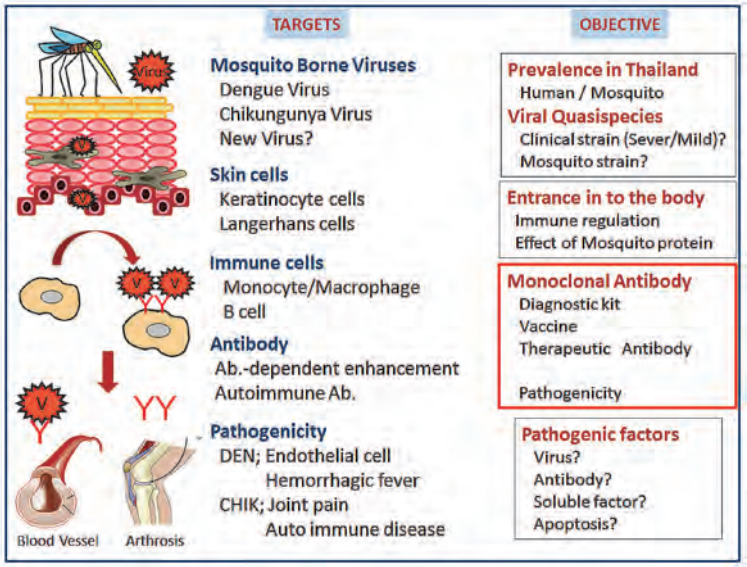
Research Group

Director Professor (SUP)	Yoshiharu Matsuura, DVM, Ph. D
Associate Professor	Tamaki Okabayashi, DVM, Ph. D.
Postdoctoral Fellow	Orapim Puiprom, Ph.D.
Research Fellow	Panjaporn Chaichana, M.Sc.
Research Fellow	Nantarat Chantawat, M. Sc.

The MOCID interest focuses on several tropical infectious diseases that are of human health importance in Thailand, especially mosquito-borne viral infectious diseases such as dengue fever/dengue hemorrhagic fever and chikungunya fever. We focus on the development of rapid diagnosis kits for the viral diseases, and the studies of the prevalence of the viral infections in human and mosquitoes and the pathogenesis of the viruses. We would like to raise young scientists' interest and research skills on the infectious diseases through our collaborating with Mahidol University by using clinical samples.

Our main research project

1) Epidemiology of mosquito-borne virus infections in human and mosquitoes in Thailand.
2) Generation and characterization of human and mouse monoclonal antibodies against dengue virus and chikungunya virus.
3) Development of diagnosis kits for the viral diseases.



Recent Publications

1. Low levels of antibody-dependent enhancement in vitro using viruses and plasma from dengue patients.Chaichana P, Okabayashi T, Puiprom O, Sasayama M, Sasaki T, Yamashita A, Ramasoota P, Kurosu T, Ikuta K.***PLoS One***. 2014 Mar 18;9(3):e92173.
2. Antibody germline characterization of cross-neutralizing human IgGs against 4 serotypes of dengue virus. Pitaksajjakul P, Benjathummarak S, Pipattanaboon C, Wongwit W, Okabayashi T, Kuhara M, Misaki R, Fujiyama K, Ramasoota P. ***Biochem Biophys Res Commun***. 2014 Mar 14.
3. Detection and characterization of enteric viruses in flood water from the 2011 thai flood. Ngaosuwankul N, Thippornchai N, Yamashita A, Vargas RE, Tunyong W, Mahakunkijchareon Y, Ikuta K, Singhasivanon P, Okabayashi T, Leaungwutiwong P. ***Jpn J Infect Dis***. 2013;66(5):398-403
4. Characterization of chikungunya virus infection of a human keratinocyte cell line: role of mosquito salivary gland protein in suppressing the host immune response.
   Puiprom O, Morales Vargas RE, Potiwat R, Chaichana P, Ikuta K, Ramasoota P,Okabayashi T. ***Infect Genet Evol***. 2013 Jul;17:210-5.
5. Dengue virus neutralization and antibody-dependent enhancement activities of human monoclonal antibodies derived from dengue patients at acute phase of secondary infection.Sasaki T, Setthapramote C, Kurosu T, Nishimura M, Asai A, Omokoko MD, Pipattanaboon C, Pitaksajjakul P, Limkittikul K, Subchareon A, Chaichana P, Okabayashi T, Hirai I, Leaungwutiwong P, Misaki R, Fujiyama K, Ono K, Okuno Y, Ramasoota P, Ikuta K. ***Antiviral Res***. 2013 Jun;98(3):423-31.

BIKEN Endowed Department of Dengue Vaccine Development

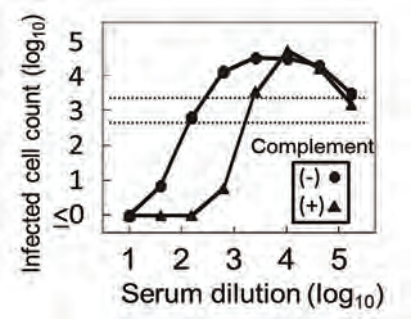
Research Group

Endowed Chair Professor Ph.D. Eiji Konishi
Endowed Assistant Professor Ph.D. Atsushi Yamanaka

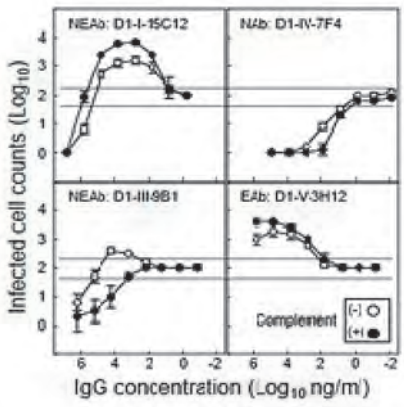
BIKEN Endowed Department of Dengue Vaccine Development was established in Faculty of Tropical Medicine, Mahidol University, Thailand, in 2011 by endowment from The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan to Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

Dengue fever is the most important mosquito-borne viral disease, which is distributed in tropical regions and producing an estimated 300,000 patients daily. Dengue hemorrhagic fever is its severer form and has a mortality up to 20% if an appropriate treatment is not done. Unfortunately, no approved vaccines or specific antivirals have been developed.

Our department will carry out basic research studies on (1) mechanisms involved in pathogenesis of dengue fever and dengue hemorrhagic fever, (2) virulence, transmission and evolution of dengue viruses, and (3) dengue vaccine development using several strategies.



World-first efficacy trial for evaluating a candidate dengue vaccine suggested a need to improve the current neutralization test using Vero cells recommended by WHO. We have established an antibody assay system using K562 cells bearing Fcγ receptors to seek more accurate antibody levels required for disease protection. A patient sample showed neutralizing activity with dengue type 1 virus-infected cell counts $<10^3$ PFU/ml and enhancing activity with infected cell counts $>10^3$ PFU/ml depending on dilution factor as shown above. The assay also indicated the presence of both complement-dependent and independent antibodies.



Most mouse monoclonal antibodies generated using dengue type 1 virus antigen displayed both neutralizing and enhancing activities depending on concentration (NEAb), whereas some showed only enhancing (EAb) or neutralizing activities (NAb). One obstacle hampering dengue vaccine development is a concern of deterioration caused by vaccine-induced enhancing antibodies. An antigen possessing epitopes that can effectively induce NAb should contribute to development of a safe and efficacious dengue vaccine.

Recent Publications

1. A mouse monoclonal antibody against dengue virus type 1 Mochizuki strain targeting envelope protein domain II and displaying strongly neutralizing but not enhancing activity. Yamanaka A, Kotaki T, Konishi E. ***J Virol***. 2013 Dec;87(23):12828-37.
2. Genetic and evolutionary analysis of cell-fusing agent virus based on Thai strains isolated in 2008 and 2012. Yamanaka A, Thongrungkiat S, Ramasoota P, Konishi E. ***Infect Genet Evol***. 2013 Oct;19:188-94.
3. Comparison of infection-neutralizing and -enhancing antibody balance induced by two distinct genotype strains of dengue virus type 1 or 3 DNA vaccines in mice. Sjatha F, Takizawa Y, Kotaki T, Yamanaka A, Konishi E. ***Microbes Infect***. 2013 Nov;15(12):828-36.
4. Memory B cells: a proposed new immunological correlate for protective efficacy of Japanese encephalitis vaccine. Konishi E. ***Expert Rev Vaccines***. 2013 Aug;12(8):871-3.
5. A review of successful flavivirus vaccines and the problems with those flaviviruses for which vaccines are not yet available. Ishikawa T, Yamanaka A, Konishi E. ***Vaccine***. 2014 Mar 10;32(12):1326-37.

Animal Resource Center for Infectious Diseases

Research Group

Head, Professor	Masahito Ikawa, Ph.D.
SA Associate Professor	Ayako Isotani, Ph.D.
Assistant Professor	Yuhkoh Satouh, Ph.D.
Assistant Professor	Yoshitaka Fujihara, Ph.D.
Assistant Professor	Haruhiko Miyata, Ph.D.

To study microbial diseases, it is important to analyze the interactions between the host and pathogenic organisms. Animal models are indispensable in current microbiological and immunological research, particularly since through the use of molecular biology and biotechnology, we can generate gene-manipulated mice that can aid our understanding of the mechanisms of infection. For these purposes, experimentally infected animals should be managed in a suitable, safe and controlled manner. Animal Resource Center for Infectious Diseases is a unique facility that was established in 1967 to meet these requirements. The center is separated into three areas: one for animal experiment for microbial disease model (BSL2 and BSL3), an SPF area. The section for animal experiment for disease model is completely air-conditioned and maintained at a negative air pressure to minimize the risk of contamination. Each sub-area has an individual pass-through type autoclave to sterilize all materials before their removal. The exhaust air is filtered to avoid exterior dissemination of pathogenic microbes. These measures ensure that disease model animals can be handled safely without accidental cross-contamination.
Before gaining access to this restricted facility, researchers must take an official orientation and submit a research plan for committee review. The condition of the animals is regularly inspected.

Services such as the generation of gene-manipulated animals, in vitro fertilization, and cryopreservation of mouse strains are available at our facility. (Table 1)

Figure 1: Biosafety level 3 room (Building A)
The room for disease model animal and experimentation at biosafety level 3. Hemorrhageic fever with renal syndrome-causing virus (HFRSV) was isolated in this area. In addition, animal experiments for Creutzfeldt-Jakob Disease (CJD), severe acute respiratory syndrome (SARS) and Acquired Immune Deficiency Syndrome (AIDS) can be handled in this facility.

Table 1) No. of mouse lines produced/preserved

period	Tg mice	KO mice	Cryopreservation
1995-2000	228	50	261
2001-2003	104	57	443
2004-2006	43	69	331
2007-2009	22	74	216
2010-2011	31	79	220
2012-2013	41	159	377
total	469	488	1848

Tg, transgenic; KO, knock-out

DNA-chip Development Center for Infectious Diseases

Research Group

Head, Professor	Hiroshi Nojima, Ph.D.
Assistant Professor	Daisuke Okuzaki, Ph.D.
Associate Professor (SUP)	Norikazu Yabuta, Ph.D.

Facility Management: The establishment of infectious diseases is driven by the gene expression of pathogenic organisms within the infected host cells. To understand parasite pathogenesis and pathophysiology and to develop new methods to prevent and treat infectious diseases, it is necessary to identify the pathogenic genes that are expressed in the infected host cells and to determine how they induce disease at the genetic level. This process requires an analysis of the transcriptional patterns of both the genes of the pathogenic organism and the responsive genes of the host genome.

The DNA-chip Development Center for Infectious Diseases is a unique facility that was established in 2004 to analyze the transcriptional dynamics and variations involved in infectious diseases. Two research approaches are employed in this facility:

(1) *Transcriptome analysis using DNA-chip analyzers*

The high-density DNA microarray system in this facility permits comprehensive transcriptional analysis of gene expression in the human or mouse host, and in various pathogenic organisms. Two DNA microarray systems (Agilent- and Affymetrix-type) are available in this center. Our real-time PCR analysis system (ABI, PRISM7900HT-2) and Nano-counter are useful for more accurate quantitative analysis of the transcriptional levels of particular genes. In addition, a novel DNA microarray system (Genopal of Mitsubishi Rayon Co. Ltd.) is currently being used in this center. An example of its practical use is the establishment of a blood RNA-based system for the diagnosis of autoimmune diseases such as vasculitis. This system employs a "focused microarray" that examines the expression of ~200 blood cell-specific and disease-related genes.

Fig. 1: High-density DNA microarray system.

(2) *Proteome analysis using mass spectrometry*

Comprehensive translational analyses are also very important in furthering our understanding of infectious diseases. The MS/MS spectrometer installed in this facility allows the analysis of the expression, interactions, and modifications of proteins from humans, mice, and pathogenic organisms. This center is also capable of recent technical innovations, such as the mass spectrometric detection of pathogenic organisms to facilitate the development of novel diagnostic systems for infectious diseases.

Fig. 2: MS/MS spectrometer.

Recent Publications

1. Nagi-Miura N, Okuzaki D, Torigata K, Sakurai MA, Ito A, Ohno N, Nojima H. CAWS administration increases the expression of interferon γ and complement factors that lead to severe vasculitis in DBA/2 mice. ***BMC Immunol***. 2013 Sep 24;14:44.
2. Muso E, Okuzaki D, Kobayashi S, Iwasaki Y, Sakurai MA, Ito A, Nojima H. Ficolin-1 is up-regulated in leukocytes and glomeruli from microscopic polyangiitis patients. ***Autoimmunity***. 2013 Dec;46(8):513-24.
3. Okuzaki D, Kimura S, Yabuta N, Ohmine T, Nojima H. LeukoCatch, a quick and efficient tool for the preparation of leukocyte extracts from blood. ***BMC Clin Pathol***. 2011 Aug 17;11:9.
4. Okuzaki D, Fukushima T, Tougan T, Ishii T, Kobayashi S, Yoshizaki K, Akita T, Nojima H. Genopal™: a novel hollow fibre array for focused microarray analysis. ***DNA Res***. 2010 Dec;17(6):369-79.
5. Tougan T, Okuzaki D, Nojima H. Chum-RNA allows preparation of a high-quality cDNA library from a single-cell quantity of mRNA without PCR amplification. ***Nucleic Acids Res***. 2008 Sep;36(15):e92.

Center for genetic analysis of biological responses

Research Group

<Production laboratory for genetically-manipulated animals>
Head, Professor — Masahito Ikawa, Ph.D.
SA Associate Professor — Kazuo Yamagata, Ph.D.
SA Assistant Professor — Jun Ueda, Ph.D.
<Resource laboratory for genetically-manipulated animals>
Guest Professor — Kenichi Yamamura, M.D., Ph.D.
SA Associate Professor — Ayako Isotani, Ph.D.
Assistant Professor — Haruhiko Miyata, Ph.D.
<Laboratory for analysis of genetically-manipulated animals>
Professor — Shizuo Akira, M.D., Ph.D.
Professor — Hisashi Arase, M.D., Ph.D.
Professor — Taroh Kinoshita, Ph.D.
<Laboratory for analysis of genetically-manipulated animals>
Professor — Hitoshi Kikutani, M.D., Ph.D.
Professor — Hiroshi Nojima, Ph.D.
Professor — Nobuyuki Takakura, M.D., Ph.D.
Professor — Masato Okada, Ph.D.
Assistant Professor — Yoshitaka Fujihara, Ph.D.
<Laboratory for promotion of collaborative research>
Guest Professor — Yoichiro Iwakura, Ph.D.
Guest Professor — Nobuaki Yoshida, M.D., Ph.D.
Assistant Professor — Naohisa Goto, Ph.D.
Assistant Professor — Yuhkoh Satouh, Ph.D.

Our bodies are kept homeostatically stable by functions of proteins produced from many genes. In other words, our health is basically maintained in accordance with the balance of our gene products. Many diseases can therefore be traced to a defect in, or malfunction of, various genes. In order to find and develop new drugs or new therapy, it is very important to identify the function of each gene in vivo. However, at present, we do not have enough information about the function of various genes to clarify their relationships to each other, or to analyze the relationships to specific diseases systematically.

Gene-disrupted animals can be a powerful tool in helping us understand the role of certain genes in vivo. Such animals can be produced and used as a model for various human diseases as well as to screen new drugs. With this in mind, preparation of gene-disrupted mouse lines of entire genes is planned and progressing as research projects on a national level in many countries. The production of gene-manipulated animals may have another aspect. These animals are considered to be variable animal resources protected by patents to develop new drugs and therapeutic methods. It is highly important for Japan to make a reasonable contribution in this area. Under these circumstances, we created a consortium of three research institutes from three universities (The Research Institute for Microbial Diseases, Osaka University: The Institute of Medical Science, University of Tokyo: Center for Animal Resources and Development, Kumamoto University), placing the headquarters at Kumamoto University. With this consortium, we are sharing our specialties with each other and aiming to produce many gene-manipulated animals closely focused on human diseases. In our center, we are mainly focusing on genes related to reproduction, infection and allergy, taking advantage of an existing disease screening system in our university, including features such as fluorescent colored sperm and eggs (Figure 1). Through these gene-manipulated animals, we aim to perform translational research for the establishment of new therapeutic systems and to aid in the discovery of new drugs.

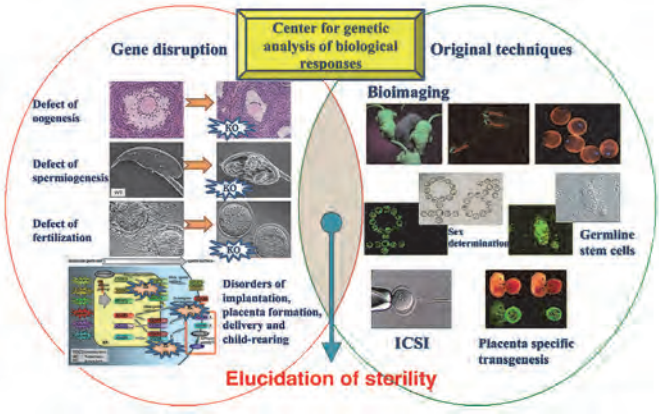


Figure 1. Strategy for elucidating sterility

Recent Publications

1. Generation of mutant mice by pronuclear injection of circular plasmid expressing Cas9 and single guided RNA. Mashiko D, Fujihara Y, Satouh Y, Miyata H, Isotani A, Ikawa M. ***Sci Rep***. 2013 Nov 27;3:3355.
2. MiR-200b and miR-429 Function in Mouse Ovulation and Are Essential for Female Fertility. Hasuwa H, Ueda J, Ikawa M, Okabe M. ***Science***. 2013 Jul 5;341(6141):71-3.
3. Expression of TEX101, regulated by ACE, is essential for the production of fertile mouse spermatozoa. Fujihara Y, Tokuhiro K, Muro Y, Kondoh G, Araki Y, Ikawa M, Okabe M. ***Proc Natl Acad Sci U S A***. 2013 May 14;110 (20):8111-6.
4. Visualization of the moment of mouse sperm-egg fusion and dynamic localization of IZUMO1. Satouh Y, Inoue N, Ikawa M, Okabe M. ***J Cell Sci***. 2012 Nov 1;125(Pt 21):4985-90.
5. Testis specific PDILT is required for quality control of sperm membrane protein ADAM3 and male fertility. Tokuhiro K, Ikawa M, Benham AM, Okabe M. ***Proc Natl Acad Sci U S A***. 2012 March 6;109 (10):3850-3855.

The Biken History Museum

Head, Professor Professor Hiroshi Nojima, Ph.D.

The Research Institute for Microbial Diseases (RIMD) was established in 1934 by combining two institutions, namely the Takeo Tuberculosis Institute and the Osaka Leprosy Institute. To commemorate the 70th anniversary of its establishment, a plan of the Biken History Museum was proposed, and it was opened on December 17, 2010.

The Research Institute for Microbial Diseases (RIMD) originated from three institutions, namely Osaka Medical School, the Takeo Tuberculosis Institute (donated by Mr. Jiemon Takeo), and the Osaka Leprosy Institute (donated by an anonymous benefactor). In 1929, Dr. Chozaburo Kusumoto (the president of Osaka Medical School) and Dr. Tenji Taniguchi (professor of Bacterial Serology, Osaka Medical School) were concerned about the frequent introduction of non-native infectious diseases like cholera and the plague into the Kansai district via the Kobe port, a major international port of Japan at that time; given its central location of the Kansai district, it readily served as a gateway from which these diseases could spread throughout the rest of Japan. The concerns of Dr. Chozaburo Kusumoto and Dr. Tenji Taniguchi were heightened by their experiences with the Great Kanto Earthquake in 1923, which had revealed to them how rapidly infectious diseases could spread.

Fig.1. A picture of Biken History Museum before the opening ceremony.

These concerns led them to strongly encourage the Governor of the Osaka Prefecture, Mr. Zenzaburo Shibata, and the Osaka business community to establish public institutions on infectious diseases in the Kansai district, particularly in Osaka. In response to this campaign, the businessman Mr. Gendo Yamaguchi donated 200,000 yen (the equivalent now of several hundred million yen), which made it possible to construct the main building of RIMD at the Nakanoshima campus of Osaka Medical School (Dojima, Osaka City) in February 1934. After its construction, the researchers of the three institutions mentioned above moved into the RIMD building and continued their active research.

Fig. 2. The portrait bust of Mr. Jiemon Takeo, 10th (left) and 11th (right).

Both members and non-members of Osaka University can visit the Museum free of charge from 9:00 a.m. to 5 p.m. on working days. A gorgeous pamphlet will be presented by writing the visitors name at the information desk of RIMD which is located near the Museum entrance.

Inside the museum, you will find the portrait bust of Mr. Jiemon Takeo, 10th (left) and 11th (right), Koch' s microscope donated by the German government, influenza and SARS virus model made by Kaiyodo Co. Ltd. (Kadoma, Osaka).

Fig. 3. Koch' s microscope.

Fig. 4. A plastic model of In fluenza virus made by Kaiyodo Co. Ltd.

Fig. 5. A plastic model of Severe Acute Respiratory Syndrome (SARS) virus made by Kaiyodo Co. Ltd.

Central Instrumentation Laboratory

Head	Professor	Hiroaki Miki, Ph.D.
	Associate Professor	Shinji Higashiyama, Ph.D.
	Assistant Professor	Kazunobu Saito, Ph.D.
	Specially Appointed Assistant Professor	Miki Morimatsu, Ph.D.

The Central Instrumentation Laboratory was established in the Research Institute for Microbial Diseases in 1959 for the mutual advantage of researchers, by bringing machines from each laboratory when equipment was lacking. Nowadays, various precise and high performance machines are available in the laboratory at all times. There are ultracentrifuges, transmission and scanning electron microscopes, a Biacore, cell analyzer/sorters, an automatic plasmid DNA separation device, DNA sequencers, mass spectrometers etc. Also, cell storage large tanks equipped with automatic liquid nitrogen supply system and a specified chemical treatment room are also present. In addition, professional technicians are employed to maintain and manage the devices, as well as to provide service, education and training for newcomers. In-house services such as flow cytometer cell sorting, mass spectrometry protein identification, electron microscope image capture, and DNA sequencing services are available at the institute in response to requests from researchers. These services play a very important role in the institute since experimental machines become more and more complicated and researchers have difficulty handling the variety of experimental machines by themselves.

Radioisotope Laboratory

Head	Professor	Hiroaki Miki, Ph.D.
	Associate Professor	Shinji Higashiyama, Ph.D.

The Radioisotope (RI) Laboratory is adjacent to the main building of the Institute in 1967, and was extended by branch laboratories with a combined space of about 600 sq. m. during the establishment of the North building in 1979 and the Central Laboratory for Biological Hazardous Microbes in 1983. In 1998, a radiation exposure room was established on the first basement level of the South building. The Genome Information Research Center radioisotope laboratory joined in 2007. The main RI Laboratory, the North building RI Laboratory and the Genome Information Research Center radioisotope laboratory were closed in 2010 and 2011. The new RI Laboratory was established in the Immunology Frontier Research Center building in 2011.

The RI Laboratory is designed for biomedical experiments with radioisotopes and plays an important role in the Institute. Its facilities include an 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 radioisotopes. About 200 researchers use this laboratory every year.

Central Laboratory for Biological Hazardous Microbes

Head Professor Tatsuo Shioda, D. Med. Sc.

This laboratory was set up in 1983 to ensure the safe handling of biologically hazardous microbes, such as hemorrhagic fever with renal syndrome (HFRS) virus. Since then, all experimental studies using such microbes, including the human immunodeficiency virus (HIV), have been carried out in this laboratory. The laboratory is a three-story building that is 550 m^2 in area. The first floor is reserved for experiments using radioisotopes. 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 a negative pressure. Air is released from the facility through high-quality outlet filters to minimize contamination of the outside environment. Each room is equipped with safety cabinets and autoclaves for the sterilization of used materials before their disposal. The entire laboratory was renovated from 2005 to 2007 to increase number of pathogens simultaneously used in this laboratory.

In 2012 and 2013, 45 and 53 researchers, respectively, were approved by the Biosafety Committee to use this laboratory. The microbes used included HIV, human and avian influenza viruses, SARS corona virus, and scrapie agent.

Library

Head Professor Hiroaki Miki , Ph.D.

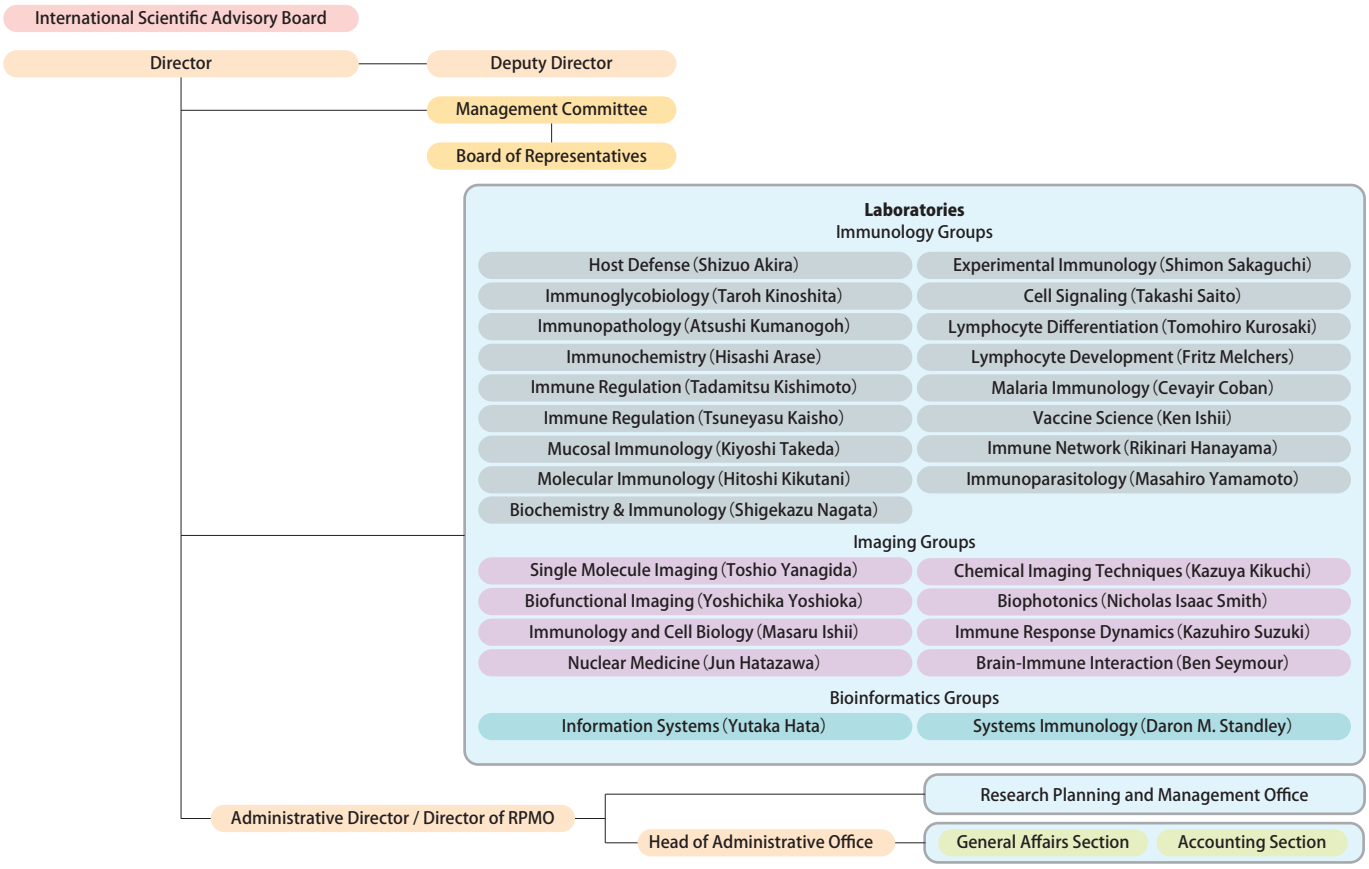
The RIMD library collects academic books and jounals on microbiology and immunology, as well as work on related scientific fields such as cell biology, genetics, histology, developmental biology, biochemistry, pharmacology pathology, microbiology and oncology. In particular, we have collected rare books on parasitology that cannot be found at other institutes. These books are frequently accessed by visitors to the RIMD library. Due to the construction of a new building for the Integrated Life Science Building, the RIMD library was moved to a temporary library room set up on the 1st floor of the south building of RIMD on December 2007. At July 2010, the RIMD library was moved to a new space located at the 1st floor of the new RIMD main building. Since the temporary library space was quite small, we were forced to discard most of the old books and journals. For this reason, we now only hold journals published after 1991, all of the back issues of Biken Journal, and 14,000 books. We now purchase 18 and 23 journals published in English and Japanese, respectively. Most of the books and journals are kept in the stock room set up on the 1st floor of the north building, but textbooks and newly arrived journals after 2010 are arranged on the front shelf of the bureau. Most of the materials in the RIMD library are registered on the online index at the main Library of Osaka University, which can also be accessed by libraries throughout Japan via the Interlibrary Loan (ILL) system. One librarian handles the RIMD library together with two professors, two associate professors who act as members of RIMD library committee.

World Premier International Research Center

Immunology Frontier Research Center

●Uniqueness and Objectives

Immunology has always been a scientific strength of Japan. In particular, Osaka University has been historically known for its leading immunology researchers including Prof. Shizuo Akira.
The Osaka University Immunology Frontier Research Center (IFReC), directed by Prof. Akira, is an example of this reputation. IFReC was selected by the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) as one of the nation's exclusive World Premier International (WPI) Research Center Initiative Programs. Established on October 1st of 2007, the center is expected to engage in high level research that will make it an international leader in the field of immunology. Immunology investigates the mechanisms that protect the body against microbial infection. Because the immune system is essential for eliminating infectious pathogens from the body, its malfunction gives rise to various disorders such as autoimmune diseases, allograft rejection during transplantation, and allergies.
The scientific aim of IFReC is to unveil a comprehensive understanding of the dynamic immune system by employing a variety of imaging technologies and Bioinformatics to immunology.

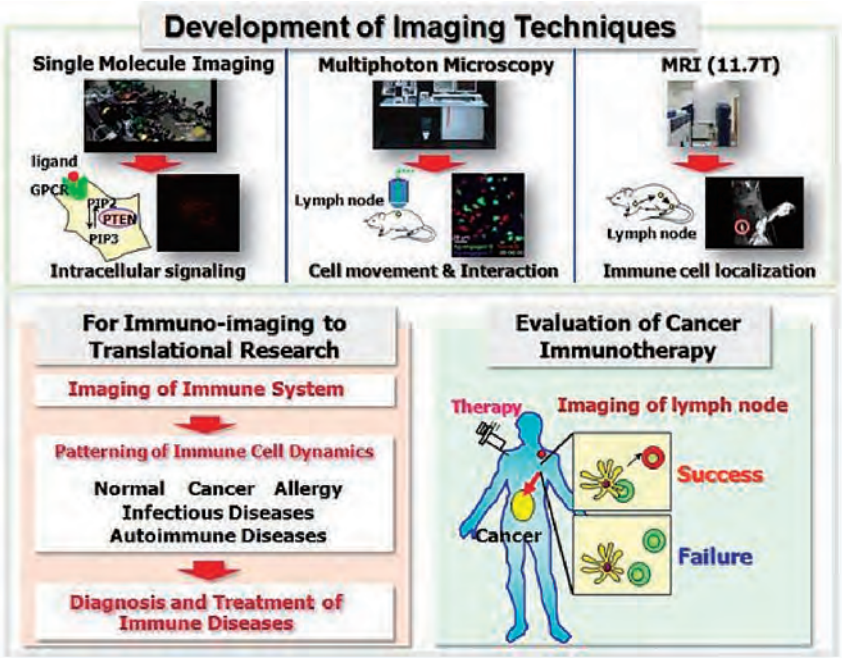


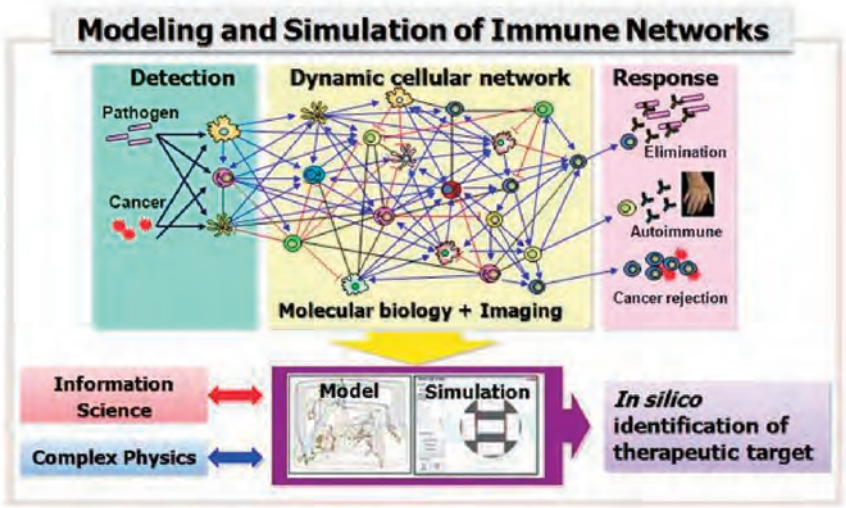
●Research fields / Expected Achievements

To date, research in immunology has either been carried out by isolating immune cells from the body and examining these cells in vitro or by using in vitro cultured cell lines. Although such studies have provided many new insights on the immunology system, we still do not understand the system well enough to predict how it will respond when a certain pathogen invades. One approach to resolving this problem is to study immune responses in a spatiotemporal manner. Given the importance of the spatiotemporal organization of the lymphoid organs in an immune response and the importance of understanding how single immune cells behave, combining immunology techniques with imaging techniques is vital for advancing immunology to a new frontier.

To meet our needs and expectations, new imaging techniques will need to be developed by applying an interdisciplinary effort that combines people from disparate fields like physics, computer science, and immunology.

By integrating the immunology and imaging fields, we will be able to understand the dynamic interactions of immune cells and their activation. This will lead to new and more efficient development strategies for vaccines and immune therapies when combating infectious diseases, cancers and autoimmunity disorders.





Management Expenses Grants

(unit : thousand yen)

Classification	2009	2010	2011	2012	2013
Personnel	859,673	887,150	863,168	877,581	932,979
Non-Personnel	548,947	704,408	567,143	477,809	527,783
Total	1,408,620	1,591,558	1,430,311	1,355,390	1,460,762

Other Grants

(unit : thousand yen)

Classification	2009	2010	2011	2012	2013
Contract Research	1,040,180	908,861	711,772	634,565	601,976
Donations for Research	343,772	689,654	765,777	730,868	262,716
Miscellaneous	2,090	4,506	4,082	4,283	4,426
Total	1,386,042	1,603,021	1,481,631	1,369,716	869,118

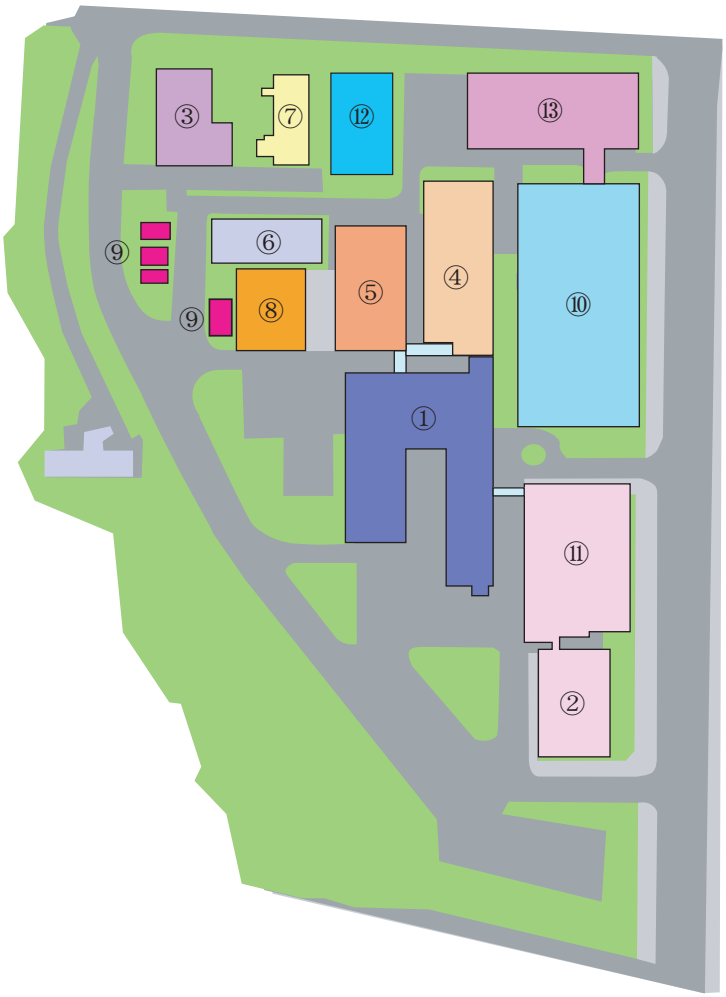
Grants-in-Aid for Scientific Research

(unit : thousand yen)

Classification	2009	2010	2011	2012	2013
MEXT Research Grants	688,999	453,744	466,212	439,280	436,020
Health and Labor Sciences Research Grants	118,789	107,632	87,913	130,669	245,040
Health and Labor other Research Grants	13,988	0	0	0	0
Global COE Program Grants	120,037	85,441	74,992	76,651	0
Total	941,813	646,817	629,117	646,600	681,060

Building Area

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



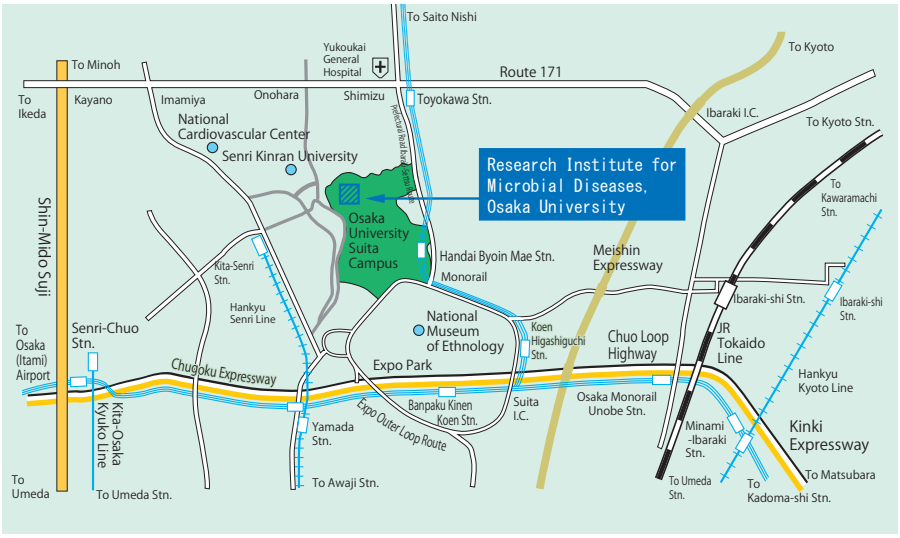
① Main building (left) and
⑩ Integrated Life Science building (right)

② South building

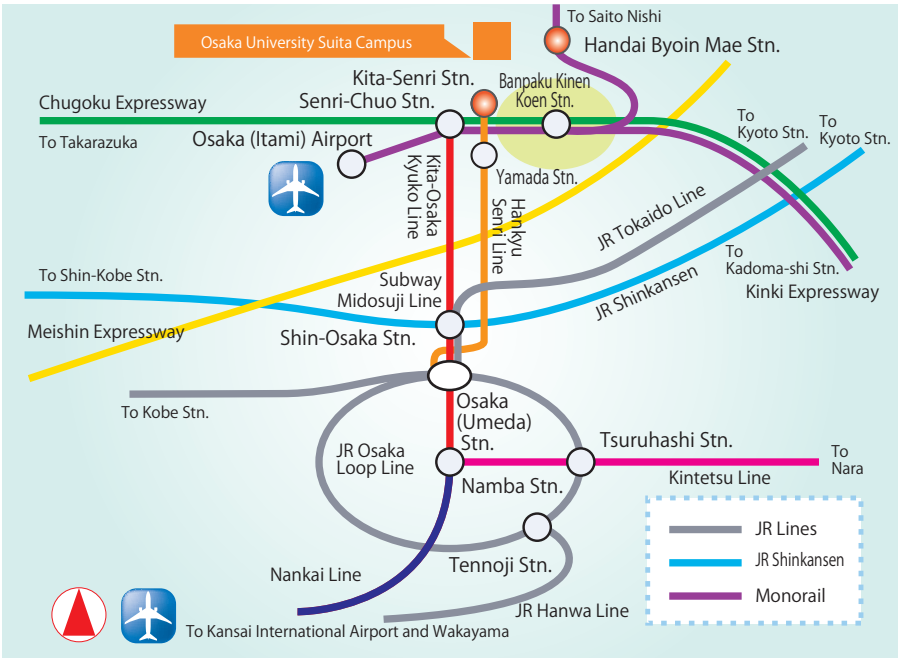
⑦ Central Laboratory for Biological Hazardous Microbes
and
⑤⑥ Animal Resource Center

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

Location

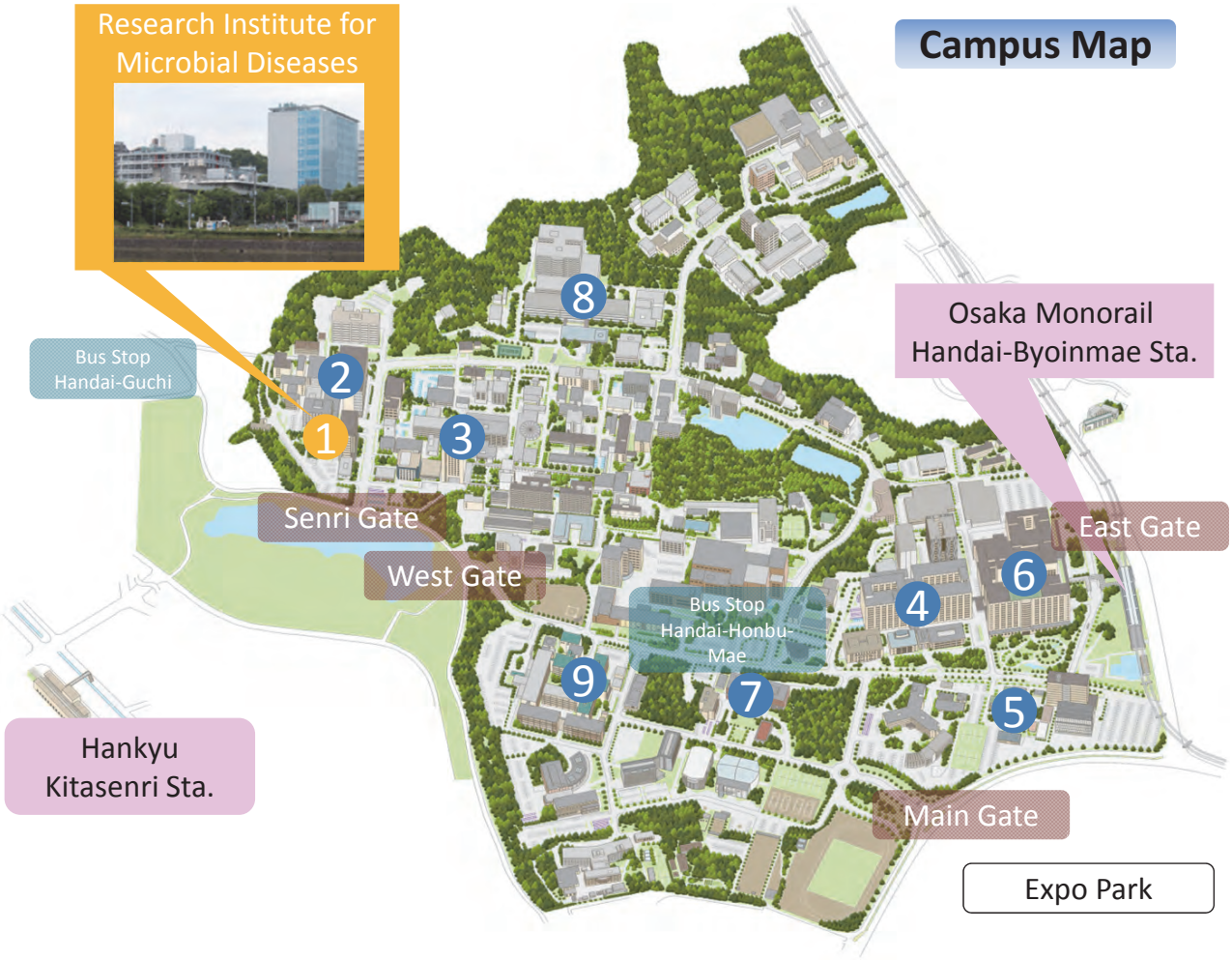


Transportation access



●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”.
●Bus : 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).

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

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