Osaka University

Research Institute for Microbial Diseases

2012–2013

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 Infections
  - 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
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Shohei Otani, M.D., Professor
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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 Yoneyama, 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
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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

Department Heads

2012,04,01

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	Associate Professsor Masahiro Yamamoto Ph.D.
Division of Host Defense	
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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.
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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	Professor Kazuyoshi Ikuta Ph.D.
Genome Infomation Research Center	Head, Professor Teruo Yasunaga Ph.D.
Department of Experimental Genome Research	Professor Masaru Okabe Ph.D.
Department of Genome Informatics	Professor Teruo Yasunaga Ph.D.
Department of Infection Metagenomics	
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 Malariology	
Laboratory of Combined Research on Microbiology and Immunology	Associate Professor Hiroki Nagai Ph.D.
Animal Resource Center for Infectious Diseases	Head, Professor Masaru Okabe Ph.D.
DNA-chip Development Center for Infectious Diseases	Head, Professor Hiroshi Nojima Ph.D.
Center for Genetic Analysis of Biological Responses	Head, Professor Masaru Okabe 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 Masato Okada Ph.D.
Radioisotope Laboratory	Head,Professor Masato Okada Ph.D.
Central Laboratory for Biological Hazardous Microbes	Head, Professor Tatsuo Shioda Ph.D.
Library	Head, Professor Toshihiro Horii Ph.D.
Administration	Head, Katsumi Uedono

＊SA : Specially Appointed

Staff

2012,04,01

Professor	16
Endowed Chair Professor	1
SA Professor	4
Associate Professor	14
Endowed Chair Associate Professor	0
SA Associate Professor	4
SA Lecturer	4
Assistant Professor	30
Endowed Chair Assistant Professor	1
SA Assistant Professor	10
Educational Support Staff	3
Technical Staff	3
Administrative Staff	21
SA Researcher	47
Part-time General & Technical staff	48
Total	206

SA：Specially Appointed

Graduate Students

2012,04,01

	Doctor Course	Master Course
Graduate School of Medicine	21	3
Graduate School of Science	3	11
Graduate School of Pharmaceutical Science	0	1
Graduate School of Dentistry	0	0
Graduate School of Frontier Biosciences	8	4
Total	32	19

Research Fellows & Research Students

2012,04,01

Special research students	0
Research Students	7
Visiting Research Scholars	1
JSPS Research Fellows	3
Total	11

Department of Molecular Bacteriology

Research Group

Professor	Yasuhiko Horiguchi, D.V.M., D.Agr.Sci.
Assistant Professor	Shigeki Kamitani, D.M.Sc.
Assistant Professor	Hiroyuki Abe, Ph.D.
SA Researcher	Aya Fukui, Ph.D.
SA Researcher	Hirono Toshima, 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, act specifically on a particular cell or 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 include *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 these toxins. Together, these approaches will help to clarify the structure and function of these bacterial toxins.

(2) *Analysis of the pathogenesis of whooping cough*

Bordetella pertussis is a bacterial pathogen that infects the human respiratory tract and causes whooping cough, which is characterized by paroxysmal cough. Two important questions remain in terms of the pathogenesis of *B. pertussis* infection: Why does *B. pertussis* infect humans but not other mammals? How does the bacteria cause the paroxysmal cough? We are examining the pathology of the disease and function of bacterial virulence factors using an animal model of the infection.

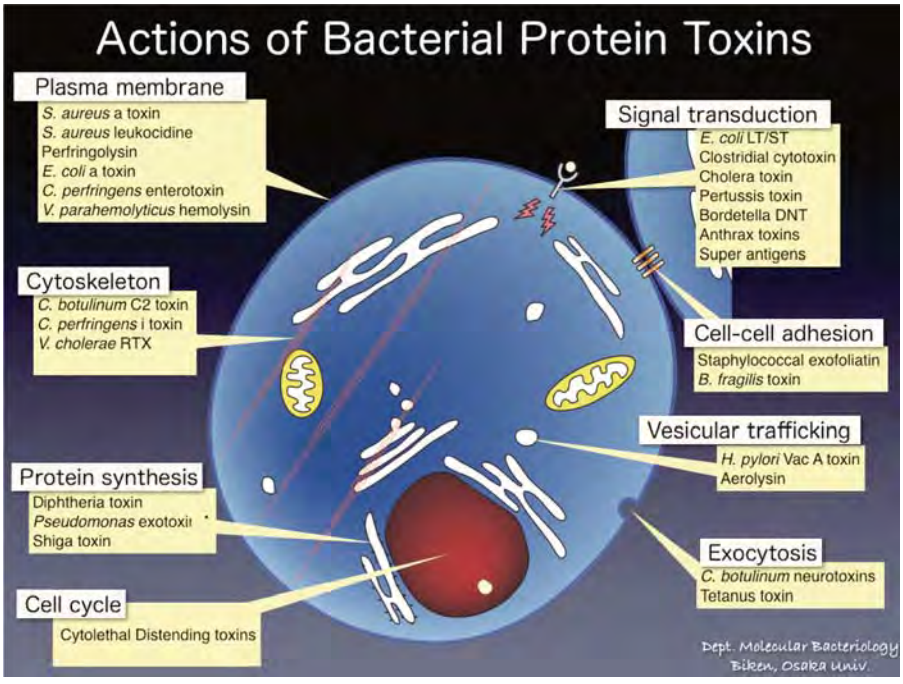


Fig. 1: Bacterial protein toxins with various activities that influence particular cellular functions. Many bacterial protein toxins exert their toxicity by modifying important functions of the host cells. The relevant physiological functions of the cells can be determined by dissecting the actions of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

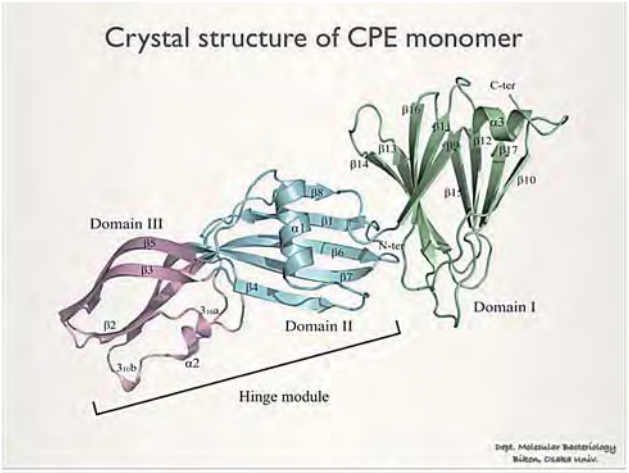


Fig. 2: Overall structure of *Clostidium perfringens* enterotoxin

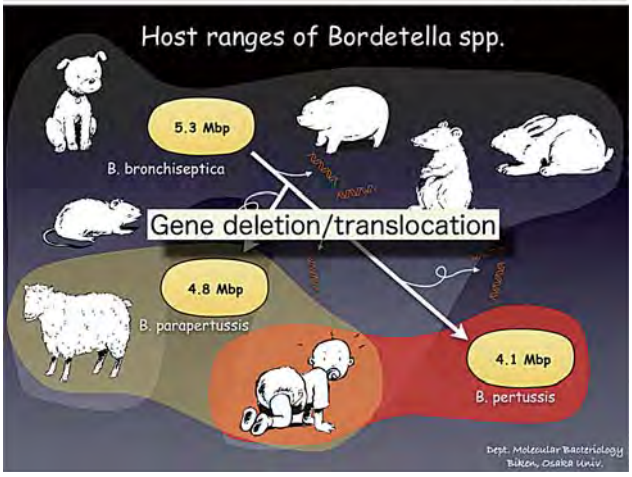


Fig. 3: *B. pertussis, B. parapertussis*, and *B. bronchiseptica* are closely related pathogenic bacteria. *B. bronchiseptica* with the largest genome shows the broadest host range, whereas *B. pertussis* with the smallest genome shows the narrowest range. *B. pertussis* is believed to have evolved from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

Recent Publications

1. Horiguchi, Y. "Swine Atrophic Rhinitis Caused by Pasteurella Multocida Toxin and Bordetella Dermonecrotic Toxin." ***Curr Top Microbiol Immunol*** (2012).
2. Kitadokoro, K., K. Nishimura, S. Kamitani, A. Fukui-Miyazaki, H. Toshima, H. Abe, Y. Kamata, Y. Sugita-Konishi, S. Yamamoto, H. Karatani, and Y. Horiguchi. "Crystal Structure of Clostridium Perfringens Enterotoxin Displays Features of {Beta}-Pore-Forming Toxins." ***J Biol Chem*** 3;286(22) (2011):19549-55.
3. Kamitani, S., S. Ao, H. Toshima, T. Tachibana, M. Hashimoto, K. Kitadokoro, A. Fukui-Miyazaki, H. Abe, and Y. Horiguchi. "Enzymatic Actions of Pasteurella Multocida Toxin Detected by Monoclonal Antibodies Recognizing the Deamidated Alpha Subunit of the Heterotrimeric Gtpase Gq." ***Febs J 278, no. 15*** (2011): 2702-12.
4. Kimura, J. , H. Abe, S. Kamitani, H. Toshima, A. Fukui, M. Miyake, Y. Kamata, Y. Sugita-Konishi, S. Yamamoto, and Y. Horiguchi. "Clostridium Perfringens Enterotoxin Interacts with Claudins Via Electrostatic Attraction." ***J Biol Chem 285, no. 1*** (2010): 401-08.
5. Kamitani, S, K. Kitadokoro, M. Miyazawa, H. Toshima, A. Fukui, H. Abe, M. Miyake, and Y. Horiguchi. "Characterization of the Membrane-Targeting C1 Domain in Pasteurella Multocida Toxin." ***J Biol Chem 285, no. 33*** (2010): 25467-75.

Department of Viral Infections

Research Group

Professor	Tatsuo Shioda, D.Med.Sc.
Assistant Professor	Jun-ichi Sakuragi, D.Med.Sc.
Assistant Professor	Emi E. Nakayama, M.D., D.Med.Sc.

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 observation 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 cyclophilin A 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 (Fig. 1, left). We also have found that sequence variations in the N-terminal half of the viral capsid protein (Fig. 1, right) determine viral sensitivity to TRIM5α-mediated restriction, which indicates that there is an interaction between TRIM5α and the virus capsid. We found that HIV-2 replication levels in infected individuals were associated with capsid variations and suggested that viral sequence analysis can predict AIDS progression. Furthermore, we succeeded in improving the simian-tropic HIV-1 virus and the methods of monkey genome analysis. These new developments will greatly facilitate the generation of an HIV-1 animal model, which would be a highly useful tool in research aiming to understand AIDS pathogenesis and to develop an effective vaccine. We are seeking to identify the binding surface between the viral capsid protein and TRIM5α because this information may be useful for the development of new antiretroviral drugs.

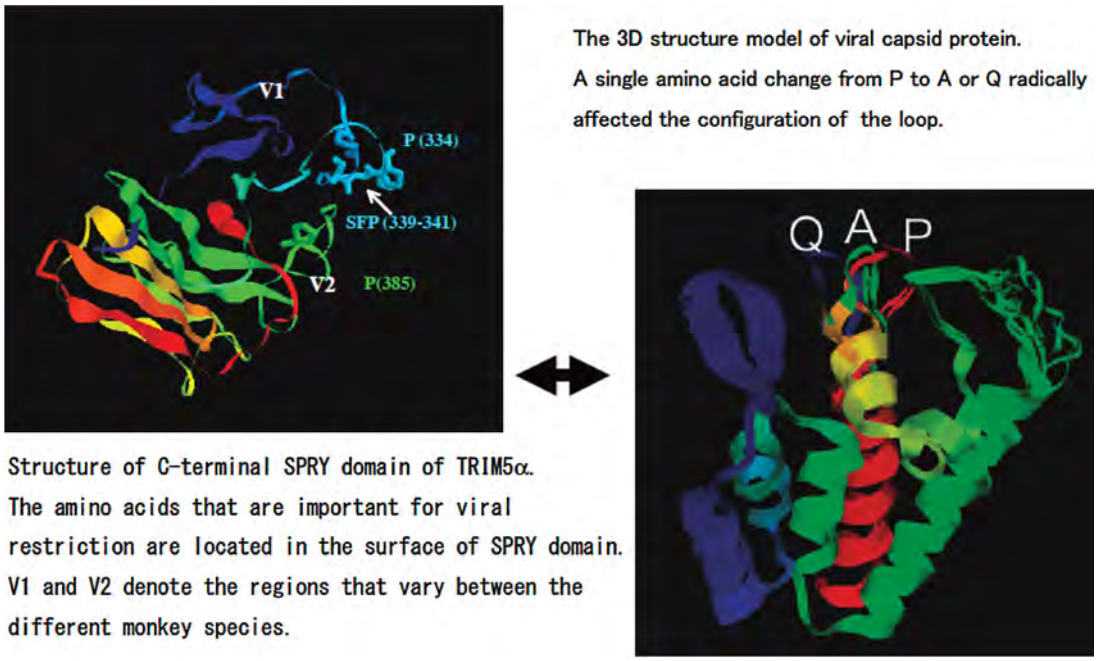


Fig. 1. Structural models of TRIM5α (left) and the viral capsid (right).

(2) Host factors that participate in antiretroviral drug side-effects

Human genetic variations affect differences in human phenotypes. In collaboration with Thai groups, we are analyzing the relationship between human genomic variation and antiretroviral therapy side-effects, 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 genome recombination processes involved in viral diversification. We have constructed HIV-1 Gag cleavage site mutants to enable the steady-state observation of virion maturation steps to study Gag processing, RNA dimerization, virion morphology, and infectivity precisely. We revealed that the maturation of the viral RNA/protein plays critical roles for 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 detailing the fine mapping of DLS, we discovered the possibility of a long-range interaction, which had 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). Because 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.

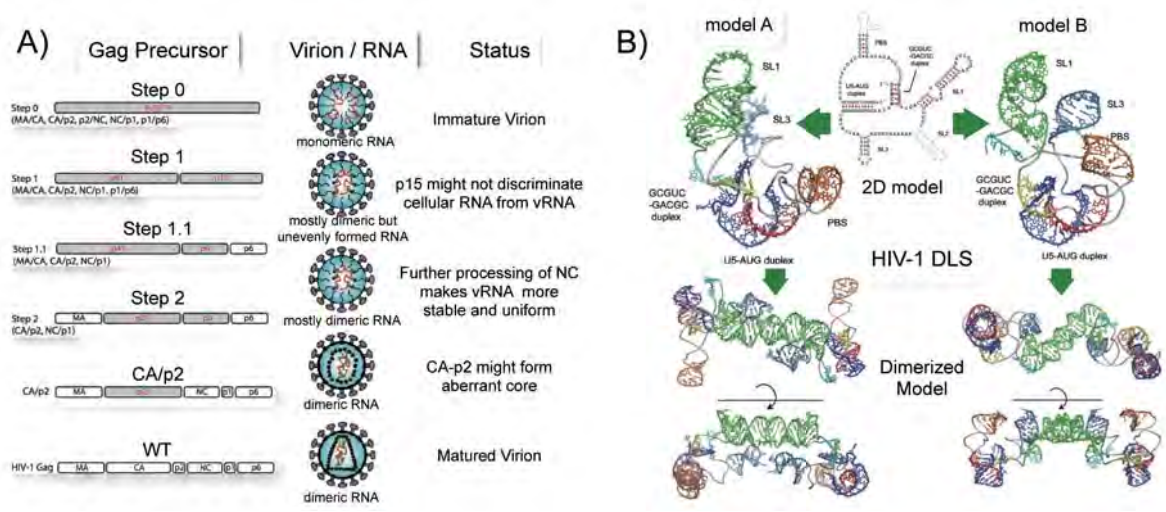


Fig. 2 HIV-1 genome RNA dimerization.

Recent Publications

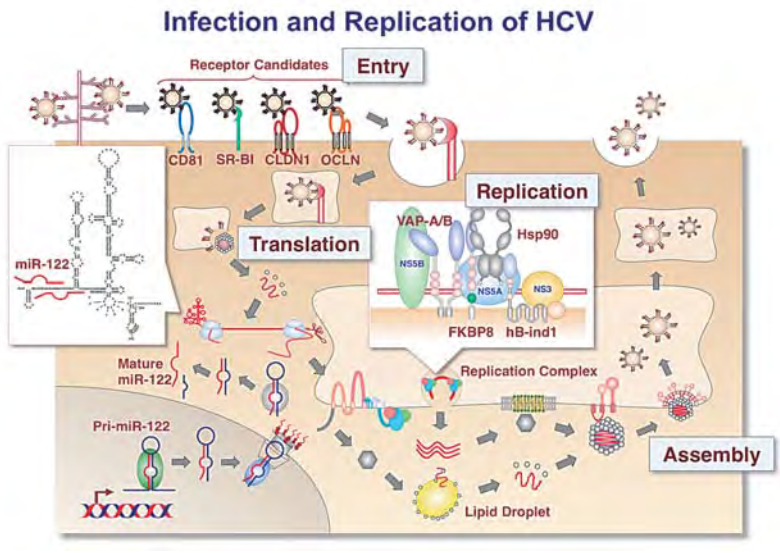
1. A proposal for a new HIV-1 DLS structural model. Sakuragi JI, Ode H, Sakuragi S, Shioda T, Sato H. ***Nucleic Acids Res***. In press.
2. Role of human TRIM5α in intrinsic immunity. Nakayama EE, Shioda T. ***Front Microbiol***.2012;3:97.
3. TRIM5α and Species Tropism of HIV/SIV. Nakayama EE, Shioda T. ***Front Microbiol***. 2012; 3:13.
4. The relationship between HIV-1 genome RNA dimerization, virion maturation and infectivity. Ohishi M, Nakano T, Sakuragi S, Shioda T, Sano K, Sakuragi JI. ***Nucleic Acids Res***. 2011 Apr;39(8):3404-17.
5. HLA-Cw*04 allele associated with nevirapine-indued rash in HIV-infected Thai patients. Likanonsakul S, Rattanatham T, Feangvad S, Uttayamakul S, Prosithsirikul W, Tunthanathip P, Nakayama EE, Shioda T. ***AIDS Res Ther***. 2009 Oct 21;6:22.

Department of Molecular Virology

Research Group

Professor	Yoshiharu Matsuura	DVM, PhD
Assistant Professor	Toru Okamoto	PhD
Assistant Professor	Takasuke Fukuhara	MD, PhD
SA Assistant Professor	Hiroto Kambara	PhD
SA Researcher	Chikako Ono	PhD
SA Researcher	Takashi Motomura	MD, PhD
Postdoctoral Fellow	Hiroshi Kato	DVM, PhD

We are working towards understanding the molecular mechanisms of entry, replication, immune escape, and pathogenesis of hepatitis C virus (HCV) and the development of a novel viral vector for gene delivery.

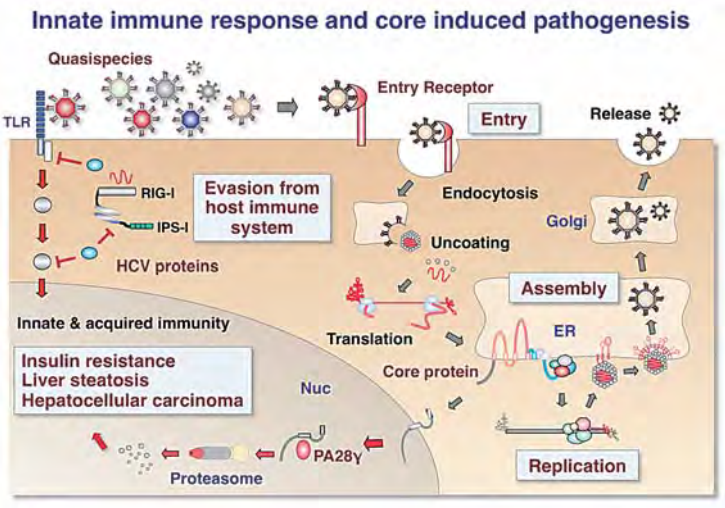


1. Studies on the molecular biology of HCV replication and pathogenesis

More than 3% of the worldwide population is infected with HCV, and 80% of these individuals will develop persistent infection. Persistent HCV infection often leads to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The incidence of hepatitis C has significantly decreased since the introduction of a screening system for anti-HCV antibodies in 1999; however, more than two million people have already been infected with HCV in Japan. Use of combination therapy with pegylated-IFNα, ribavirin, and HCV protease inhibitor has greatly increased the proportion of patients achieving a sustained virological response. Nevertheless, 30% of patients with HCV genotype 1 exhibit no response to the combination therapy. Although IL28B genetic polymorphism, viral RNA mutations, and expression of ISGs, including IP-10, are associated with the response to IFN-based antiviral therapy, the mechanism has not been elucidated.

HCV exhibits quasispecies heterogeneity. As such, its infection mechanisms are difficult to assess by surrogate systems, such as pseudotype and recombinant viruses derived from a single HCV clone. *In vitro* replication of genotype 2a HCV (HCVcc) was recently established. Several receptor candidates, including hCD81, SR-BI, Claudin1, and Occludin, for HCV entry were identified by using the surrogate viruses and HCVcc. However, sera from persistently infected individuals show a high level of neutralization antibodies to the artificial viruses, suggesting that these antibodies do not play a crucial role in HCV clearance.

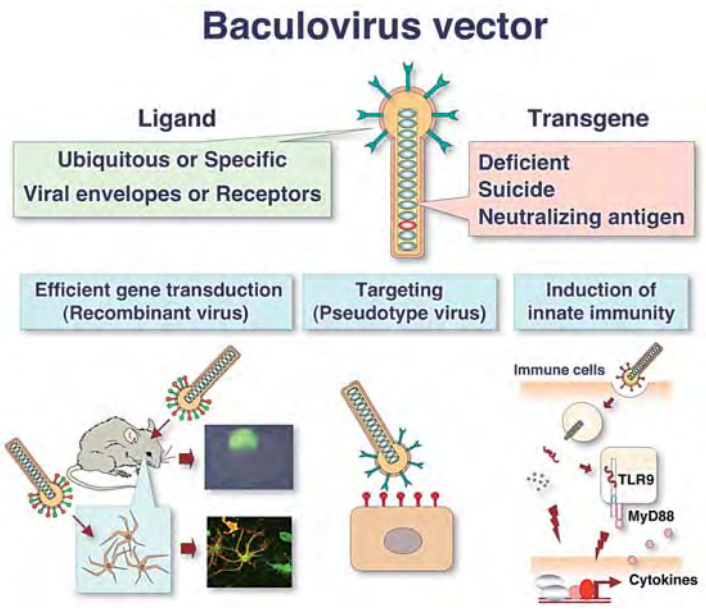
HCV particles are internalized into cells through endocytosis. HCV NS3/4A protease was shown to cleave adaptor molecules involved in the TLR- and RIG-I-dependent signaling pathways. After uncoating, a viral RNA is translated into a large precursor polyprotein composed of 3,000 amino acids. This viral polyprotein is cleaved by signal peptidase (SP), signal peptide peptidase (SPP), and viral-encoded proteases, resulting in at least 10 viral proteins. The liver-specific microRNA miR-122 enhances translation through a genetic interaction with the 5' UTR of HCV-RNA. However, the molecular mechanism of the enhancement has not been clarified. The formation of a multichaperone complex with NS5A is essential for the replication of viral RNA. Viral RNA and core proteins



are recruited around a lipid droplet, and viral capsids are formed.
Although novel innovative agents have shown significant antiviral activity in patients with HCV, drug-resistant viruses easily emerge. Therefore, host factors indispensable for HCV replication are ideal targets for the development of new therapeutics for chronic hepatitis C with a broad spectrum and a low possibility of emergence of breakthrough viruses against antiviral drugs. HCV belongs to the family of *Flaviviridae*, which includes flavivirus such as Japanese encephalitis virus (JEV) that has a robust cell culture system and a small animal model. We are 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 of replication-deficient viral infectious diseases, such as HCV. Furthermore, the development of novel viral vectors is essential for future gene therapy. We are studying the baculovirus *Autographa californica* nucleopolyhedrovirus (AcNPV) as a versatile viral vector for gene delivery in vitro and in vivo. AcNPV is an insect virus possessing a 134 kb double-stranded circular DNA genome. Due to its strong promoters, baculovirus is commonly used as a tool for the large-scale production of recombinant protein in insect cells. Baculovirus is capable of entering various mammalian cells to facilitate the expression of foreign genes under the control of mammalian promoters without replication of the 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, the possibility of generating replication-competent revertants expressing baculoviral gene products, which can often lead to harmful immune responses against mammalian cells, is significantly lower than that with other viral vectors currently in use. Furthermore, intranasal inoculation with AcNPV induces a strong innate immune response, protecting mice from lethal challenges of influenza viruses.



We demonstrated that the internalization of viral DNA *via* membrane fusion by the envelope glycoprotein in the endosome is required for the induction of innate immune response by AcNPV through a TLR9/MyD88-dependent pathway. This finding raises the possibility that AcNPV may be harnessed therapeutically to induce the host immune response against various infectious diseases, especially those caused by pathogens invading from the respiratory tract.

Recent Publications

1. Abe T, Fukuhara T, Wen X, Ninomiya A, Moriishi K, Maehara Y, Takeuchi O, Kawai T, Akira S, Matsuura Y. CD44 participates in the IP-10 induction in cells replicating HCV RNA through an interaction with TLR2 and hyaluronan. ***J Virol***. 2012.
2. Kataoka C, Kaname Y, Taguwa S, Abe T, Fukuhara T, Tani H, Moriishi K, Matsuura Y. Baculovirus GP64-mediated entry into mammalian cells. ***J Virol***. 2012 Mar;86(5):2610-20.
3. Kambara H, Fukuhara T, Shiokawa M, Ono C, Ohara Y, Kamitani W, Matsuura Y. Establishment of a novel permissive cell line for the propagation of hepatitis C virus by expression of microRNA miR122. ***J Virol***. 2012 Feb;86(3):1382-93.
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Department of Immunoparasitology

Research Group
Associate Professor Ph.D. Masahiro Yamamoto
Assistant Professor Ph.D. Miwa Sasai

Our laboratory studies immmunoparasitology using an apicomplexan protozoan parasite called *Toxoplasma gondii* as a research model to analyze the "host-to-pathogen" interaction.

1) What is *Toxoplasma*?

Toxoplasma gondii (T. gondii) is a eukaryotic pathogen that causes life-threatening toxoplasmosis, a condition that includes encephalitis in immunocompromised individuals, such as those suffering from AIDS or being treated by chemotherapy, and congenital diseases following primary infection during pregnancy in humans and animals. *T. gondii* is an obligate intracellular eukaryotic parasite capable of proliferating exclusively inside a parasitophorous vacuole, which is formed during host cell invasion.

Taxonomically, *T. gondii* belongs to the Apicomplexa phylum that is defined by the presence of an apical complex including secretory organelles, such as conoids, micronemes, and rhoptries (Figure 1A). Among them, the large bulb-shaped organelles called rhoptries contain various proteins that are secreted into the host cytoplasm or in the forming parasitophorous vacuole during parasite entry to co-opt the host cell for growth and survival (Figure 1B and C).

2) Downregulation of host immune responses by *T. gondii*.

T. gondii is divided into three major lineages (types I, II, and III). Stimulation of innate immune cells, such as macrophages and dendritic cells, by *T. gondii*-derived components results in strong production of proinflammatory cytokines. However, "live" infection with type I or type III, but not type II, parasites reduces cytokine production. We revealed that a single amino acid substitution of the kinase domain of a rhoptry protein ROP16 is one molecular mechanism by which type II parasites fail to suppress the host innate immune response (Figure 2). In terms of virulence, in mice, the type III parasite is avirulent compared with the type I and type II parasites. Another ROP16 family member, ROP18, was identified as the gene responsible for the genotype-dependent virulence mechanism. We revealed that ROP18 targets a host transcription factor, ATF6b, and degrades the protein in a proteasome-dependent fashion (Figure 3).

Effector proteins, such as ROP16 and ROP18, constitute a large family of proteins. However, the functions of most of the effectors released by this parasite in host cells remain uncertain. Moreover, the host target molecules are also unknown. Therefore, we are studying the role of the *T. gondii*-derived effector molecules by generating and analyzing gene-targeted parasite lines. We are trying to uncover the strategy used by the parasite to suppress the host immune response, which may result in the identification of novel immunological concepts.

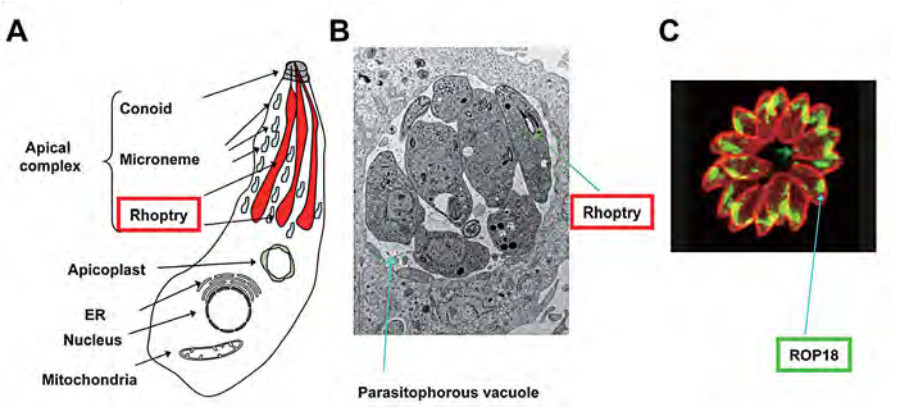


Figure 1 Toxoplasma gondii and the rhoptry protein
(A) A schematic figure of *T. gondii*. The apical complex includes rhoptries (red). (B) Electron micrograph of *T. gondii* residing in a parasitophorous vacuole (blue) in an infected host cell. (C) Immunofluorescent image of *T. gondii* showing detection of the rhoptry protein ROP18.

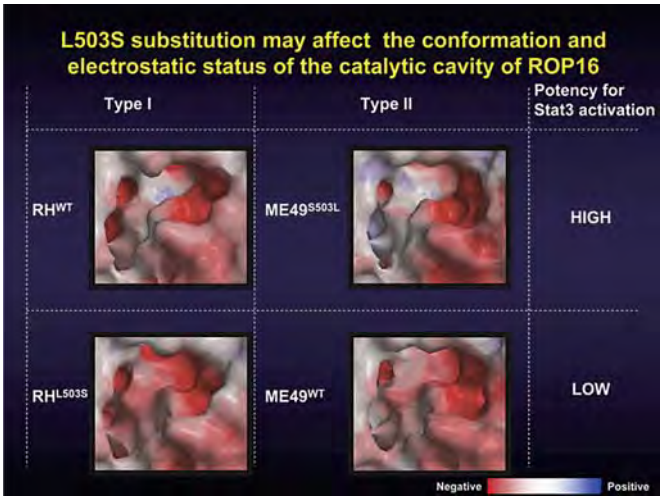
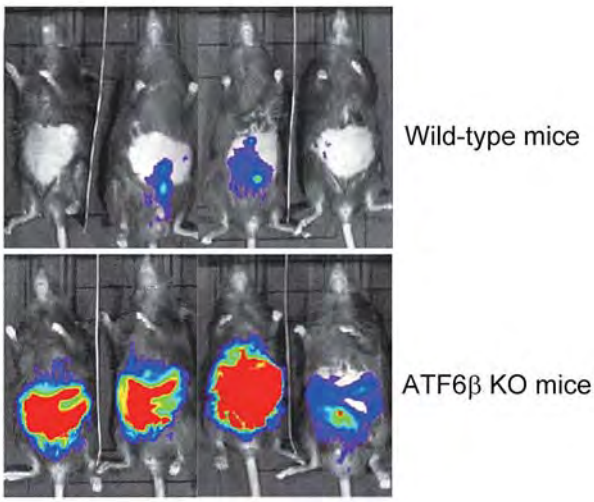


Figure 2. Single amino acid substitution of ROP16 determines *T. gondii* infection-induced suppression of innate immune response.



FFigure 3. ATF6β KO mice are highly sensitive to rop18-KO parasites. Luciferase-expressing *T. gondii* lacking ROP18 were infected in wild-type or ATF6β-KO mice. Luciferase emission was monitored with a live imaging analyzer.

Recent Publications

1. **Yamamoto M**, Takeda K. Inhibition of ATF6β-dependent host adaptive immune response by a Toxoplasma virulence factor ROP18. ***Virulence***. (2012) In press.
2. **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.
3. Sayama K*, **Yamamoto M* (* Equal contribution)**, Shirakata Y, Hanakawa Y, Hirakawa S, Dai X, Tohyama M, Tokumaru S, Shin MS, Sakurai H, Akira S, Hashimoto K. E2 polyubiquitin-conjugating enzyme Ubc13 in keratinocytes is essential for epidermal integrity. ***J Biol Chem***. (2010) 285:30042-9.
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.
5. **Yamamoto M**, Uematsu S, Okamoto T, Matsuura Y, Sato S, Kumar H, Satoh T, Saitoh T, Takeda K, Ishii KJ, Takeuchi O, Kawai T, Akira S. Enhanced TLR-mediated NF-IL6 dependent gene expression by Trib1 deficiency. ***J Exp Med***. (2007) 204:2233-9.

Department of Molecular Immunology

Research Group

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Assistant Professor	Shuhei Sakakibara, Ph.D.
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SA Researcher	Kentaro Morita, Ph.D.
SA Researcher	Takeharu Minamitani, Ph.D.
SA Researcher	Chiau-Yuang Tsai, Ph.D.

Molecular mechanisms involved in the regulation of immune responses

T-cells are activated by antigenic peptides bound to the MHC complex on antigen-presenting cells. Once activated, the T-cells differentiate into functional helper or effector T-cells. Antigen-stimulated B-cells differentiate into antibody-forming cells or memory B-cells with the help of antigen-specific T-cells. Thus, T- and B-cell differentiation processes require physiological interactions between T-cells and antigen-presenting cells and between T-cells and B-cells, respectively. Such cell–cell interactions are mediated by various costimulatory molecules, including CD40, CD40 ligand, B-7, and CD28. In addition, it was recently revealed that several members of the Semaphorin family are crucially involved in immune cell interactions. We are currently studying the roles of these molecules in the regulation of immune responses.

A) Mechanisms of immune regulation by Semaphorin molecules

The Semaphorin family contains axonal guidance factors that function during neuronal development. However, studies by our laboratory have shown that several Semaphorin molecules play crucial roles at various stages of immune responses (Figure 1). For instance, Sema4D/CD100 is involved in the activation of B-cells and dendritic cells, whereas Sema4A plays roles in T-cell priming and Th1 differentiation. The interaction between Sema6D and its receptor Plexin-A1 mediates cellular immune responses by activating dendritic cells and bone homeostasis by inducing osteoclastgenesis. Furthermore, we recently demonstrated that Sema7A on activated T-cells activates macrophages to produce inflammatory cytokines and triggers inflammatory responses through α1β1 integrin (Figure 2).

B) Elucidation of the molecular mechanism by which B-cells survive and differentiate into effecter cells

B-cells differentiate into antibody-secreting cells and memory B-cells to respond to nonself antigens. Signals through the B-cell antigen receptor (BCR) and members of the TNF receptor family, such as CD40 and BAFF-R, on the B-cell surface are required for B-cell activation, survival, and differentiation. Our group has been studying the roles of the molecules that are involved in the signaling pathways downstream of BCR and CD40. We recently identified a PKC-related serine/threonine kinase, PKN1, which is associated with the TRAF family, as a negative regulator of Akt in BCR signaling. Our findings showed that the PKN1-mediated regulation of Akt is critical for the selection of high-affinity B-cells.

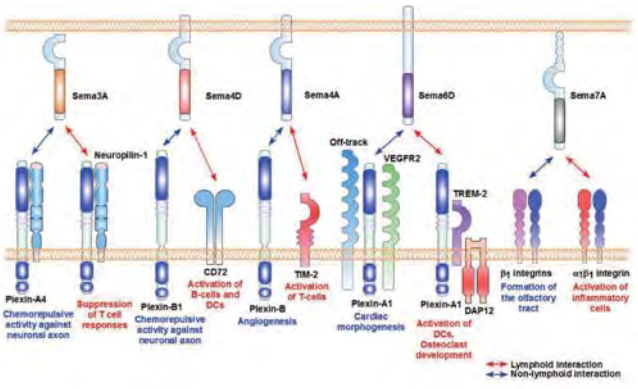

Fig.1 Representative immune Semaphorins
Semaphorins and their receptors have been shown to play roles in immune regulation by our research group

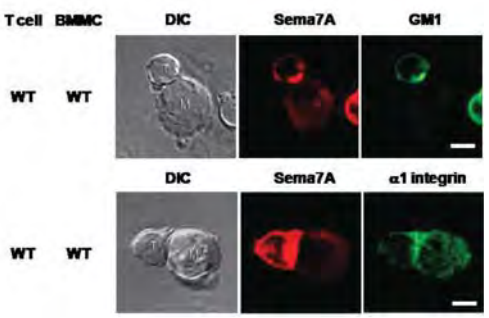

Fig.2 Accumulation of Sema7A and α1Integrin in immunological synapse by the interaction between T cells and macrophages.

Molecular pathogenesis by which γ-herpesvirus induces immune disorders

Epstein-Barr virus (EBV) is a human γ-herpesvirus that infects B-cells latently and is prevalent worldwide. EBV causes infectious mononucleosis in healthy donors and proliferative disorders in immune-suppressive conditions induced by aging, immunesuppresant therapy, and HIV infection. EBV infection is sometimes associated with B-cell malignancies, such as Burkitt’s lymphoma and Hodgkin’s lymphoma, and with some autoimmune diseases, such as systemic lupus erythematosus (SLE) and multiple sclerosis (MS). By studying EBV biology, we hope to determine how EBV leads to autoimmune diseases and human carcinogenesis. The outcomes from this study may contribute to the development of novel therapies for EBV-associated disorders (Figure 3).

A) Signaling pathways by which EBV latent membrane proteins and downstream molecules induce the cell growth and survival of infected cells

EBV infection elicits growth transformation and the immortalization of B-cells and epithelial cells. Recently, it was also suggested that EBV infects T-cells and NK-cells, leading to nasal lymphoma and chronic active EBV infection (CAEBV) associated with hemophagocytic syndrome. How does EBV transform human cells? Of the EBV latent gene products, our group is focusing on two latent membrane proteins, LMP1 and LMP2a, which are involved in EBV-induced transformation by mimicking CD40 and BCR signals, respectively. We are currently studying the mechanisms by which these molecules modify B-cell function and induce EBV-related disorders by using knock-in mice in which either or both proteins can be expressed in a cell-specific manner (Figure 4).

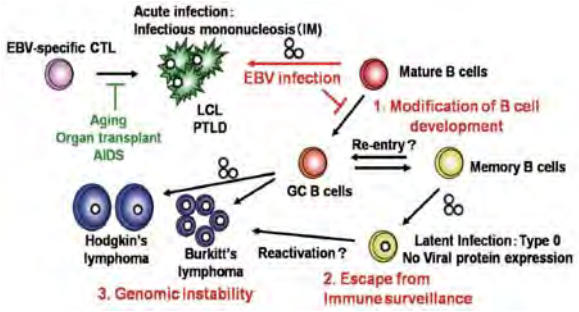

Fig.3 EBV and host immune system
The mechanism by which EBV induces human B cell growth transformation is closely associated with the vulnerability of the host immune system.

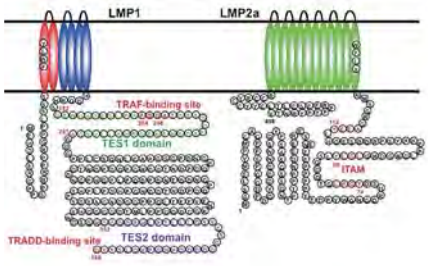

Fig.4 Schematic structure of EBV LMP1 and LMP2a.

B) Elucidation of the virus-host interaction using a mouse model of γ-herpesvirus infection with MHV68

Intranasal inoculation of mice with MHV68, which is closely related to EBV, results in an acute productive infection of the lung and latent infection in several cell types, including B-cells and macrophages. Because of the limited susceptibility of experimental animals to EBV infection, MHV68 infection of mice should be a useful animal model for understanding EBV pathogenesis. In fact, MHV68 infection induces emerging autoreactive antibody that can trigger a complement-dependent inflammation. We have been investigating various immune responses against MHV68 to determine the molecular mechanisms by which γ-herpesvirus induces autoantibody production and lymphoma development. Currently, we are investigating the relationship between infection and the generation of autoreactive B-cells in MHV68-infected mice. In addition, we have generated recombinant strains of MHV68, in which desired gene cassettes, such as genes for chicken ovalbumin (OVA) and/or modified fluorescent proteins (mVenus and mCherry), can be inserted. Together with knockout or transgenic mice, this technique would be of help to achieve our research goals.

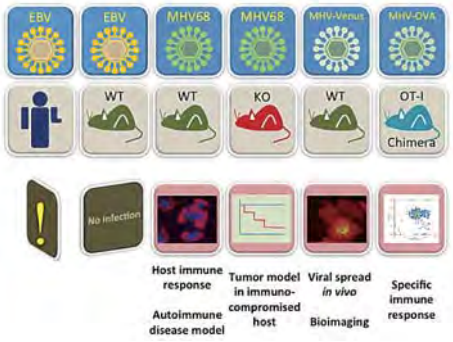

Fig.5 Mouse model of γ-herpesvirus infection with recombinant MHV68 and gene-modified mice

Recent Publications

1. Takamatsu H, Takegahara N, Nakagawa Y, Tomura M, Taniguchi M, Friedel RH, Rayburn H, Tessier-Lavigne M, Yoshida Y, Okuno T, Mizui M, Kang S, Nojima S, Tsujimura T, Nakatsuji Y, Katayama I, Toyofuku T, Kikutani H, Kumanogoh A. Semaphorins guide the entry of dendritic cells into the lymphatics by activating myosin II. ***Nat Immunol***. 2010 Jul;11(7):594-600.
2. Mizui M, Kumanogoh A, Kikutani H. Immune semaphorins: novel features of neural guidance molecules. ***J Clin Immunol***. 2009 Jan;29(1):1-11.
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5. Takegahara N, Takamatsu H, Toyofuku T, Tsujimura T, Okuno T, Yukawa K, Mizui M, Yamamoto M, Prasad DV, Suzuki K, Ishii M, Terai K, Moriya M, Nakatsuji Y, Sakoda S, Sato S, Akira S, Takeda K, Inui M, Takai T, Ikawa M, Okabe M, Kumanogoh A, Kikutani H. Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. ***Nat Cell Biol***. 2006 Jun;8(6):615-22.

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	Morihisa Fujita, D. Sc.
SA Assistant Professor	Noriyuki Kanzawa, D. Med. Sc.
Postdoctoral Fellow (SUP)	Yetao Wang, M.D., D. Med. Sc.

Research Projects

We mainly focus on two research areas involved in disease, immune defense, and homeostasis. The first research area concerns how glycosylphosphatidylinositol (GPI)-anchored proteins are synthesized, processed, transported, and secreted, and how GPI defects lead to the onset of diseases. The second research area concerns how the pH levels of the intracellular organelles are regulated, and how these pH levels regulate many biological functions.

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

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. It is essential for the development of higher animals and for the growth of yeasts and protozoan parasites. Protein modifications due to attachment of the GPI-anchor serve as protein localization and sorting signals. Our current project is to identify and clarify the functions of all of the genes involved in the biosynthesis of the GPI-anchor in the 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) (Fig. 1). We expect that these studies will reveal why many proteins are modified with the GPI-anchor.

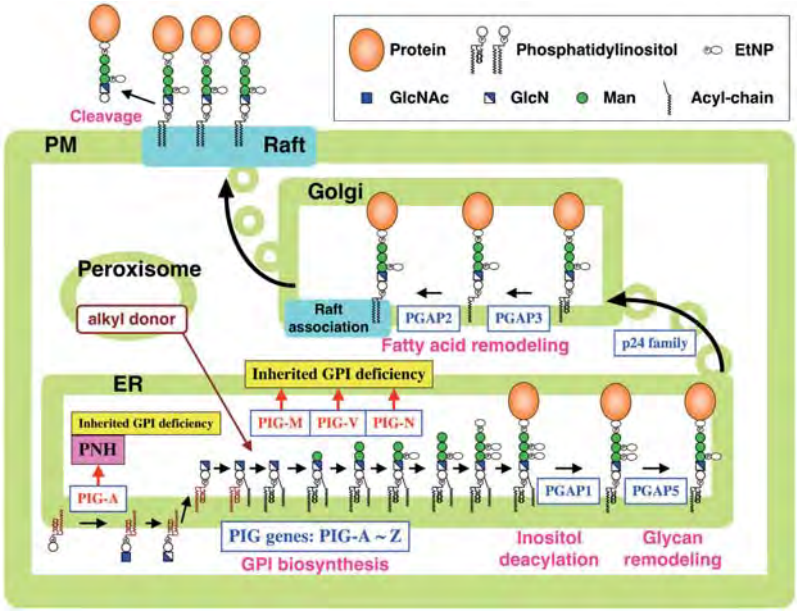

Fig. 1 GPI-anchor biosynthesis and transport/ remodeling of GPI-APs. PIG genes are involved in the biosynthesis of the GPI-anchor in the ER. During biosynthesis, an alkyl-lipid generated in the peroxisome is utilized. Thereafter, GPI-APs are transported to the plasma membrane and enriched in rafts. PGAP 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 affects the sorting of GPI-APs because it alters the physical characteristics of the GPI-anchor.

2) Molecular genetics of acquired paroxysmal nocturnal hemoglobinuria (PNH) and inherited GPI deficiencies.

PNH is an acquired hematopoietic stem cell disorder in which clonal cells that are defective in GPI biosynthesis are expanded. As a result, abnormal erythrocytes that lack CD59 and DAF/CD55 predominate. CD59 and DAF/CD55 are widely distributed GPI-anchored proteins that inhibit the activation of complement on the host cell surface. Their absence on erythrocytes makes these cells very sensitive to complement and lysis during infections and other events. We propose a three-step model of PNH pathogenesis. Step 1 involves the generation of GPI-deficient hematopoietic stem cells due to the somatic mutation of the PIG-A gene. Step 2 involves the immunological selection of GPI-deficient hematopoietic stem cells. In this step, GPI-deficient cells not only survive, but they also proliferate much more frequently than usual to compensate for anemia. This elevated proliferation rate may increase the chance that additional genetic mutations are acquired, which leads to Step 3, in which a subclone bearing the growth phenotype is generated (Fig. 2). We identified HMGA2 as the candidate gene for Step 3.

Together with our colleagues in England, we identified inherited GPI deficiency (IGD) caused by the PIGM mutation, a mannosyltransferase-encoding gene that plays an essential role in GPI biosynthesis. Recently, through an analysis of patients' genomes using a second-generation sequencer, other IGDs, the responsible genes of which were PIGV, PIGN, and PIGA, were reported. Among these, PIGV was shown to be one of the genes responsible for Mabry syndrome, a hyperphosphatasia mental retardation syndrome. Over 25 genes are responsible for the biosynthesis and transport of GPI-anchored proteins (Fig. 1). It is likely that new IGDs with similar symptoms will be found. We will analyze these patients and establish a new disease category of "IGD" .

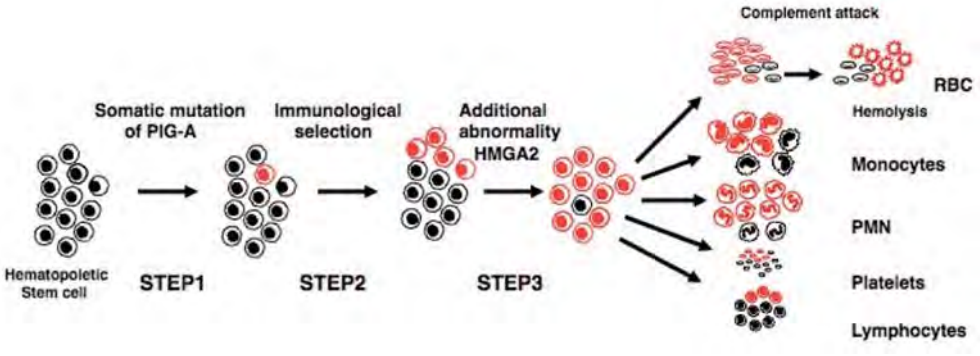

Fig. 2 Pathogenesis of PNH. Step 1 involves the generation of GPI-deficient hematopoietic stem cells due to the somatic mutation of the *PIG-A* gene. Step 2 involves the immunological selection of GPI-deficient hematopoietic stem cells. In this step, GPI-deficient cells survive and proliferate much more frequently than usual to compensate for anemia. This elevated proliferation may increase the chance that additional genetic mutations occur. Step 3 involves the generation of a subclone bearing the growth phenotype.

Each intracellular organelle is compartmentalized by lipid bilayers and possesses unique environment, proteins, and lipids in which it fully exercises its functions. One of the major factors influencing the environment is the pH. Organelles in the secretory and endocytosis pathways have acidic environments in their lumens. This acidic environment is believed to be critical in many biological processes, such as transport, processing and glycosylation of both proteins and lipids, as well as in the onset and pathology of diseases that affect pH regulation. Despite their significance, the mechanisms by which abnormal phenotypes are caused are largely unknown. We recently reported the first establishment of mutant cells in which the Golgi pH was dysregulated and identified a gene responsible for the mutants and its functions (Ref. 5). We aim to clarify the mechanisms and biological significance of acidic pH regulation through establishing and analyzing mutant cells defective in organelle pH regulation, and to understand the disease pathologies to allow drug discovery.

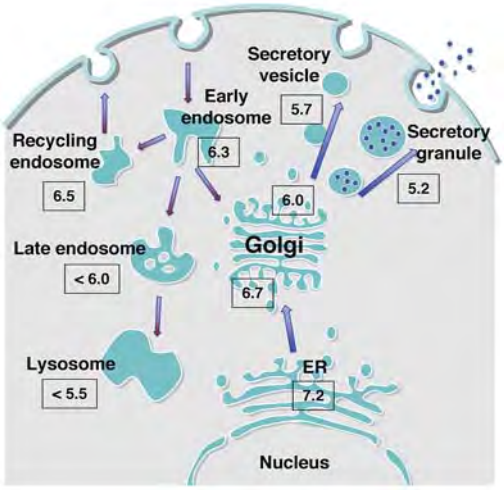

Fig. 3 pH regulation of the intracellular organelles. Luminal pH levels of organelles in secretory and endocytosis pathways are acidic, as indicated in the boxes (values are approximate).

Recent Publications

1. Murakami Y, Kanzawa N, Saito K, Krawitz PM, Mundlos S, Robinson PN, Karadimitris A, Maeda Y, Kinoshita T. Mechanism for release of alkaline phosphatase caused by glycosylphosphatidylinositol deficiency in patients with hyperphosphatasia mental retardation syndrome. ***J Biol Chem***. 2012 Feb 24;287(9):6318-25.
2. Fujita M, Watanabe R, Jaensch N, Romanova-Michaelides M, Satoh T, Kato M, Riezman H, Yamaguchi Y, Maeda Y, Kinoshita T. Sorting of GPI-anchored proteins into ER exit sites by p24 proteins is dependent on remodeled GPI. ***J Cell Biol***. 2011 Jul 11;194(1):61-75.
3. Kanzawa N, Maeda Y, Ogiso H, Murakami Y, Taguchi R, Kinoshita T. Peroxisome dependency of alkyl-containing GPI-anchor biosynthesis in the endoplasmic reticulum. ***Proc Natl Acad Sci U S A***. 2009 Oct 20;106(42):17711-6.
4. Fujita M, Maeda Y, Ra M, Yamaguchi Y, Taguchi R, Kinoshita T. GPI glycan remodeling by PGAP5 regulates transport of GPI-anchored proteins from the ER to the Golgi. ***Cell***. 2009 Oct 16;139(2):352-65.
5. Maeda Y, Ide T, Koike M, Uchiyama Y, Kinoshita T. GPHR is a novel anion channel critical for acidification and functions of the Golgi apparatus. ***Nat Cell Biol***. 2008 Oct;10(10):1135-45.

Department of Host Defense

Research Group		
	Professor (SUP)	Shizuo Akira
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	SA Associate Professor (SUP)	Satoshi Uematsu
	Assistant Professor	Tatsuya Saitoh
	SA Assistant Professor	Yutaro Kumagai
	SA Assistant Professor (SUP)	Takashi Satoh

The innate immune system senses invading microbial pathogens, such as bacteria, viruses, and parasites, and plays an essential role in inducing inflammatory responses and assisting adaptive immune responses. Pattern-recognition receptors (PRRs) expressed on innate immune cells, such as macrophages and dendritic cells, recognize pathogen-associated molecular patterns, which are conserved molecular features of microbial pathogens. Upon recognition of pathogen-associated molecular patterns, PRRs initiate signaling pathways responsible for innate immune responses. We are seeking to clarify the complex regulatory mechanisms of the innate immune system.

1) Understanding signaling pathways via PRRs and their roles in immune responses

So far, Toll-like receptors (TLRs), RIG-I-like receptors, and Nod-like receptors have been identified as PRRs. We are using molecular biology and bioinformatics tools to identify molecules involved in PRR signaling. We are also generating mice deficient in relevant genes to understand their in vivo function with respect to immune responses. In particular, we focus on the recognition mechanism of virus- or bacteria-derived nucleic acids by PRRs and their signaling pathways (Fig. 1).

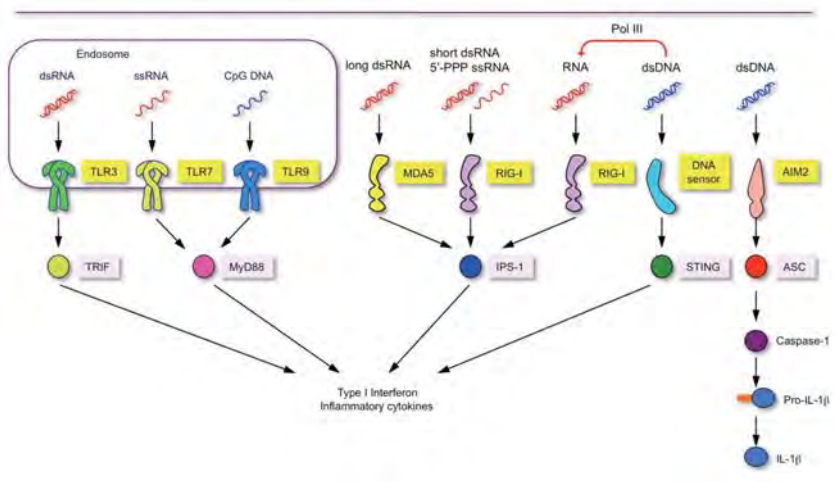

Fig. 1: PRRs that mediate the sensing of foreign nucleic acids and their signaling pathways
TLR3, TLR7, and TLR9 are exclusively localized to endosomes and recognize viral double-stranded (ds) RNA, single-stranded (ss) RNA of RNA viruses, and DNA derived from viruses or bacteria, respectively. Whereas TLR3 signals through an adapter TRIF, TLR7 and TLR9 use MyD88 as an adapter to induce type I interferon and inflammatory cytokines. In the cytoplasm, RIG-I-like receptors, such as RIG-I and MDA5, recognize different RNA species derived from RNA viruses as indicated and signal through an adapter IPS-1. Double-stranded DNA is recognized by AIM2 in the cytoplasm, which promotes caspase-1–dependent IL-1β production through recruitment of an adapter ASC. Although STING appears to mediate a type I interferon-inducing pathway in response to dsDNA, cytosolic dsDNA sensors upstream of STING are not well understood.

2) Regulation of innate immune responses by membrane trafficking

Dynamic membrane traffic plays a crucial role in the regulation of innate immune responses. We found that the autophagy proteins ATG16L1 and ATG9a and the interferon-inducible protein Viperin regulate innate immune responses, such as the production of inflammatory cytokines and type I interferon, in vivo. We seek to find new mechanisms of membrane trafficking involved in the regulation of innate immune responses.

3) Analysis of transcriptional networks in innate immune cells

We aim to understand the transcriptional networks in innate immune cells by comprehensively comparing gene expression profiles in dendritic cells derived from mice deficient for molecules involved in PRR signaling. We are comprehensively analyzing promoter sequences of various genes to identify transcription factors that regulate innate immune responses. Furthermore, we are investigating histone modifications to understand the role of histones in innate immune responses and macrophage polarization (M1 vs. M2 macrophages) (Fig. 2).

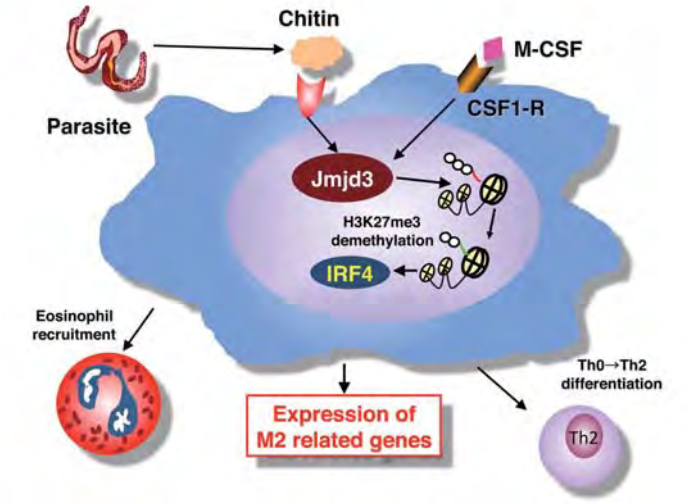

Fig. 2: Regulation of M2 macrophage polarization by Jmjd3
Macrophages are functionally polarized into M1 and M2 cells. M1 macrophages are critical for clearing bacterial, viral, and fungal infections, whereas M2 macrophages play an important role in responses to parasite infection, tissue remodeling, angiogenesis, and tumor progression. Jmjd3 is a H3K27 demethylase catalyzing H3K27me3 (trimethylated) to HeK27me1 (monomethylated). It is induced in macrophages by TLR stimuli. Jmjd3 is essential for M2 macrophage polarization in response to helminth infection and chitin, although Jmjd3 is dispensable for M1 responses. Jmjd3 deficiency affects H3K27 trimethylation in only a limited number of genes. Among them, IRF4 is a key transcription factor that controls M2 macrophage polarization. Thus, Jmjd3 plays a critical role in regulating M2 macrophage development, leading to antihelminth host responses.

4) Regulation of the stability of mRNA encoding inflammatory cytokines

The expression of genes encoding inflammatory cytokines is controlled by transcription factors and molecules that mediate the mRNA stability. We identified a TLR-inducible CCCH-type zinc finger-containing protein, Regnase-1 (Zc3h12a), as a molecule that degrades IL-6 mRNA. Mice lacking this gene spontaneously exhibit severe autoimmune symptoms. Currently, we are investigating the roles of other proteins belonging to this protein family in the regulation of inflammatory responses (Fig. 3).

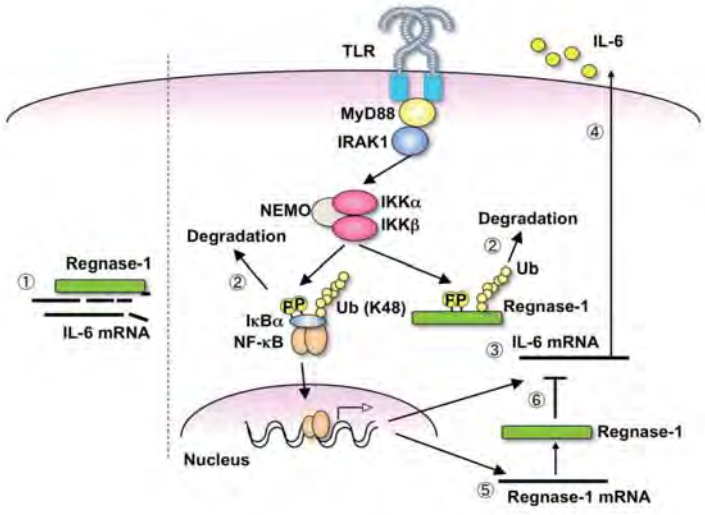

Fig. 3: Regulation of inflammatory responses by Regnase-1
Regnase-1 is an RNase that regulates the decay of mRNA encoding inflammatory cytokines (such as IL-6) and suppresses inflammation. Regnase-1 constitutively degrades IL-6 mRNA under the unstimulated condition (1). Activation of the IKK complex by TLR engagement leads to the phosphorylation and degradation of I□B□ and Regnase-1 (2). Transcription of IL-6 mRNA is induced (3) and IL-6 is produced (4). Regnase-1 mRNA is also transcribed and Regnase-1 protein is expressed (5). Regnase-1 promotes the degradation of IL-6 mRNA to suppress IL-6 production (6).

Recent Publications

1. Iwasaki H, Takeuchi O, Teraguchi S, Matsushita K, Uehata T, Kuniyoshi K, Satoh T, Saitoh T, Matsushita M, Standley DM, Akira S. The IκB kinase complex regulates the stability of cytokine-encoding mRNA induced by TLR-IL-1R by controlling degradation of regnase-1. ***Nat Immunol***. 2011 Oct 30;12(12):1167-75.
2. Saitoh T, Satoh T, Yamamoto N, Uematsu S, Takeuchi O, Kawai T, Akira S. Antiviral protein Viperin promotes Toll-like Receptor 7- and Toll-like Receptor 9-mediated type I interferon production in plasmacytoid dendritic cells. ***Immunity***. 2011 Mar 25;34(3):352-63.
3. Kawai T, Akira S. Toll-like Receptors and their crosstalk with other innate receptors in infection and immunity. ***Immunity***. 2011 May 27;34(5):637-50.
4. Tsuchida T, Zou J, Saitoh T, Kumar H, Abe T, Matsuura Y, Kawai T, Akira S. The ubiquitin ligase TRIM56 regulates innate immune responses to intracellular double-stranded DNA. ***Immunity***. 2010 Nov 24;33(5):765-76.
5. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, Miyake T, Matsushita K, Okazaki T, Saitoh T, Honma K, Matsuyama T, Yui K, Tsujimura T, Standley DM, Nakanishi K, Nakai K, Akira S. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. ***Nat Immunol***. 2010 Oct;11 (10):936-44.

Department of Cell Biology

Research Group

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Assistant Professor	Hiroto Mizushima, Ph.D.
SA Researcher	Takashi Nakamura, Ph.D.
SA Researcher	Tomoya Hikita, Ph.D.
SA Researcher	Mizuho Sato, Ph.D.

We are studying the mechanisms of cell growth and differentiation that involve growth factors and adhesion molecules present at the cell-cell contact sites. In particular, we are focusing on the mode of action of HB-EGF, a membrane-anchored protein from the EGF family of growth factors, and the molecules that bind to HG-EGF, namely the tetraspanin family. 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.

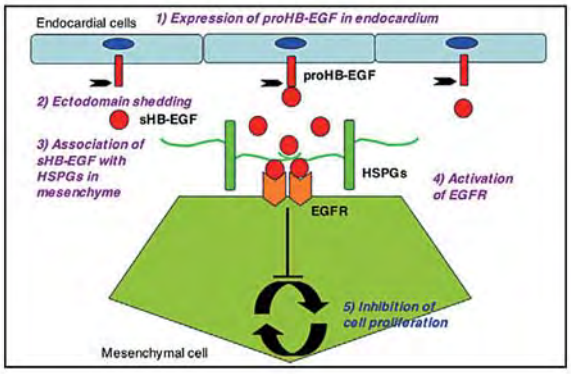
1) Mode of action of HB-EGF

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 its activity; therefore, this process is tightly regulated. HB-EGF is secreted by various tissues and cells, and it functions in several physiological processes. For example, it maintains heart muscle function, suppresses cell proliferation in the 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, but 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 depend 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.

2) Role of HB-EGF in cancer malignancy

HB-EGF is expressed in cancer cells and/or cancer-derived stroma (Ichise et al. 2010; Murata et al. 2011), and it is involved



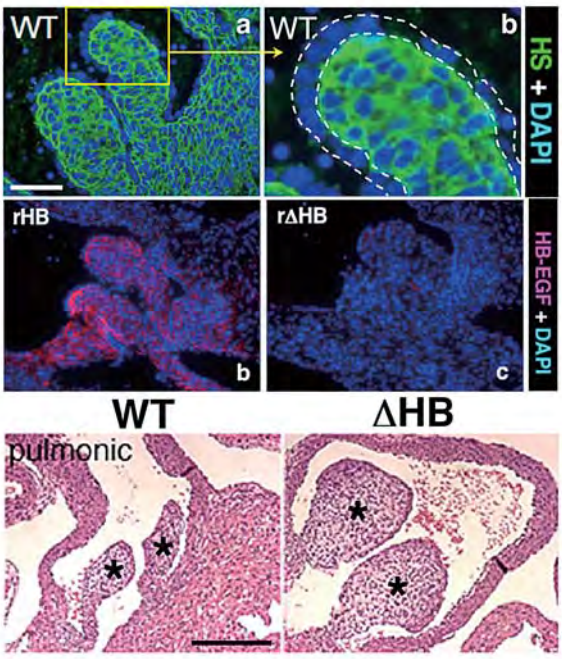


Fig. 1 The role of HB-EGF in heart valve formation.
In heart valve development, HB-EGF is expressed and secreted from endocardial cells. Secreted HB-EGF associates with heparan sulfate proteoglycans (HSPGs) in the valve mesenchyme (*upper right*)(Fig. 1). When EGFR on the surface of mesenchymal cells 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 with hyperproliferation of mesenchymal cells (*lower right*) (Iwamoto et al., 2010).

in tumor growth, invasion, and metastasis (Fig. 2, 3). To develop a novel strategy for cancer therapy, we are analyzing the role of HB-EGF in cancer malignancy.

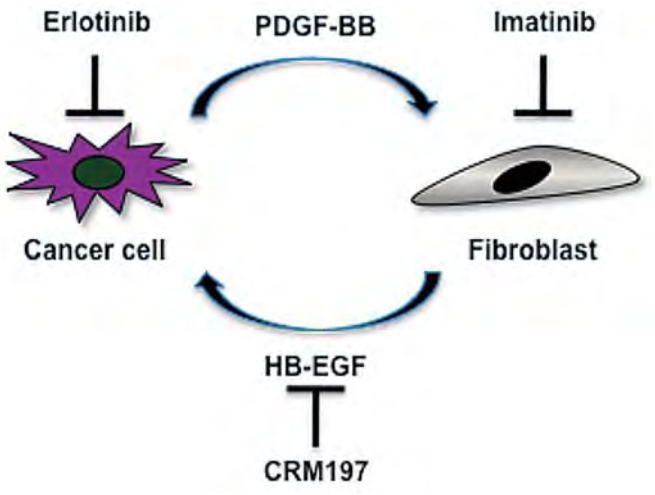


Fig. 2. Cancer cell–stromal fibroblast interactions in the cervix (Murata et al. 2011).

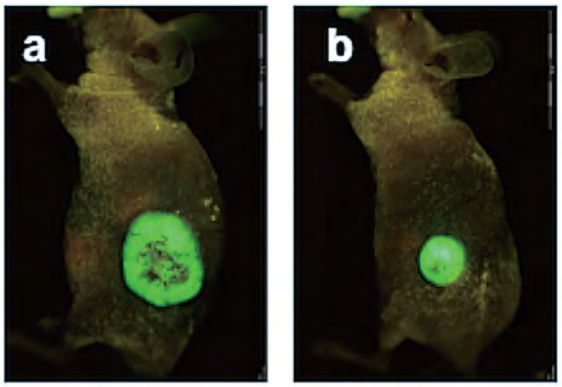


Fig. 3. Cancer-derived fibroblasts enhance tumorigenesis of cervical cancer cells in nude mice (Murata et al. 2011). Fluorescence imaging of tumors injected with cervical cancer cells with fibroblasts (a) or with cervical cancer cells alone (b).

3) Development of anticancer drugs targeting HB-EGF

We are developing new anticancer drugs targeting HB-EGF. Preclinical and clinical studies of an anti-HB-EGF monoclonal antibody (Miyamoto et al. 2011) and a nontoxic mutant protein of diphtheria toxin CRM197 (BK-UM) are in progress (Fig. 4).

4) CD9 and tetraspanin function

CD9, a member of the tetraspanin superfamily, 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.

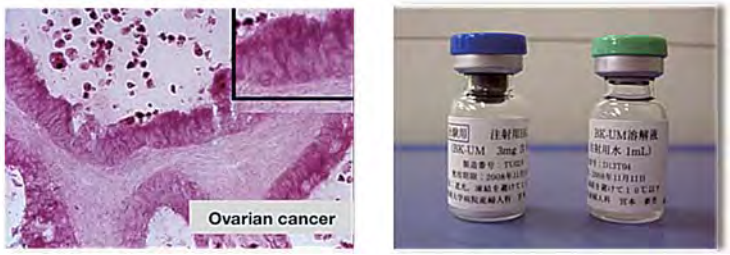


Fig. 4. Enhanced expression of HB-EGF in ovarian cancer tissue (right) and BK-UM, a therapeutic for ovarian cancer under development by our laboratory.

Recent Publications

1. 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.
2. 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.
3. 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.
4. 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.
5. Mizushima H, Wang X, Miyamoto S, Mekada E. Integrin signal masks growth-promotion activity of HB-EGF in monolayer cell cultures. ***J Cell Sci***. 2009 Dec 1;122(Pt 23):4277-86.

Department of Immunochemistry

Research Group

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Assistant Professor	Masako Kohyama, Ph.D., SUP
SA Assistant Professor	Kouyuki Hirayasu,Ph.D.
SA Researcher	Fumiji Saito, Ph.D.
SA Researcher	Jing Wang, Ph.D.

Research Projects

Our department analyzes how pathogens, such as viruses, have acquired the ability to evade the immune system and how host immune systems have acquired resistance to various pathogens. In particular, we seek to elucidate a fundamental mechanism of host defense against various pathogens by analyzing the immune regulatory receptors. Of particular interest are "paired receptors," which consist of activating and inhibitory receptors expressed on some immune cells (Figure 1). Results from the identification of host ligands and their corresponding viral ligands have led us to suggest that these "paired receptors" coevolved with the pathogens. We have also found that these receptors mediate viral entry into cells. Our studies will help to explain the fundamental mechanisms of immune evasion by pathogens and the host factors that influence resistance to various infections. This research will help to build the foundation required for the development of new vaccines and therapies for infectious diseases.

(1) Analysis of recognition by "paired receptors"

Immune cells express various receptors comprised of highly homologous activating and inhibitory receptors. The inhibitory receptors recognize self-antigens, such as MHC molecules. In contrast, the activating receptors generally do not recognize self-antigens, and the ligands for activating receptors remain unclear. We have found that one of these "paired receptors" recognizes cytomegalovirus protein and showed that "paired receptors" play an important role in determining host resistance to pathogens (Figure 2). We are continuing to analyze the functions of these receptors using various pathogens, including viruses, parasites, and bacteria, to elucidate the interactions between pathogens and the host immune system.

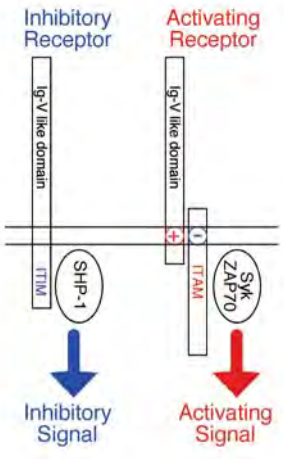

Figure 1. Paired receptors
Paired receptors consist of inhibitory and activating receptors that are highly homologous to each other. The inhibitory receptors transduce inhibitory signals via immunoreceptor tyrosine-based inhibition motif (ITIM) in their cytoplasmic domain, whereas the activating receptors transduce activating signals by association with immunoreceptor tyrosine-based activation motif (ITAM)-bearing adaptor molecules.

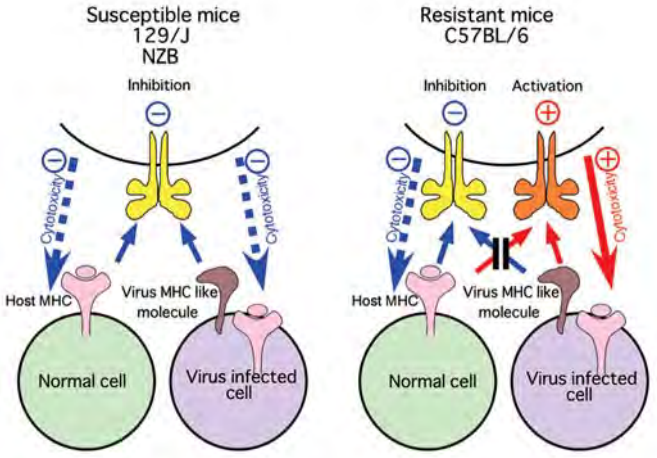

Figure 2. Recognition of cytomegalovirus-infected cells by inhibitory and activating paired receptors
Cytomegalovirus have MHC-like molecules that serve as ligands for inhibitory receptors expressed on the natural killer (NK) cells of susceptible mouse strains. As a result, virus-infected cells are not killed by NK cells, despite their low MHC expression (left). In contrast, NK cells from resistant mouse strains express activating rather than inhibitory receptors that recognize the viral MHC-like molecules. Therefore, these cells can efficiently eliminate virus-infected cells (right) (Arase et al. *Science* 2002).

(2) Entry mechanism of virus into cells

Several viruses that show persistent infection downregulate the immune response by expressing ligands for inhibitory receptors. Interestingly, we have found that some viruses exploit inhibitory receptors to enter the cells. Interactions between immune receptors and viral proteins play an important role in the entry mechanism of herpes simplex virus (HSV) (Figure 3). Because there is a possibility that other viruses may use similar receptors to enter cells, we will further investigate the molecular mechanisms involved in viral entry into cells.

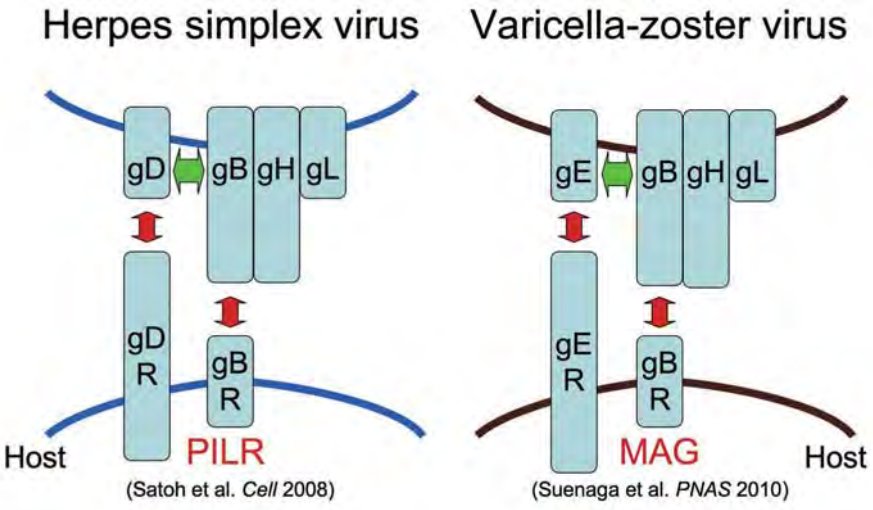

Figure 3. Entry mechanism of virus into cells

Some viruses express ligands for inhibitory receptors and downregulate the immune response. PILRα, one of the inhibitory paired receptor, was found to recognize herpes simplex virus (HSV)-infected cells. Molecular cloning of the ligands for PILRα revealed that PILRα recognizes HSV glycoprotein B, which plays an essential role in HSV infection. Interactions between PILRα and glycoprotein B were involved in viral entry into cells. On the other hand, glycoprotein B of varicella-zoster virus associated with myelin-associated glycoprotein (MAG, Siglec-4), another component of a paired receptor, which mediated VZV entry into cells. In this way, paired receptors were found to play important roles not only in immune regulation but also in viral entry into cells.

Recent Publications

1. Kogure A, Shiratori I, Wang J, L Lanier L, Arase H. PANP is a novel O-glycosylated PILRα ligand expressed in neural tissues. ***Biochem Biophys Res Commun*** 2011 Feb 18; 405(3): 428-433.
2. Suenaga T, Satoh T. Somboonthum P, Kawaguchi Y, Mori Y, and Arase H. Myelin-associated glycoprotein mediates membrane fusion and entry of neurotropic herpesviruses. ***Proc Natl Acad Sci USA***. 2010 Jan 12; 107(2): 866-71.
3. Wang J, Fan Q, Satoh T, Arii J, Lanier LL, Spear PG, Kawaguchi Y, Arase H.Binding of herpes simplex virus glycoprotein B (gB) to PILRα depends on specific sialylated O-linked glycans on gB. ***J Virol***. 2009 Dec ; 83(24): 13042-5.
4. Satoh T, Arii J, Suenaga T, Wang J, Kogure A, Uehori J, Arase N, Shiratori I, Tanaka S, Kawaguchi Y, Spear PG, Lanier LL, Arase H. PILRα is a herpes simplex virus-1 entry co-receptor that associates with glycoprotein B. ***Cell***. 2008 Mar 21; 132(6): 935-44
5. Wang J, Shiratori I, Satoh T, Lanier LL, Arase H. An essential role of sialylated O-linked sugar chains in the recognition of mouse CD99 by paired immunoglobulin-like type 2 receptor (PILR). ***J Immunol***. 2008 Feb 1; 180(3): 1686-93

Department of Molecular Genetics

Research Group

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Assistant Professor (SUP)	Daisuke Okuzaki, Ph.D.
SA Researcher	Yoko Naito, 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 function (Fig. 1).

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. Two miRNAs, miRNA-372 and -373, function as potential novel oncogenes in testicular germ cell tumors by inhibiting *LATS2* expression. This observation suggests that Lats2 is an important tumor suppressor (Voorhoeve *et al*., Cell, 2006). 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. Then, 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 an enhanced growth rate, centrosome fragmentation (Fig. 2), misalignment of the chromosome at M phase, abnormal chromosome segregation, and aberrant cytokinesis. These results indicate the essential role that Lats2 plays in proper M phase progression (Yabuta *et al*., J. Biol. Chem., 2007). (C) 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 (Fig. 3; GW182 is a component of P-body) (Ref. 4). In the ALB pathway, Lats2 is phosphorylated by Aurora-A. Phosphorylated Lats2 localizes to the chromosomes and the central spindle, and regulates Lats1 and Aurora-B, thereby executing proper chromosomal segregation during mitosis (Fig. 4). (D) Lats2 phosphorylates and regulates ASPP1/p53 complexes, thereby undergoing apoptosis in malignant tumor cells with aneuploidy and polyploidy (Ref. 5). Moreover, Lats2 also phosphorylates the transcription factor Snail, thereby regulating the epithelial-mesenchymal transition (EMT) (Ref. 2).

(2) GAK（cyclin G-associated kinase）/cyclin G 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

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

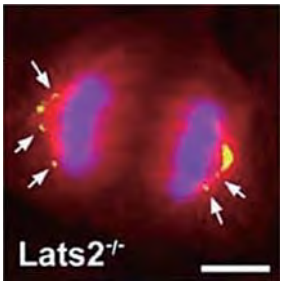

Fig. 2: Centrosome fragmentation in *Lats2*$^{-/-}$ MEFs.

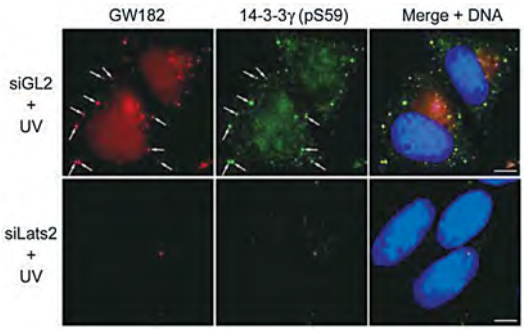

Fig. 3: Lats2 is required for P-body formation after UV irradiation.

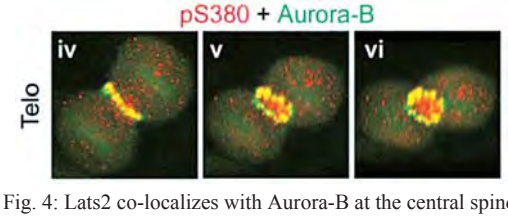

Fig. 4: Lats2 co-localizes with Aurora-B at the central spindle.

has a kinase domain (Fig. 5) whose function has remained unclear. We have discovered the following: (a) GAK forms a complex with PP2A B'γ and cyclin G (cyclin G1 and cyclin G2), which regulate the dephosphorylation activity of PP2A (Fig. 6; Naito *et al*., Cell Cycle, 2012). (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). (c) GAK knockdown by siRNA causes cell cycle arrest at metaphase, which indicates that GAK is required for proper mitotic progression. This impaired mitotic progression was found to be due to the activation of the spindle assembly checkpoint (SAC), which senses protruding, misaligned, or abnormally condensed chromosomes in knockdown cells (Fig. 7; Shimizu *et al*., J. Cell Sci., 2009). (d) GAK-kd$^{-/-}$ mice exhibit neonatal lethality with pulmonary dysfunction (Fig. 8). Because an EGFR inhibitor, Gefitinib (Iressa), also efficiently inhibits the kinase activity of GAK, the side effects of Gefitinib, including interstitial pneumonia, may be responsible for the inhibition of GAK (Ref. 1).

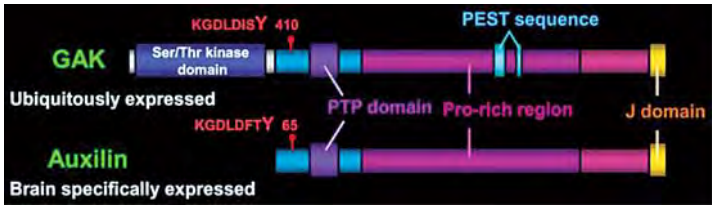

Fig. 5: GAK is similar to auxilin except for bearing a kinase domain.

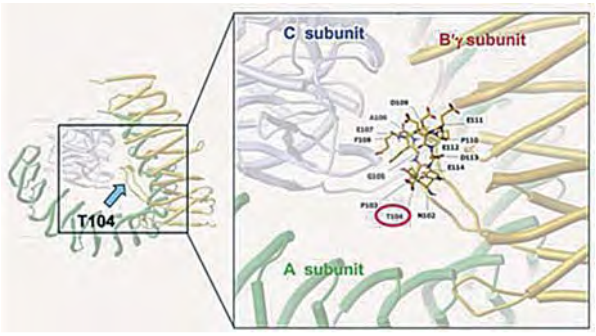

Fig. 6: T104 of PP2A B'γ by GAK is an important phosphorylation site for the activity of PP2A complex.

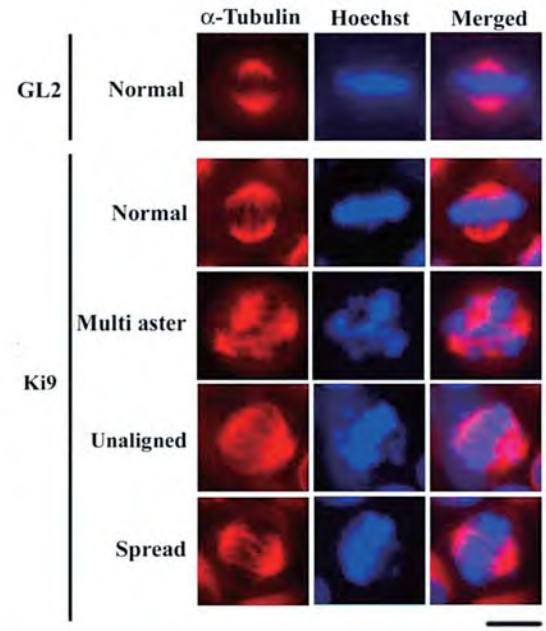

Fig. 7: GAK knockdown by siRNA (Ki9) generates abnormal chromosomes.

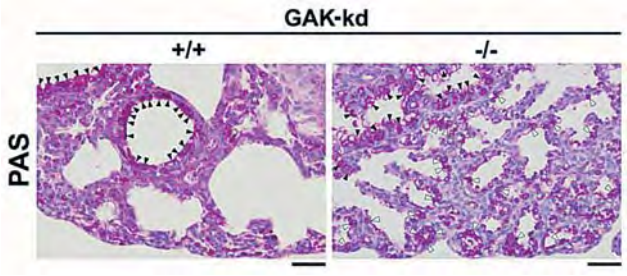

Fig. 8: Severe defects of fetal lung maturity in GAK-kd$^{-/-}$ mice.

Recent Publications

1. Tabara H, Naito Y, Ito A, Katsuma A, Sakurai MA, Ohno S, Shimizu H, Yabuta N, Nojima H. Neonatal lethality in knockout mice expressing the kinase-dead form of the gefitinib target GAK is caused by pulmonary dysfunction. ***PLoS ONE***. 2011, 6(10): e26034.
2. Zhang K, Rodriguez-Aznar E, Yabuta N, Owen RJ, Mingot JM, Nojima H, Nieto MA, Longmore GD. Lats2 kinase potentiates Snail1 activity by promoting nuclear retention upon phosphorylation. ***EMBO J***. 2011 Sep 27;31(1):29-43.
3. Yabuta N, Mukai S, Okada N, Aylon Y, Nojima H. The tumor suppressor Lats2 is pivotal in Aurora A and Aurora B signaling during mitosis. ***Cell Cycle***. 2011, 10(16):2724-36.
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5. Aylon Y, Ofir-Rosenfeld, Y., Yabuta N, Lap, E. Nojima H, Lu, X. and Oren M. The Lats2 tumor suppressor augments p53-mediated apoptosis by promoting the nuclear proapoptotic function of ASPP1. ***Genes Dev***. 2010 Nov 1;24(21):2420-9.

Department of Oncogene Research

Research Group

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Associate Professor	Chitose ONEYAMA, D.Sc.
SA Researcher	Kentaro KAJIWARA, D.Sc.
SA Researcher	Kyoko KAKUMOTO, D.Sc.

Research Projects

Cancers arise, evolve, and develop progressively due to the accumulation of mutations and/or modifications in the genomic DNA. Loss-of-function mutations in “tumor suppressor genes” induce cell immortalization, whereas 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, loss of cell communication, morphological changes, and increased production of matrix proteases and growth factors that participate in invasion, metastasis, and angiogenesis. These cellular events 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 gain-of-function mutations of proto-oncogenes. As a representative proto-oncogene, we have focused on the c-Src proto-oncogene, which encodes a nonreceptor tyrosine kinase. To date, we have analyzed its physiological roles in development and the mechanisms by which it is modulated by its specific regulators, such as Csk and Cbp. 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 activates downstream components, such as the MAPK pathway and Rho family GTPases, thereby inducing gene expression and cytoskeletal rearrangements, respectively. Consequently, promotion of cell growth and phenotypic changes that are involved in cell transformation are induced (Fig. 1). Although the *c-src* gene is rarely mutated in human cancers, its protein is frequently hyperactivated and overexpressed. This aberrant activation of c-Src may contribute to cancer malignancy. We recently showed that the oncogenic potential of c-Src is regulated by a 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.

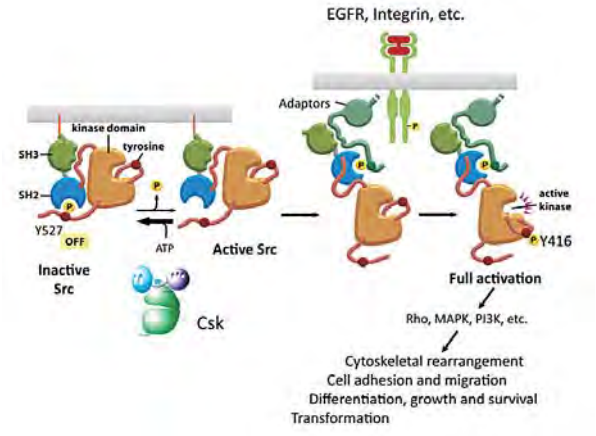


Fig. 1. Function and regulation of c-Src.

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 using a newly developed c-Src inducible system. Proteomic analysis identified Arhgef5, which is a Dbl family member GEF for Rho GTPase, as a critical c-Src target. A miRNA array analysis revealed that c-Src activation altered the gene expression of a limited number of miRNAs. We identified several target genes for these miRNAs and analyzed their functions. Many of the target molecules (e.g., FGFR3, mTOR, and ILK) were components of the c-Src signaling pathway; therefore, we proposed a

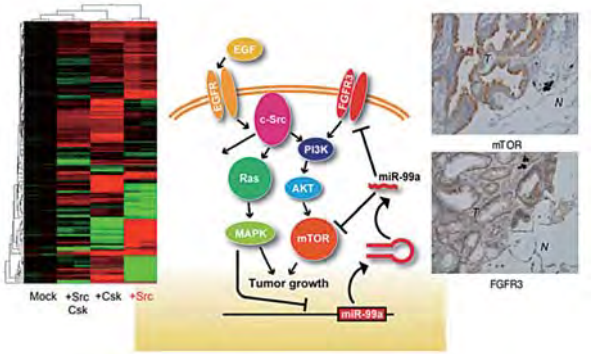


Fig. 2. A mechanism of c-Src–induced cell transformation regulated by miRNA.

new tuning system of c-Src–mediated cell transformation via miRNA. Analysis of the regulatory mechanism for c-Src–mediated miRNA expression is now underway.

III. Regulation of cell growth signaling via late endosomes/lysosomes.

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

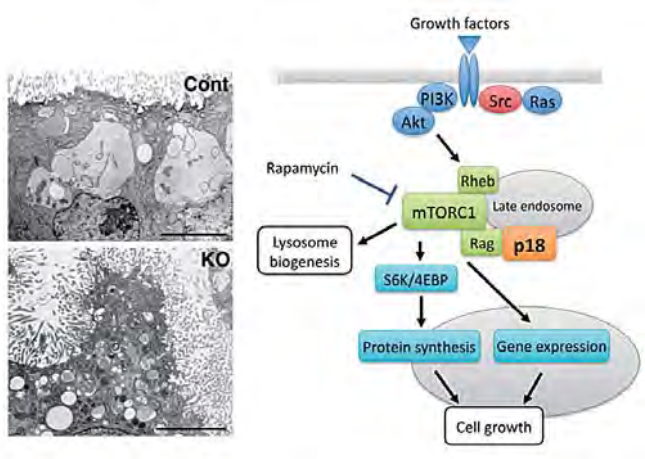


Fig. 3. Roles played by the late endosome/lysosome-anchored p18-mTORC1 pathway.

Recent Publications

1 : Takahashi Y, Nada S, Mori S, Soma-Nagae T, Oneyama C, Okada M. The late endosome/lysosome-anchored p18-mTORC1 pathway controls terminal maturation of lysosomes. ***Biochem Biophys Res Commun***. 2012 Jan 27;417(4):1151-7.

2 : Oneyama C, Morii E, Okuzaki D, Takahashi Y, Ikeda J, Wakabayashi N, Akamatsu H, Tsujimoto M, Nishida T, Aozasa K, Okada M. MicroRNA-mediated upregulation of integrin-linked kinase promotes Src-induced tumor progression. ***Oncogene***. 2012 Mar 29;31(13):1623-35.

3 : Kuroiwa M, Oneyama C, Nada S, Okada M. The guanine nucleotide exchange factor Arhgef5 plays crucial roles in Src-induced podosome formation. ***J Cell Sci***. 2011 May 15;124(Pt 10):1726-38.

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.
Postdoctoral Fellow	Yumi Kinugasa, Ph.D.
Postdoctoral Fellow	Tomomi Mohri, Ph.D.

Research Projects

The development of normal tissues and organs requires the generation of tissue-specific cells from stem cells. 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. With a few exceptions, the development of all tissues requires blood vessel formation. Our research group is analyzing the molecular mechanisms by which blood vessels form under 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. Our ultimate goal is to establish strategies to inhibit the malignant progression of various diseases.

Our specific research projects are as follows:

I. Analysis of the molecular mechanisms 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
4) Development of a system that delivers drugs into blood vessels

II. Molecular analysis of self-renewal in normal and cancer stem cells (CSCs)
1) Mechanism of stem cell reprogramming
2) Analysis of cell cycle regulation in stem cells, with a special focus on Galectin-3 and the GINS complex
3) Establishment of a strategy that can inhibit the formation of the vascular niche inhabited by CSCs

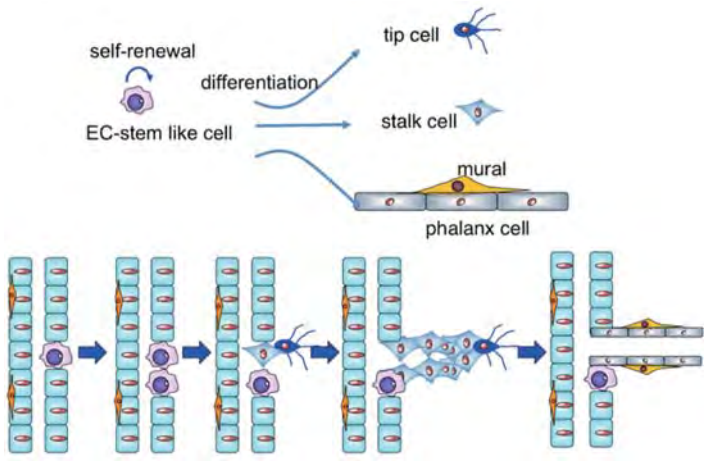


Figure 1. ESCs and angiogenesis. During angiogenesis, tip cells in the front of new vessels determine the migration direction of new vessels; stalk cells produce greater numbers of endothelial cells (ECs) for elongation; and the phalanx cells induce maturation of new blood vessels. Recently, ESCs were identified in the preexisting blood vessels. These ESCs may produce heterogeneous ECs and critically regulate the processes of pathological and physiological angiogenesis.

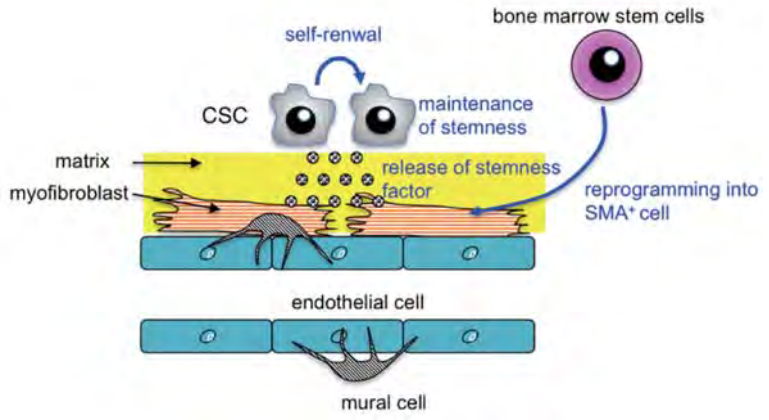


Figure 2. Vascular niche of CSCs. The vascular niche was found to contain CSCs by using PSF1, a member of DNA replication factors GINS. Molecular analysis of vascular niche formation may be highly useful for the development of new therapies for CSCs.

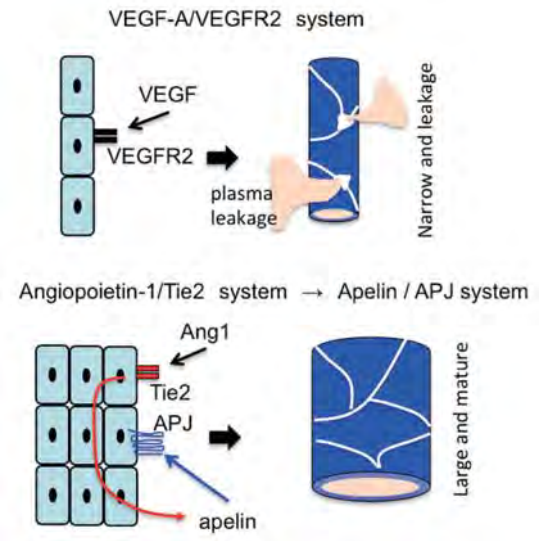


Figure 3. Implication of the Tie2/Angiopoietin-1 and APJ/apelin axis for blood vessel maturation. Angiopoietin-1 (Ang1) stimulates Tie2 on endothelial cells (ECs), which results in EC migration and proliferation. Ang1 promotes the production of apelin by ECs, thereby stimulating the apelin receptor APJ on ECs. The activation of APJ induces the proliferation and assembly of ECs, resulting in blood vessel enlargement. Apelin also induces firm cell–cell adhesion between ECs regulating VE-cadherin and claudin-5. Although VEGF induces narrow and leaky blood vessels, Ang1 and apelin regulate mature blood vessels.

Recent Publications

1. Naito H, Kidoya H, Sakimoto S, Wakabayashi T, Takakura N. Identification and characterization of a resident vascular stem/progenitor cell population in preexisting blood vessels. ***EMBO J***. 2011 Dec 16;31(4):842-55.
2. Kidoya H, Naito H, Takakura N. Apelin induces enlarged and non-leaky blood vessels for functional recovery from ischemia. ***Blood***. 2010 Apr 15;115(15):3166-74.
3. Nagahama Y, Ueno M, Miyamoto S, Morii E, Minami T, Mochizuki N, Saya H, Takakura N. (2010). PSF1, a DNA replication factor expressed widely in stem and progenitor cells, drives tumorigenic and metastatic properties. ***Cancer Res***. 2010 Feb 1;70(3):1215-24.
4. Ueno M, Itoh M, Sugihara K, Asano M, and Takakura N. (2009). Both Alleles of PSF1 are Required for Maintenance of Pool Size of Immature Hematopoietic Cells and Acute Bone Marrow Regeneration. ***Blood***. 2009 Jan 15;113(3):555-62.
5. Kidoya H, Ueno M, Yamada Y, Mochizuki N, Nakata M, Yano T, Fujii R, Takakura N. (2008). Spatial and temporal role of the apelin/APJ system in the caliber size regulation of blood vessels during angiogenesis. ***EMBO J***. 2008 Feb 6;27(3):522-34.

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	Kanami Uesugi, Ph.D.
SA Researcher	Yusuke Hirata, Ph.D.

Cells are equipped with a signal transduction system that enables them to respond appropriately to the surrounding environment, such as stimulation with various hormones/growth factors and physical interaction with other cells or the extracellular matrix. Malfunctions in the signaling system are responsible for various human diseases, such as in the case of the abnormal proliferation of cancer cells. In our laboratory, we are investigating the mechanism of intracellular signaling that regulates the proliferation, differentiation, and motility of cells at levels ranging from molecules to organisms using nematodes and mice. Our present major research interests are (1) oxidative stress signaling by reversible oxidation of proteins, and (2) cancer metastasis and Mg^{2+}-regulation by redox-responsive proteins.

(1) *Oxidative stress signaling by reversible oxidation of proteins*

We discovered thioredoxin-related protein nucleoredoxin (NRX) as a novel regulator of Wnt signaling, which plays important roles in early development and oncogenesis. We observed that NRX directly bound to the Wnt signal transducer Dishevelled (Dvl) and inhibited its function. Interestingly, the NRX-Dvl interaction was negatively regulated by the formation of intramolecular S-S bonds in NRX. Thus, NRX regulates Wnt signaling in a redox-dependent manner (Fig. 1).

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 stimulated H2O2 generation and CRMP2 oxidation, resulting in the formation of homodimers linked with S-S bonds. This oxidation of CRMP2 mediated the repulsive axon guidance induced by Semaphorin stimulation (Fig. 2).

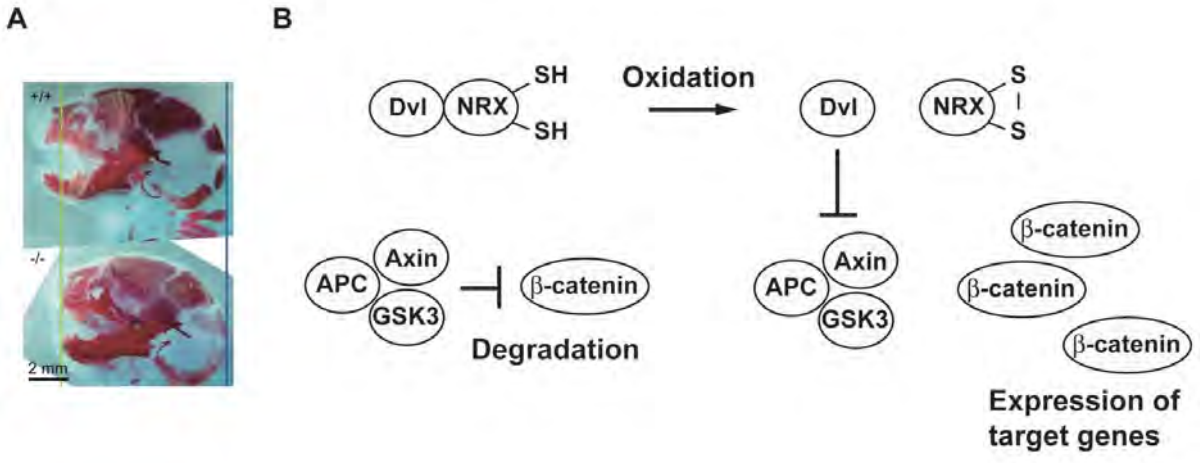


Fig. 1: Redox regulation of Wnt signaling by reversible oxidation of NRX
(A) Developmental abnormality of bones in *NRX*$^{-/-}$ mice. (B) Schematic illustration of the mechanism of redox regulation of Wnt signaling by NRX. NRX inhibits the function of Dvl. The protein complex composed of APC, Axin, and GSK3 induces the degradation of β-catenin. When NRX is oxidized, Dvl becomes free from NRX and inhibits the degradation of β-catenin, which induces the expression of its target genes.

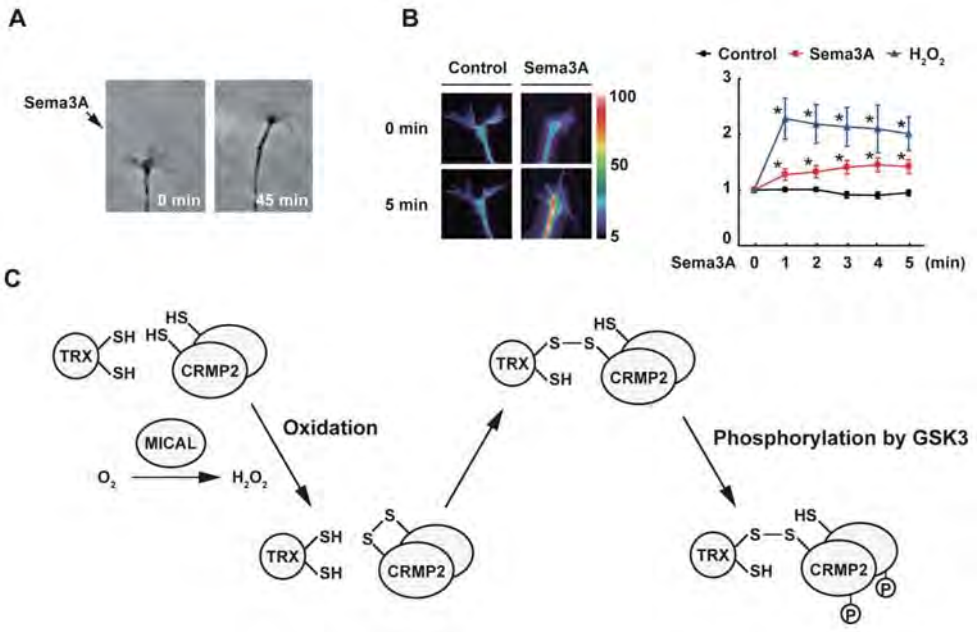


Fig. 2: CRMP2 oxidation in Semaphorin signaling
(A) Repulsive guidance of axons by Semaphorin 3A (Sema3A). (B) Increase of H_2O_2 in neuronal growth cones by Sema3A. (C) Schematic illustration of the mechanism of Semaphorin signaling via CRMP2 oxidation. Sema3A stimulation generates H_2O_2 and oxidizes CRMP2. Oxidized CRMP2 transiently forms protein complexes with TRX, which induces CRMP2 phosphorylation by GSK3.

(2) *Cancer metastasis and Mg^{2+}-regulation by redox-responsive protein*

We identified PRL, a tyrosine phosphatase with unknown function, as a novel S-S bond-containing protein. PRL is consistently overexpressed in various human metastatic cancers and can promote experimental metastasis. We searched for novel binding proteins for PRL and identified magnesium-exporting protein (MagEx), an integral membrane protein that regulates the intracellular Mg^{2+} level by exporting Mg^{2+}. The activity of MagEx was inhibited by its interaction with PRL.

Recent Publications

1. Morinaka A, Funato Y, Uesugi K, Miki H. Oligomeric peroxiredoxin-I is an essential intermediate for p53 to activate MST1 kinase and apoptosis. ***Oncogene***. 2011 Oct 6;30(40):4208-18.
2. Morinaka A, Yamada M, Itofusa R, Funato Y, Yoshimura Y, Nakamura F, Yoshimura T, Kaibuchi K, Goshima Y, Hoshino M, Kamiguchi H, Miki H. Thioredoxin mediates oxidation-dependent phosphorylation of CRMP2 and growth cone collapse. ***Sci Signal.*** 2011 Apr 26;4(170):ra26.
3. Funato Y, Terabayashi T, Sakamoto R, Okuzaki D, Ichise H, Nojima H, Yoshida N, Miki H. Nucleoredoxin sustains Wnt/β-catenin signaling by retaining a pool of inactive dishevelled protein. ***Curr Biol***. 2010 Nov 9;20(21):1945-52.
4. Hayashi T, Funato Y, Terabayashi T, Morinaka A, Sakamoto R, Ichise H, Fukuda H, Yoshida N, Miki H. Nucleoredoxin negatively regulates Toll-like receptor 4 signaling via recruitment of flightless-I to myeloid differentiation primary response gene (88). ***J Biol Chem***. 2010 Jun 11;285(24):18586-93.
5. Yoshimura Y, Terabayashi T, Miki H. Par1b/MARK2 phosphorylates kinesin-like motor protein GAKIN/KIF13B to regulate axon formation. ***Mol Cell Biol***. 2010 May;30(9):2206-19.

Department of Molecular Protozoology

Research Group

Professor	Toshihiro Horii, Ph. D.
Invited Professor	Kazuyuki Tanabe, Ph. D.
Assistant Professor	Nobuko Arisue, Ph. D.
Assistant Professor	Takahiro Tougan, Ph. D.
SA Assistant Professor	Nirianne M. Q. Palacpac, Ph. D.
SA Researcher	Masanori Yagi, 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 succumb to the disease annually. Controlling malaria has become more challenging since the emergence of drug-resistant malaria parasites. This development has intensified 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 an amino acid sequence present in the serine repeat antigen (SERA) of malaria parasites. We and coresearchers in malaria-endemic areas have demonstrated that naturally acquired immunity against malaria exclusively correlates with the development of anti-SERA IgG3 antibodies. We have also shown that many types of animals, including chimpanzees, develop antibodies upon vaccination with SE36 and that these antibodies inhibit the growth of malaria parasites.

Together with the Kanonji Institute of The Research Foundation of Osaka University, we constructed a system by which the SE36 malaria vaccine can be mass produced. In 2005, we conducted a phase I clinical trial in Japan with SE36 to assess its safety and immunogenicity. All vaccine-administered volunteers sero-converted and showed no serious adverse events. We are moving towards further phase Ib clinical trials in an endemic region in Uganda. This project is being conducted in collaboration with The Research Foundation for Microbial Diseases of Osaka University.

We are also studying the function of the SERA molecule in the parasite and characterizing the host immune response against SERA. In addition, we have started a new research project for *P. vivax* vaccine development by collaborating with colleagues in Uganda, Thailand, Indonesia, and the Solomon Islands.

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

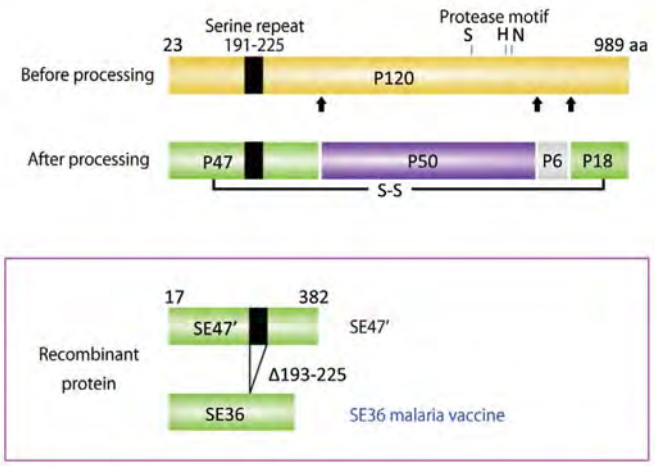


Fig. 2. Processed fragments of P. *falciparum* SERA and the structure of recombinant SE36

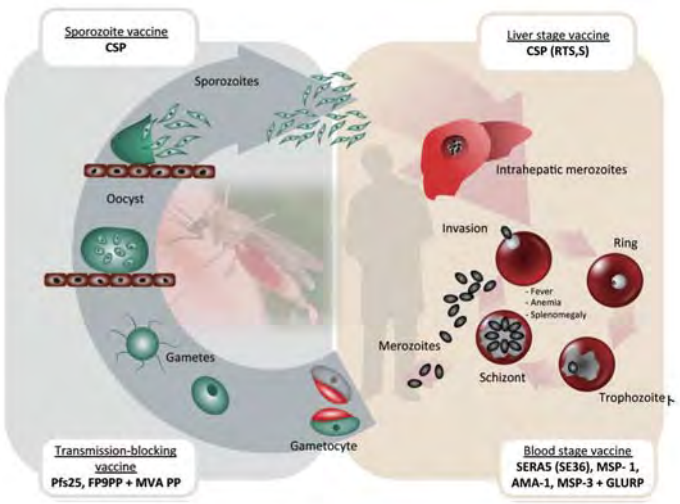


Fig. 3. *P. falciparum* life cycle and vaccine targets

Fig. 4. 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.

(2) *Approaches for understanding theevolution of malaria parasites*
Malaria parasites have many unique features, such as a highly restricted host range, complex life cycle, and host immune evasion systems. To understand the molecular basis of those features, we utilized several genetic approaches, such as nuclear and organelle genome analyses, as well as population genetic analyses of the antigen coding genes. The findings from these studies can be effectively utilized in vaccine development.

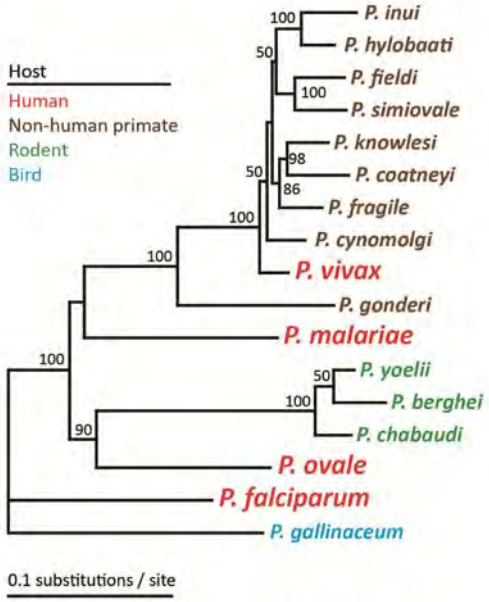


Fig. 5. Evolutionary tree of malaria parasites inferred from 30 protein-encoding genes from the apicoplast genome. Four human parasites do not show a monophyletic relationship revealing host-switching during parasite evolution.

Recent Publications

1. Arisue N, Hashimoto T, Mitsui H, Palacpac NM, Kaneko A, Kawai S, Hasegawa M, Tanabe K, Horii T. The *Plasmodium* apicoplast genome: conserved structure and close relationship of *P. ovale* to rodent malaria parasites. ***Mol Biol Evol***. 2012 Mar 6. (in press)
2. 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. Geographic differentiation of polymorphism in the *Plasmodium falciparum* malaria vaccine candidate gene SERA5. ***Vaccine***. 2012 Feb 21;30(9):1583-93.
3. Palacpac NM, Arisue N, Tougan T, Ishii KJ, Horii T. *Plasmodium falciparum* serine repeat antigen 5 (SE36) as a malaria vaccine candidate. ***Vaccine***. 2011 Aug 11;29(35):5837-45.
4. Arisue N, Kawai S, Hirai M, Palacpac NM, Jia M, Kaneko A, Tanabe K, Horii T. Clues to evolution of the SERA multigene family in 18 *Plasmodium* species. ***PLoS One***. 2011 Mar 15;6(3):e17775.
5. Horii T, Shirai H, Li J, Ishii KJ, Palacpac NM, Tougan T, Hato M, Ohta N, Bobogare A, Arakaki N, Matsumoto Y, Namazue J, Ishikawa T, Ueda S, Takahashi M. Evidences of protection against blood-stage infection of *Plasmodium falciparum* by the novel protein vaccine SE36. ***Parasitol Int***. 2010 Sep;59(3):380-6.

Department of Virology

Research Group

Professor	Kazuyoshi Ikuta, Ph.D.
Assistant Professor	Takeshi Kurosu, D.V.M., Ph.D.
Assistant Professor	Yohei Watanabe, D.V.M., Ph.D.
SA Assistant Professor	Tadahiro Sasaki, Ph.D.
SA Assistant Professor	Azusa Asai, Ph.D.
SA Researcher	Yuji Inoue, Ph.D.
SA Researcher	Yuta Kanai, D.V.M., Ph.D.
SA Researcher	Mitsuhiro Nishimura, Ph.D.

Research in our department focuses on several viruses that target immune and respiratory regions, as well as prions that target the central nervous system. We seek to understand the mechanisms by which these pathogens replicate and induce pathogenesis, and to apply our understanding to the development of methods to control these agents, remove them from blood products, and diagnosis their infection rapidly.

(1) Infections to the immune system

Dengue illness, which is transmitted to humans by mosquitoes, is among the most important viral infectious diseases in tropical and subtropical regions around the world. There are four antigenically distinct dengue virus serotypes. Severe dengue cases, such as dengue hemorrhagic fever and dengue shock syndrome, mostly occur by secondary infection with a serotype different from the primary infected serotype. Although individuals infected with dengue virus produce high titers of neutralizing antibodies, some of these antibodies may play a role in the antibody-dependent enhancement of the secondarily infected serotype, which could make vaccine development difficult.

We seek to characterize the mechanism by which dengue virus derived from South Asian countries induces dengue illness in order to develop an antidengue viral drug. For its in vivo evaluation, we are also developing animal models for dengue viral infection. In addition, we are preparing human monoclonal antibodies to understand the viral-induced pathogenicity and to develop therapeutic antibodies.

(2) Infections to the respiratory system

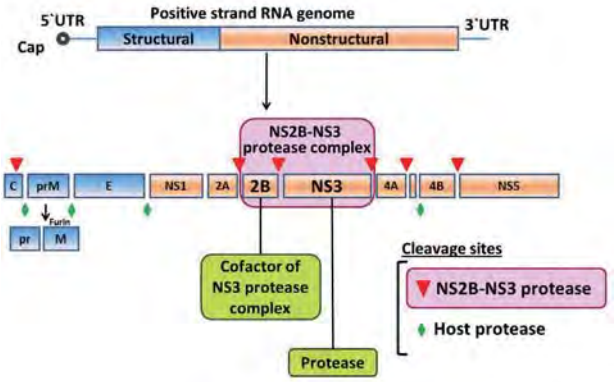
Influenza virus induces typical acute infection in the respiratory region. In addition to seasonal influenza viruses, a swine-origin pandemic virus appeared in 2009. Currently, the possible global emergence of a pandemic virus from highly pathogenic avian influenza virus, such as H5N1, is a public concern worldwide. Together with Alexandria University, we are studying H5N1 circulating in Egypt. Recently, we found that some of the new H5 sublineages in Egypt have acquired an enhanced binding capacity to human-type receptors. The purpose of our search is to ascertain the pandemic potential of these H5N1 viruses in Egypt. We have prepared several human neutralizing monoclonal antibodies against influenza virus. Because the epitope region recognized by one of the monoclonal antibodies is highly conserved and forms a conformational structure, we are working on the possible development of a new vaccine with this conformation in the synthetic peptide in collaboration with several companies.

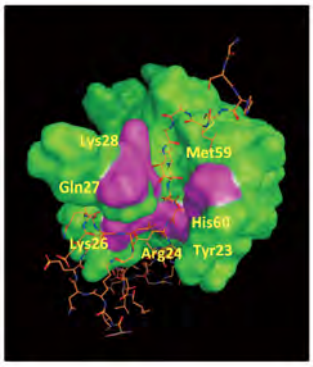
(3) Blood-borne infections

We are working to establish how to remove infectious agents, such as parvovirus B19, SARS-corona virus, hepatitis E virus, and prions, from blood products in collaboration with a company.

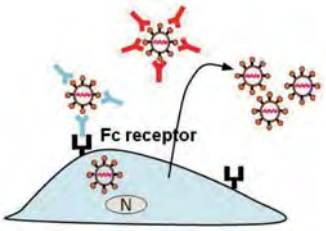
(4) Rapid diagnosis kits

There are many techniques for the diagnosis of viral infections, including immunofluorescence, ELISA, Western blot, and PCR assays. We are currently working with several companies towards the development of rapid diagnosis kits against several infectious diseases.

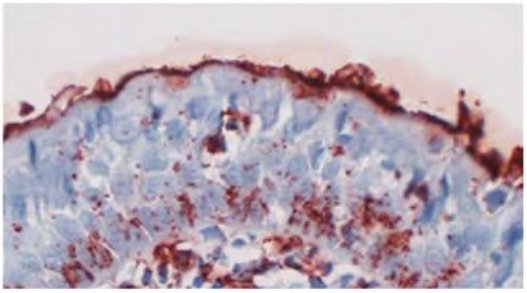


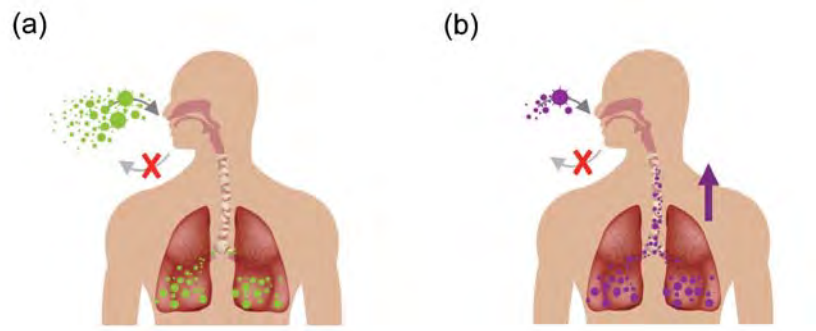


Dengue virus is produced as a single polyprotein and is cleaved into 10 proteins for viral replication. The dengue virus protease complex, which is essential for cleavage, is composed of the NS3 protease and NS2B cofactor. Small molecules that interfere with the interaction between NS2B and NS3 impair protease activity and inhibit viral replication. NS2B (frame structure) interacts with the pocket site of NS3 (green). The key residues of NS3 that interact with the newly developed small compound are indicated in pink.



Dengue viral infection induces strong humoral immunity and antibody production. These antibodies include neutralizing antibodies and negatively regulating antibodies by antibody-dependent enhancement. Human monoclonal antibodies are being prepared by using immune cells from patients to investigate dengue virus-induced pathogenicity and to develop antibody therapeutics.





Some of the new H5 sublineages in Egypt have acquired an increased attachment to and infectivity in the human lower respiratory tract (H5N1 virus attached to human tracheal epithelia is stained red in the panel). We are currently investigating the mechanism(s) underlying the possible emergence of pandemic H5N1 viruses in Egypt and its risk in the field, in collaboration with Alexandria University. (a) Pathology of the classical virus lineage (H5N1) in humans. The virus lineage only has binding affinity for α2,3 sialylglycan and needs to reach the lower respiratory tract. (b) Pathology of the new H5 sublineage that has emerged in Egypt. Expansion of receptor usage (increased α2,6 sialylglycan binding) enables the new sublineage to bind more efficiently to epithelia in the lower respiratory tract.

Recent Publications

1. Yasugi M, Nakamura S, Daidoji T, Ramadhany R, Yang C-S, Yasunaga T, Iida T, Horii T, Ikuta K, Takahashi K, Nakaya T. Frequency of D222G and Q223R hemagglutinin mutants of pandemic (H1N1) 2009 influenza virus in Japan between 2009 and 2010. ***PLoS One*** 2012;7(2):e30946. Epub 2012 Feb 17.
2. Watanabe Y, Ibrahim MS, Suzuki Y, Ikuta K. The changing nature of avian influenza A virus (H5N1). ***Trends Microbiol***. 2012 Jan;20(1):11-20. Epub 2011 Dec 5.
3. Watanabe Y, Ibrahim MS, Ellakany HF, Kawashita N, Mizuike R, Hiramatsu H, Sriwilaijaroen N, Takagi T, Suzuki Y, Ikuta K. Acquisition of human-type receptor binding specificity by new H5N1 influenza virus sublineages during their emergence in birds in Egypt. ***PLoS Pathog***. 2011 May:7(5):e1002068. Epub 2011 May 26.
4. Mizuike R, Sasaki T, Baba K, Iwamoto H, Shibai Y, Kosaka M, Kubota-Koketsu R, Yang CS, Du A, Sakudo A, Tsujikawa M, Yunoki M, Ikuta K. Development of two types of rapid diagnostic test kits to detect the hemagglutinin or nucleoprotein of the swine-origin pandemic influenza A virus H1N1. Clin. ***Vaccine Immunol***. 2011 Mar;18 (3):494-9. Epub 2011 Jan 12.
5. Kurosu T, Khamlert C, Phanthanawiboon S, Ikuta K, Anantapreecha S. Highly efficient rescue of dengue virus using a co-culture system with mosquito/mammalian cells. ***Biochem Biophys Res Commun***. 2010 Apr 2;394(2):398-404. Epub 2010 Mar 7.

Department of Experimental Genome Research

Research Group		
	Professor (SUP)	Masaru Okabe, Ph.D.
	Associate Professor	Takeshi Miwa, Ph.D.
	Associate Professor (SUP)	Masahito Ikawa, Ph.D.
	Assistant Professor	Hidetoshi Hasuwa, Ph.D.
	Assistant Professor	Naokazu Inoue, Ph.D.
	Assistant Professor (SUP)	Ayako Isotani, Ph.D.
	SA Assistant Professor (SUP)	Yuhkoh Satouh, Ph.D.

In the past, naturally mutated animals have been used to elucidate the mechanisms of various diseases. However, in the "postgenome project era", genetically manipulated animals play a key role in such investigations and provide important disease models. Our laboratory, in collaboration with the Animal Resource Center for Infectious Diseases, elucidates the mechanisms of mammalian reproductive systems through gene manipulation in mice (http://www.egr.biken.osaka-u.ac.jp/).

Research Projects

We have successfully produced the first genetically altered “green mouse” in the world. These green fluorescent protein (GFP)-expressing mice are highly useful for many types of research projects, such as those involving stem cell transplantation and regeneration studies. Utilizing one of these animals, we demonstrated that it was possible to label sperm with fluorescent proteins and visualize the fertilization process (Figures 1 and 2).

Using gene knockout technology, we 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. Mutant mice were male infertile because their spermatozoa lacking ADAM3 could not migrate through the utero-tubal junction (Figure 2) (1). However, mutant spermatozoa were able to fertilize eggs when they were directly deposited into the oviduct.

Peculiarly, spermatozoa with defective zona pellucida (ZP)-binding ability were able to fertilize cumulus-covered, ZP-intact eggs effectively in vivo (1). Acrosome-reacted mouse spermatozoa that were recovered from the perivitelline space could be used to fertilize other eggs (Figure 3) (2). From these results, we propose that the observation of numerous sperm binding to the ZP surface of cumulus-free eggs could have been a “red herring” that was less important than was previously supposed.

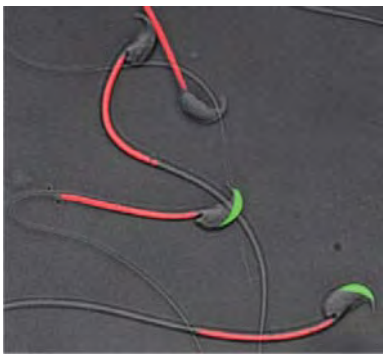


Fig. 1. Transgenic mouse spermatozoa labeled with GFP in the acrosome and red fluorescent protein (RFP) in the mitochondria. Spermatozoa lose green fluorescence after acrosome reaction.

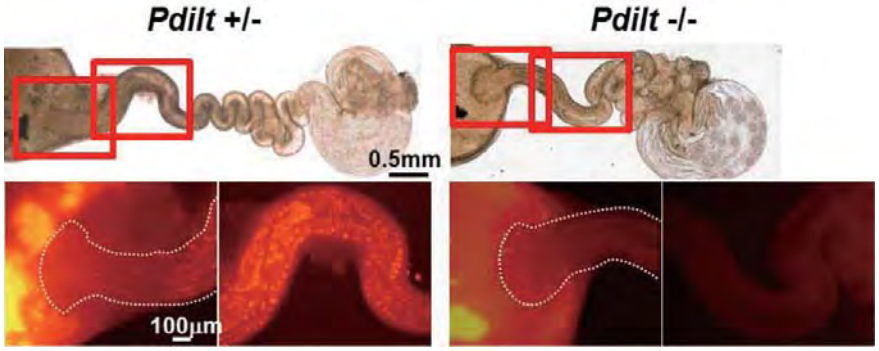


Fig. 2. *Pdilt* $^{-/-}$ spermatozoa lacking ADAM3 are unable to migrate through the utero-tubal junction.

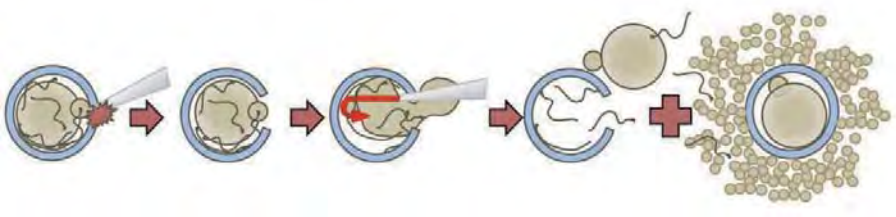


Fig. 3. Acrosome-reacted mouse spermatozoa recovered from the perivitelline space can fertilize other eggs.

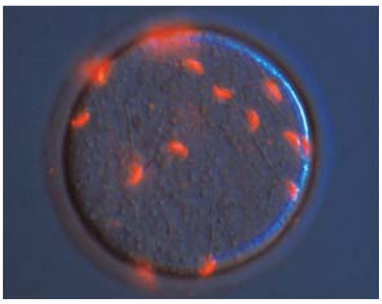


Fig. 4. Accumulation of many *Izumo1* $^{-/-}$ spermatozoa in the perivitelline space, because *Izumo1* $^{-/-}$ spermatozoa are unable to fuse with egg.

We have also reported that the sperm protein IZUMO1 is essential for fusion with eggs, and that SPESP1 is necessary to produce fully "fusion-competent" sperm (Figure 4).
In addition to studying the mechanism of the sperm-egg interaction, we are trying to invent new technologies to perform novel investigations in the field of reproduction. By transducing blastocysts with lentiviral vectors, we developed the placenta-specific gene manipulation method. Expression of soluble FLT1 in the placenta allowed us to prepare preeclampsia model mice (3).
We recently established rat embryonic stem (ES) cells and generated Mouse↔Rat chimeric animals (Figure 5). We would like to use this animal model to study body/organ size control *in vivo*. The chimeric animals may provide a method for the derivation of various organs from ES or iPS cells (4). We are also examining the role(s) of miRNA in live animals using miRNA knockout technique.

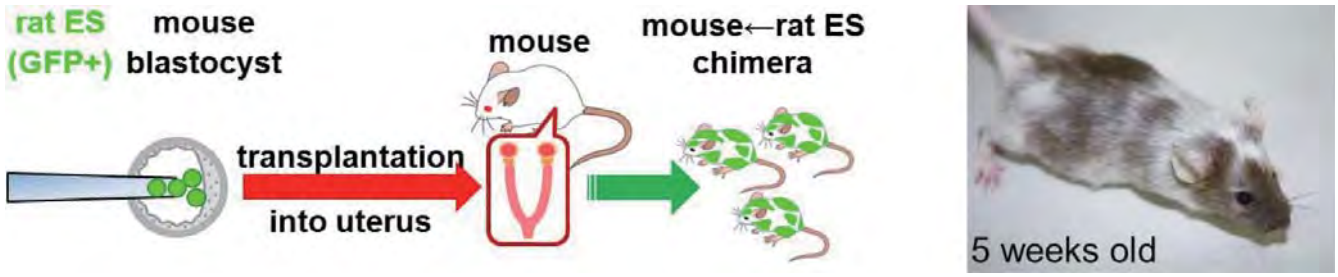


Fig. 5. Mouse↔Rat chimeric animals are generated by injecting rat ES cells into mouse blastocysts (left). Contribution of mouse (white) and rat (black) cells are visualized by coat color.

Research Projects (Miwa' s group)
We investigate the molecular biological mechanisms involved in human diseases, especially cardiovascular diseases, by using animal models. We have established a diastolic heart failure model using Dahl salt-sensitive rats. Left ventricular (LV) fibrosis and stiffening play crucial roles in the development of heart failure with preserved ejection fraction (HFpEF). Digitalis-like factors and subsequently activated Na^+/Ca^{2+} exchanger entry mode may play an important role in the development of hypertensive HFpEF (5). We have also administered carnitine to the HFpEF model (Figure 6).

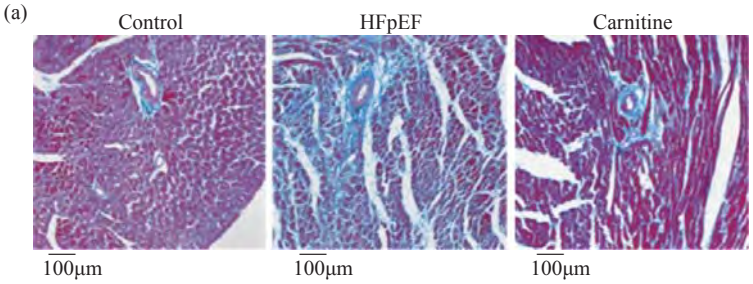


Fig. 6. Effects of carnitine on cardiac fibrosis in LV of Dahl salt-sensitive rats at age 20 weeks. The typical Azan Mallory staining of the LV in the control, HFpEF, and carnitine treatment groups. Carnitine administration significantly attenuated LV fibrosis.

Recent Publications

1. Tokuhiro K, Ikawa M, Benham AM, Okabe M. Protein disulfide isomerase homolog PDILT is required for quality control of sperm membrane protein ADAM3 and male infertility. ***Proc Natl Acad Sci U S A***. 2012 March 6; 109 (10): 3850-3855.
2. Inoue N, Satouh Y, Ikawa M, Okabe M, Yanagimachi R. Acrosome-reacted mouse spermatozoa recovered from the perivitelline space can fertilize other eggs. ***Proc Natl Acad Sci U S A***. 2011 Dec 13;108(50):20008-11.
3. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. ***Proc Natl Acad Sci U S A***. 2011 Jan 25;108(4):1451-5.
4. Isotani A, Hatayama H, Kaseda K, Ikawa M, Okabe M. Formation of a thymus from rat ES cells in xenogeneic nude mouse↔rat ES chimeras. ***Genes Cells***. 2011 Apr;16(4): 397-405.
5. 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***. 2011 Apr 13.

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.
SA Researcher	Akifumi Yamashita, Ph.D.
SA Researcher	U. Chandimal de Silva, 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, and holds 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 (Goto et al, 2007; Fig. 1). Through a comprehensive analysis of its genome, we also intend to elucidate the evolutionary pathways of the influenza virus (Fig. 2).

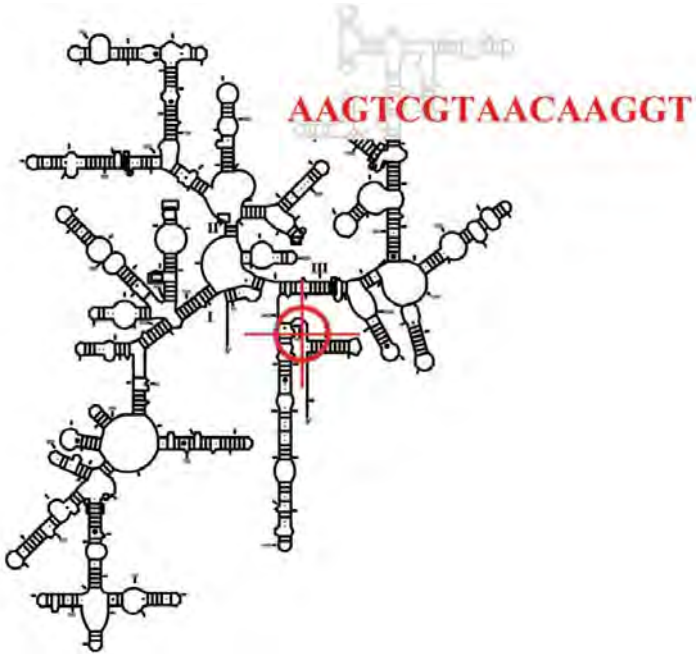


Figure 1. Large-scale genome analysis of 266 organisms. Identification of sequences conserved in almost all known genomes.

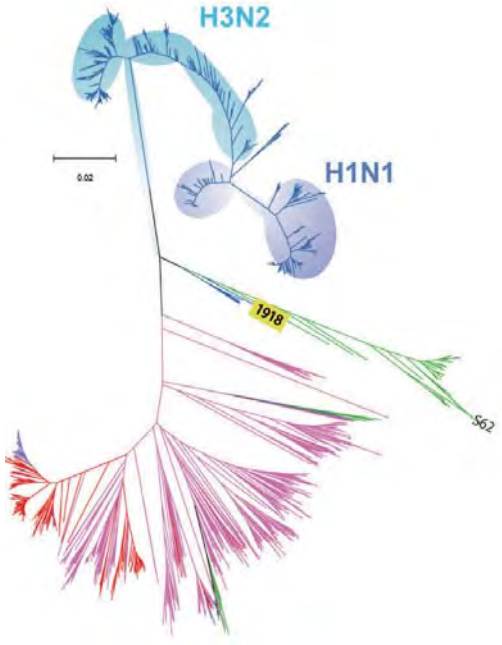


Figure 2. Comprehensive analysis of the influenza virus genome.

(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, 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 kept up to date at all times.

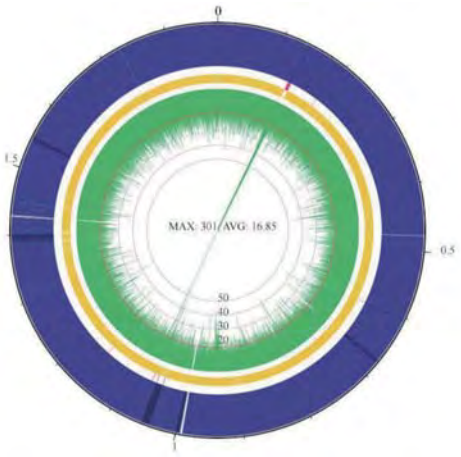


Figure 3. Next generation sequencing enables sequencing of a whole genome in one run.

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

Recent Publications

1. 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.
2. 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.
3. 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.
4. 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.
5. Goto N, Kurokawa K, Yasunaga T. Analysis of invariant sequences in 266 complete genomes. ***Gene***. 2007 Oct 15;401(1-2):172-80.

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.

Research Projects

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, 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 to amplify the genome of pathogenic microorganisms and subtract the host genome.

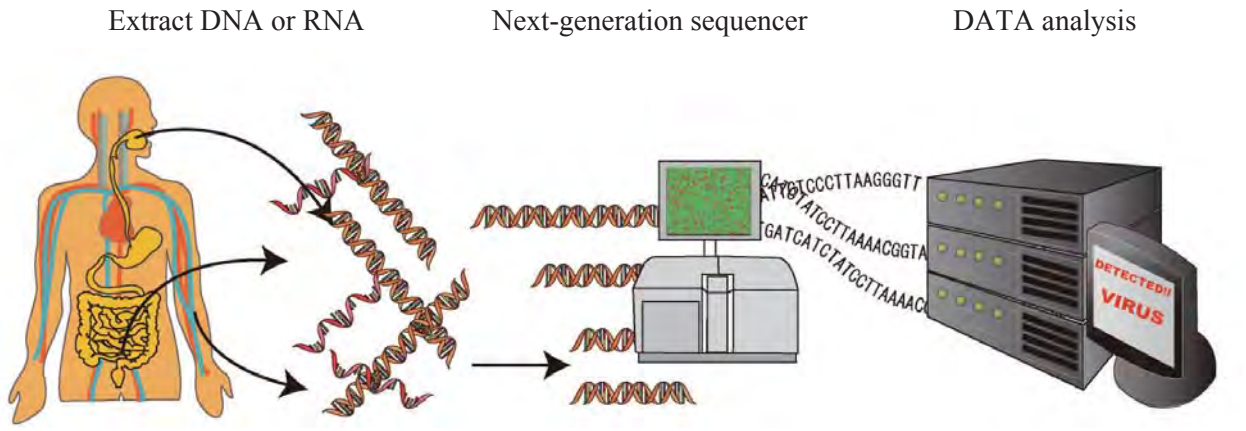


Fig. 1. Metagenomic diagnosis of infectious diseases using a next-generation sequencer.

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.

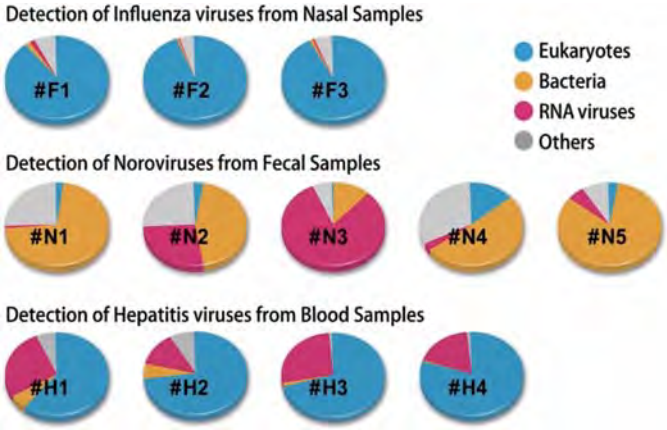


Fig. 3. Distribution of detected organisms in viral infection cases

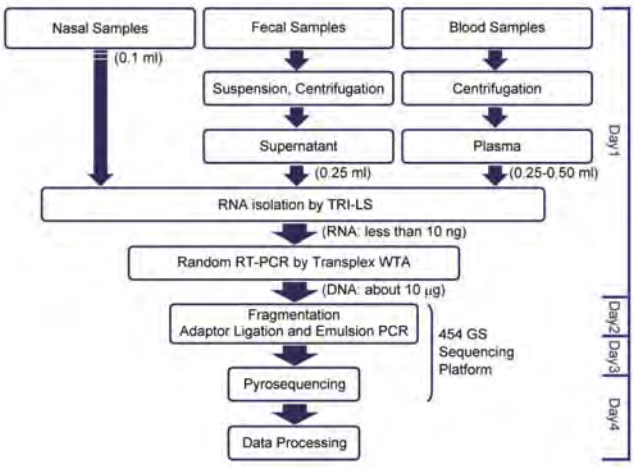


Fig. 4. Standard operating protocol for RAPID

Recent Publications

1. Yasugi M, Nakamura S, Daidoji T, Kawashita N, Ramadhany R, Yang CS, Yasunaga T, Iida T, Horii T, Ikuta K, Takahashi K, Nakaya T. Frequency of D222G and Q223R Hemagglutinin Mutants of Pandemic (H1N1) 2009 Influenza Virus in Japan between 2009 and 2010. ***PLoS One***. 2012;7(2):e30946.
2. 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;2:228.
3. Nakamura S, Nakaya T, Iida T. Metagenomic analysis of bacterial infections by means of high-throughput DNA sequencing. ***Exp Biol Med (Maywood)***. 2011;236(8):968-71.
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. Nakamura S, Maeda N, Miron IM, Yoh M, Izutsu K, Kataoka C, Honda T, Yasunaga T, Nakaya T, Kawai J, Hayashizaki Y, Horii T, Iida T. Metagenomic diagnosis of bacterial infections. ***Emerg Infect Dis***. 2008 Nov;14 (11):1784-6.

Laboratory for Clinical Research on Infectious Diseases

Research Group

Invited Professor	Kazunori Oishi M.D., Ph.D.
SA Associate Professor	Yukihiro Akeda Ph.D.
SA Researcher	Tatsuya Nakayama Ph.D.
SA Researcher	Zhenyu Piao Ph.D.
SA Researcher	Dan Takeuchi M.D., Ph.D.

The research activities in our group include the diagnosis, pathogenesis, and prevention of invasive pneumococcal diseases (IPD). We also seek to investigate the epidemiology and pathogenesis of *Streptococcus suis* infection, the pathogenesis of thrombocytopenia in dengue illness, and the mechanism of protein secretion systems of pathogenic bacteria. In addition, our group is registered as a member of the World Health Organization (WHO)/Global Outbreak Alert & Response Network (GOARN) and will join the outbreak response team in the global effort to control emerging and re-emerging infectious diseases.

1) Diagnosis, pathogenesis, and prevention of IPD

1. IPD and pneumococcal pneumonia in children and adults

We reported the effects of 23-valent pneumococcal polysaccharide vaccine (PPV) on the incidence and medical cost for all causes of pneumonia among elderly people receiving a routine influenza vaccine. We are currently conducting a prospective study of the measurement of IgG and opsonophagocytic activity to the infecting serotype in sera of pediatric patients with IPD. In particular, we are analyzing the pathogenesis of IPD before and after vaccination with 7-valent conjugate vaccine. We are also conducting a prospective, multicenter study to determine the infecting serotype by PCR using clinical specimens and the protective threshold concentration of pneumococcal antibody in adult patients with IPD and pneumococcal pneumonia.

2. Development of a pneumococcal surface protein A (PspA) vaccine

PspA can elicit protective antibodies in animals. We reported the protective effects of PspA nasal vaccine in a mouse model of secondary pneumonia after influenza virus infection. We aim to develop a cross-protective PspA vaccine.

2) Research on Streptococcus suis infections prevalent in Thailand

Streptococcus suis, an important zoonotic pathogen, causes invasive infections, such as meningitis, in humans. In a retrospective study of S. *suis* infection in 2006–2008 in Thailand, we reported that human cases were prevalent in the northern region. The causative pathogens were serotype 2 for 165 cases (92.2%) and serotype 14 for 12 cases (6.7%) (Emerg Infect Dis 2011). One case each of previously unreported serotypes 5 and 24 were confirmed in patients with liver cirrhosis (Lancet 2011). Analysis of serotype 2 infection demonstrated that 58.9% of 158 patients were associated with meningitis and the others were associated with sepsis. The sequence type (ST)1 and ST104 strains were found to be capable of causing sepsis, but only the ST1 strain commonly caused meningitis (Figure 1). In a population-based study in Phayao Province in 2010, 31 human cases were confirmed, with a case fatality of 16.1%. The estimated incidence rate was 6.2 per 100,000 in the general population (PLoS ONE, 2012). Consumption of raw pork products was confirmed in 22 cases. The incubation period was 2 days after the consumption of raw pork products. We are aiming at eliminating this disease from northern Thailand by educating people about the risks of eating raw pork dishes.

3) Mechanisms by which dengue viral infections lead to thrombocytopenia

Dengue illness is a major public health concern, particularly in tropical countries, and the mechanism of thrombocytopenia remains uncertain. Based on a clinical study in the Philippines, we reported a significant association between thrombocytopenia and increased phagocytosis of platelets in an ex vivo setting during the acute phase of secondary dengue virus infection. We recently reported increased phagocytosis of DV-induced apoptotic platelets by macrophages via a PS-recognizing pathway in secondary DV infection (Figure 2). Accelerated platelet clearance was overcome by thrombopoietin-induced enhanced thrombopoiesis in these patients (J Infect Dis, 2012).

4) Protein secretion systems of pathogenic bacteria

The development of bacterial infections requires many virulence factors. Because most such factors are proteins secreted from

pathogenic bacteria, it is essential to study the mechanisms of protein secretion systems in pathogenic bacteria during the infection process. In our laboratory, a food-borne pathogen, *Vibrio parahaemolyticus*, and a causative agent of pneumonia, *Streptococcus pneumoniae*, are used to study their protein secretion systems and secreted virulence factors. We recently identified a novel chaperone responsible for virulence factor secretion (FEMS Microbiol 2011). We have also been investigating the involvement of secretion system-associated ATPase on the protein secretion mechanism.

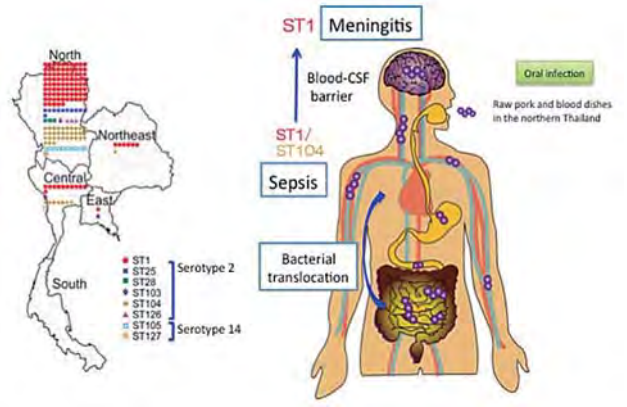


Figure 1. *Streptococcus suis* infection in Thailand: distribution and pathogenesis (Emerg Infect Dis 2011).

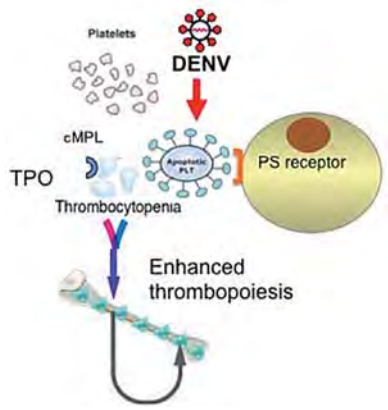


Figure 2. Thrombocytopenia and apoptotic platelet clearance in dengue (J Infect Dis, 2012).

Recent Publications

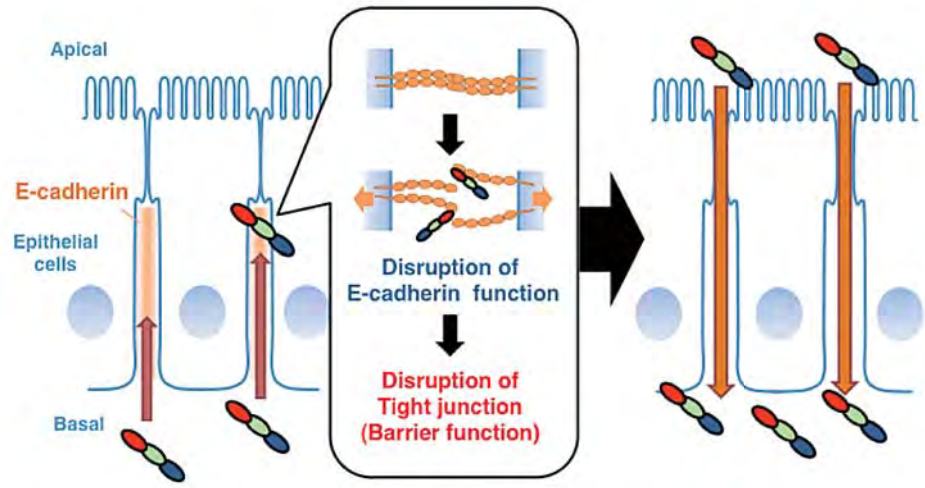
1. Alonzo MTG, Lacuesta TLV, Dimaano EM, Kurosu T, Suarez LC, Mapua CA, Akeda Y, Matias RR, Kuter DJ, Nagata S, Natividad FF, Oishi K. Platelet apoptosis and apoptotic platelet clearance by macrophages in secondary dengue virus Infections. ***J Infect Dis*** 2012 Apr;205(8):1321-9.
2. Takeuchi D, Kerdsin A, Pienpringam A, Loetthong P, Samerchea S, Pakkinee Loetthong P, Khamisra K, Wongwan N, Areeratana P, Chiranairadul P, Lertchayanti S, Petcharat S, Yowang A, Chaiwongsaen P, Nakayama T, Yukihiro 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.
3. Kerdsin A, Dejsirilert S, Sawanpanyalert P, Boonnark A, Noithachang W, Sriyakun 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.
4. 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.
5. Akeda, Y, Kodama T, Saito K, Iida T, Oishi, K, Honda T. Identification of the *Vibrio parahaemolyticus* Type III secretion system 2-associated chaperone VocC for the T3SS2-specific effector VopC. ***FEMS Microbiol Lett*** 2011 Nov; 324(2):156-64.

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 are able to damage the host severely, even at very low concentrations. Most bacterial toxins are enzymes that act catalytically and with high specificity on the 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 machinery of membrane trafficking and the functions of intracellular organelles. Studies that elucidate toxin trafficking could provide valuable information about basic cellular function, aid our understanding of the pathology induced by these toxins, and help in the development of effective therapeutic strategies against them. Our laboratory group is studying the transport mechanisms of the botulinum neurotoxin complex, which must pass through 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. ① Apically located 16S toxin is transcytosed. ② Once located in the basolateral compartment, HA of the 16S toxin binds to E-cadherin, and thereby disrupts the paracellular barrier. ③ A large amount of toxin accumulates in the basolateral area via paracellular movement.

Recent Publications

1. 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. [総説]
2. 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
3. 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.
4. Matsumura T, Jin Y, Kabumoto Y, Takegahara Y, Oguma K, Lencer WI, Fujinaga Y. The HA proteins of botulinum toxin disrupt intestinal epithelial intercellular junctions to increase toxin absorption. ***Cell Microbiol***. 2008; 10(2): 355-364.
5. Matsumura T[1], Fujinaga Y[1], Jin Y, Kabumoto Y, Oguma K. Human milk SIgA binds to botulinum type B 16S toxin and limits toxin adherence on T84 cells. ***Biochem Biophys Res Commun***. 2007; 352(4), 867-872.

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 likely serve as a scaffold for the assembly of replication machinery by providing an organization and environment facilitating viral propagation. These structures also serve as shells that protect the viruses against various cellular stress responses and allow persistent viral replication in the cytoplasm.

Our group has focused 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 successfully purified replication complexes from cells infected with hepatitis C virus or flavivirus, and performed quantitative mass spectrometry analyses. In these studies, we identified several cellular factors that were specifically recruited to 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.

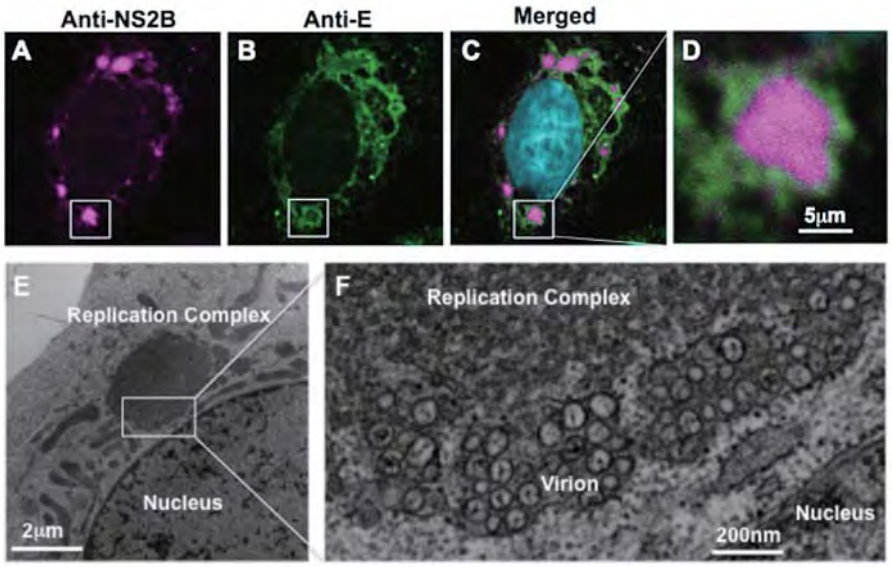


Figure: Fluorescent (A-D) and electron (E and F) microscopic images of viral replication complexes in flavivirus-infected Vero cells. At 24 hours after infection, the cells were fixed and stained with antibodies against viral nonstructural (anti-NS2B, magenta), structural (anti-E, green), and nuclear (DAPI, cyan) proteins. Unusual membrane structures, 5 to 10 nm in diameter and positive for viral antigens, were 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 the lumen.

Recent Publications

1. 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.
2. Matsunaga, K., Morita, E., Saitoh, T., Akira, S., Ktistakis, NT., Izumi, T., Noda, T., Yoshimori, T. Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. ***J Cell Biol***. 2010 190(4):511-21.
3. 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.
4. 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.
5. Kieffer, C., Skalicky JJ., Morita, E., De Domenico, I., Ward, DM., Kaplan, J., Sundquist, WI. A New Functionally Important ESCRT-III Recognition Mode for the VPS4 ATPases. Dev. ***Cell*** 2008, 15(1):62-73.

Laboratory of Genomic Research on Pathogenic Bacteria

Research Group

SA Professor	Tetsuya Iida, Ph.D.
SA Researcher	Shigeaki Matsuda, Ph.D.
SA Researcher	Hirotaka Hiyoshi, Ph.D.

This research group is studying pathogenic bacteria from the genomic perspective. 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 use DNA microarrays and other molecular methods to investigate changes in the genome expression pattern of various bacterial pathogens that occur during their interactions with the 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 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
On the basis of what is currently understood about various bacterial pathogens, we seek 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 diagnose bacterial infections rapidly, we are developing a novel system for identifying bacterial pathogens by high-throughput DNA sequencing.

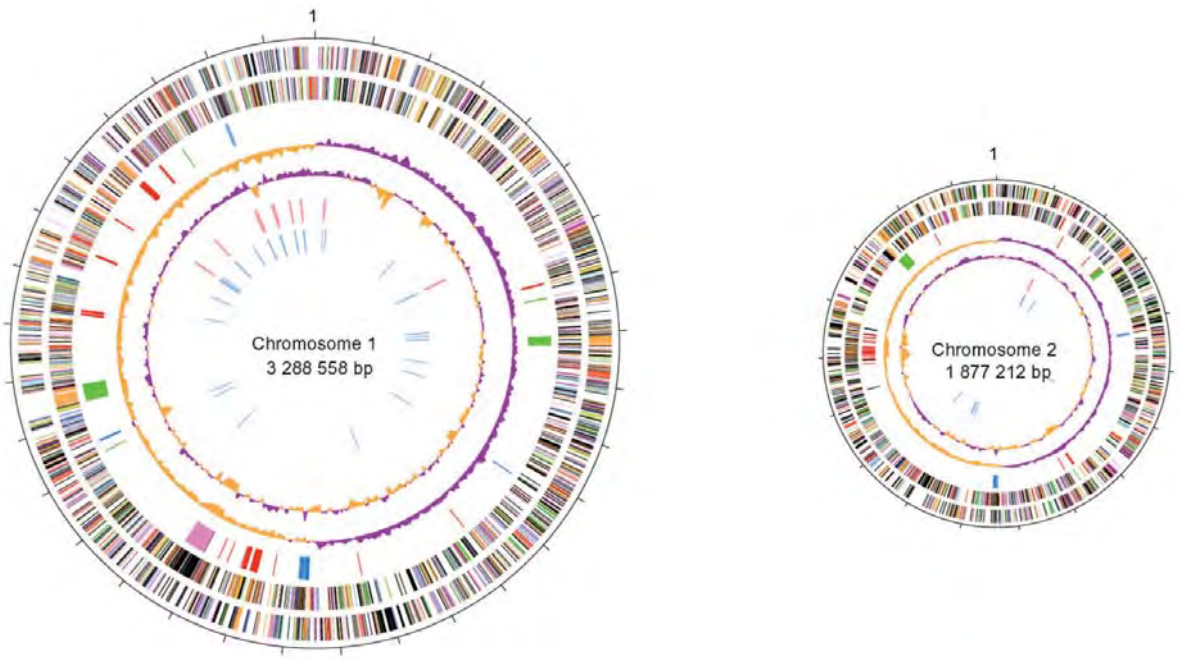


Figure 1. Whole genome sequence of *Vibrio parahaemolyticus*

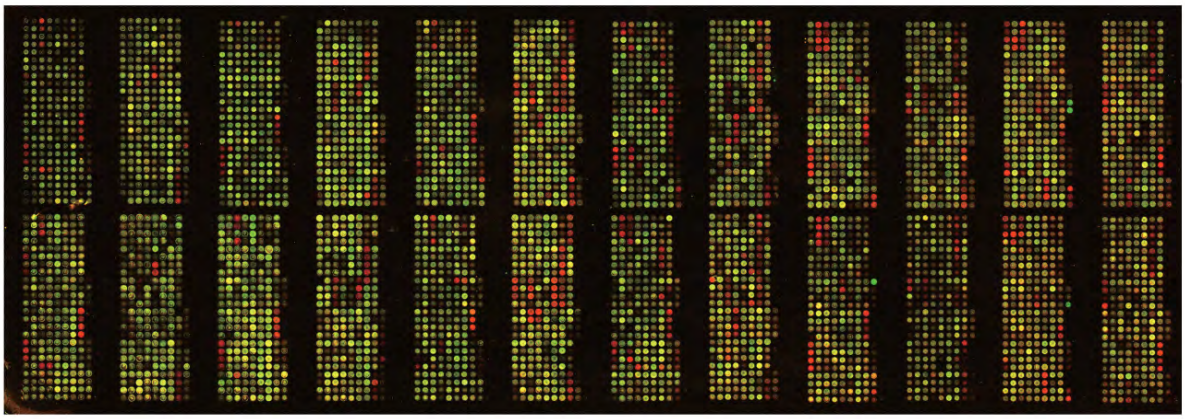


Figure 2. Characterization and comparison of the genomes of pathogenic bacteria by using DNA microarrays.

Recent Publications

1. 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.
2. 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.
3. Matsuda S, Kodama T, Okada N, Okayama K, Honda T, Iida T. Association of *Vibrio parahaemolyticus* thermostable direct hemolysin with lipid rafts is essential for cytotoxicity but not hemolytic activity. ***Infect Immun***. 2010 Feb;78(2):603-10.
4. Dryselius R, Izutsu K, Honda T, Iida T. Differential replication dynamics for large and small *Vibrio* chromosomes affect gene dosage, expression and location. ***BMC Genomics***. 2008 Nov 26;9:559.
5. Nakamura S, Maeda N, Miron IM, Yoh M, Izutsu K, Kataoka C, Honda T, Yasunaga T, Nakaya T, Kawai J, Hayashizaki Y, Horii T, Iida T. Metagenomic diagnosis of bacterial infections. ***Emerg Infect Dis***. 2008 Nov;14 (11):1784-6.

Laboratory of Combined Research on Microbiology and Immunology

Research Group

Associate Professor	Hiroki Nagai, Ph.D.
SA Associate Professor	Tomoko Kubori, Ph.D.
Postdoctoral Fellow	Andree Marie Hubber, Ph.D.
SA Researcher	Akiko Okura

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* subvert 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 from Legionella.

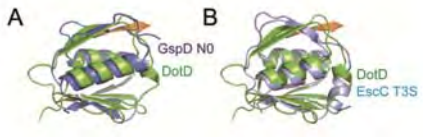


Fig. 1. T4SS core component DotD represents a conserved structural motif of the periplasmic ring.

(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.

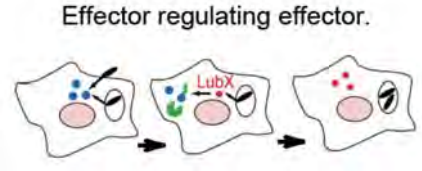


Fig. 2 Discovery of metaeffector.

Recent Publications

1. Tomoko Kubori and Hiroki Nagai. Bacterial effector-involved temporal and spatial regulation by hijack of the host ubiquitin pathway. ***Front. Microbiol***., 2011:2,145.
2. Hiroki Nagai and Tomoko Kubori. Dot/Icm type IVB secretion systems of Legionella and other gram-negative bacteria. ***Front. Microbiol***.,2011: 2,136.
3. Kubori T, Shinzawa N, Kanuka H, Nagai H. *Legionella* metaeffector exploits host proteasome to temporally regulate cognate effector. ***PLoS Pathog***. 2010;6(12),e1001216.
4. 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.
5. Kubori, T., Hyakutake, A. and Nagai, H. *Legionella* translocates an E3 ubiquitin ligase that has multiple U-boxes with distinct functions. ***Mol. Microbiol***. 2008;67(6),1307-1319.

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-premier institutes for microbiology and immunology, respectively. Taking maximal advantage of the proximity of these centers, we propose research in the combined fields of microbiology and immunology.

Research promotion

To promote combined research in microbiology and immunology, we are carrying out the following plans:

1. The Awaji international forum on infection and immunology is organized and held annually each September.
2. A research progress report is organized and held every month within the institute.
3. Large research presentations and competitions are organized and held annually within the institute.
4. Collaborative projects are organized with Institut Pasteur in France, Chonnam University in Korea, and the Research Collaboration Center on Emerging and Re-emerging Infections in Thailand. These activities facilitate research on microbiology and immunology by promoting collaboration, information exchange, and personal exchange between laboratories and by facilitating a research atmosphere.

Education promotion

To facilitate seamless research on microbiology and immunology, we direct a multidisciplinary graduate program on microbiology and immunology, for which we design the curriculum and contents. We also organize an Open House of the institute and provide guidance to new students. We organize a combined program on clinical microbiology and immunology in close cooperation with the Thailand-Japan Research Collaboration Center on Emerging and Re-emerging Infections.

Research group	Associate Professor	Yoshiko Murakami, MD. PhD.

Research projects

Dr. Murakami has an additional appointment in the Department of Immunoregulation as the leader of the paroxysmal nocturnal hemoglobinuria (PNH) group (see details on the Department page). The research projects for this group include:

1. Investigation of the pathogenesis of PNH;
2. Investigation of the pathogeneses of acquired and inherited glycosylphosphatidylinositol (GPI) deficiencies; and
3. Investigation of the functional significance of GPI-anchored proteins using the Pgap3 KO mouse in which GPI anchored proteins fail to localize to the raft due to defective GPI anchor remodeling.

Recent Publications

1. Murakami Y, Kanzawa N, Saito K, Krawitz PM, Mundlos S, Robinson PN, Karadimitris A, Maeda Y, Kinoshita T. Mechanism for release of alkaline phosphatase caused by glycosylphosphatidylinositol deficiency in patients with hyperphosphatasia mental retardation syndrome. ***J Biol Chem***. 2012 Feb 24;287(9):6318-25.
2. Murakami Y, Inoue N, Shichishima T, Ohta R, Noji H, Maeda Y, Nishimura J, Kanakura Y, Kinoshita T. Deregulated expression of HMGA2 is implicated in clonal expansion of PIGA deficient cells in paroxysmal nocturnal haemoglobinuria. ***Br J Haematol***. 2012 Feb;156(3):383-7.
3. Murakami H, Wang Y, Hasuwa H, Maeda Y, Kinoshita T, Murakami Y. Enhanced response of T lymphocytes from Pgap3 knockout mouse: Insight into roles of fatty acid remodeling of GPI anchored proteins. ***Biochem Biophys Res Commun***. 2012 Jan 27;417(4):1235-41.
4. Krawitz PM, Schweiger MR, Rödelsperger C, Marcelis C, Kölsch U, Meisel C, Stephani F, Kinoshita T, Murakami Y, Bauer S, Isau M, Fischer A, Dahl A, Kerick M, Hecht J, Köhler S, Jäger M, Grünhagen J, de Condor BJ, Doelken S, Brunner HG, Meinecke P, Passarge E, Thompson MD, Cole DE, Horn D, Roscioli T, Mundlos S, Robinson PN. Identity-by-descent filtering of exome sequence data identifies PIGV mutations in hyperphosphatasia mental retardation syndrome. ***Nat Genet***. 2010 Oct;42(10):827-9.
5. Almeida AM*, Murakami Y*, Baker A, Maeda Y, Roberts IA, Kinoshita T, Layton DM, Karadimitris A. Targeted therapy for inherited GPI deficiency. ***N Engl J Med***. 2007 Apr 19;356(16):1641-7(* equally contributed).

Fujii Group

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

Locus-specific biochemical epigenetics

Comprehensive understanding of mechanisms of epigenetic regulation requires identification of molecules bound to genomic regions of interest in vivo. However, non-biased methods to identify molecules bound to specific genomic loci in vivo are limited. To perform biochemical and molecular biological analyses of specific genomic regions, we developed the insertional chromatin immunoprecipitation (iChIP) technology to purify the genomic regions of interest.

The scheme of iChIP is as follows:
(i) A repeat of the recognition sequence of an exogenous DNA-binding protein such as LexA is inserted into the genomic region of interest in the cell 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) to be expressed in the cell to be analyzed.
(iii) The resultant cell is stimulated, if necessary, and crosslinked with formaldehyde or other crosslinkers.
(iv) The cell is lysed, and crosslinked DNA is fragmented by sonication.
(v) The complexes including the exogenous DNA DB are immunoprecipitated with an antibody against the tag.
(vi) The isolated complexes retain molecules interacting with the genomic region of interest. Reverse crosslinking and purification of DNA, RNA, proteins, or other molecules allows their identification and characterization.

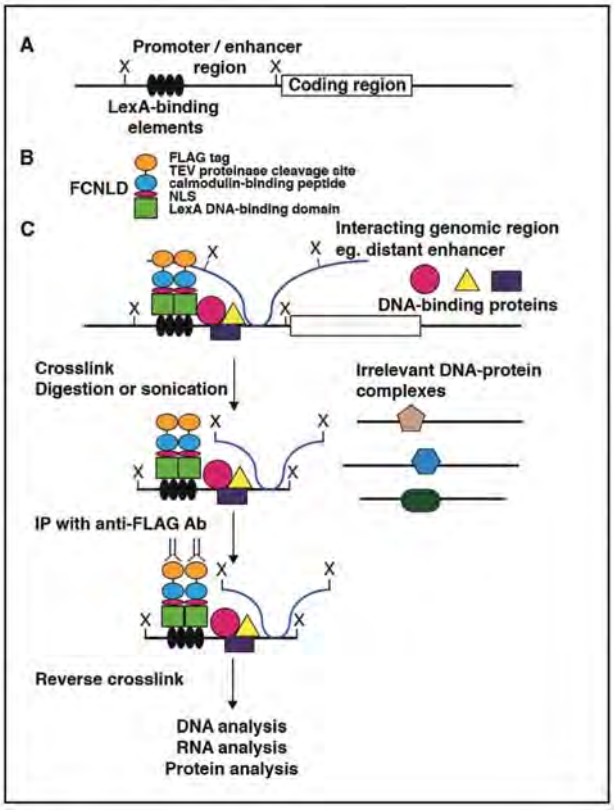


Figure. Scheme of insertional chromatin immunoprecipitation (iChIP)

We applied iChIP to direct identification of components of insulator complexes, which function as boundaries of the chromatin domain. We showed that it is feasible to directly identify proteins and RNA bound to a specific genomic region in vivo by using iChIP. We will apply iChIP to elucidation of molecular mechanisms of important epigenetic phenomena including:
(a) Lineage commitment of lymphocytes,
(b) Expression regulation of odorant receptors,
(c) Insulator function,
(d) Detection and repair of DNA double-strand breaks, and
(e) Epigenetic suppression of expression of tumor suppressor genes.

Recent Publications

1. Fujita T, Fujii H. Direct identification of insulator components by insertional chromatin immunoprecipitation. ***PLoS One***. 2011;6(10):e26109.
2. Hoshino A, Fujii H. Insertional chromatin immunoprecipitation: a method for isolating specific genomic regions. J ***Biosci Bioeng***. 2009 Nov;108(5):446-9.
3. Saint Fleur S, Hoshino A, Kondo K, Egawa T, Fujii H. Regulation of Fas-mediated immune homeostasis by an activation-induced protein, Cyclon. ***Blood***. 2009 Aug 13;114(7):1355-65.
4. Singh AP, Buscaglia CA, Wang Q, Levay A, Nussenzweig DR, Walker J, Winzeler EA, Fujii H, Fonoura BMA, Nussenzweig, V. Plasmodium circumsporozoite protein promotes the development of the liver stages of the parasite. ***Cell***. 2007 Nov 2;131(3):492-504.
5. Hoshino A, Hirst JA, Fujii H. Regulation of cell proliferation by interleukin-3-induced nuclear translocation of pyruvate kinase. ***J Biol Chem***. 2007 Jun 15;282(24):17706-11.

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

Director SA Professor Naokazu Takeda, Ph.D.

Until recently, it was believed that infectious diseases could be conquered through the development of chemotherapies and vaccines; however, the recent worldwide emergence of new infectious diseases and re-emergence of infectious diseases that were thought 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. Because many infectious diseases can spread rapidly across national borders, these diseases clearly 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) within the Thai National Institute of Health (NIH), Department of Medical Sciences of the Ministry of Public Health of Thailand in 2005. The second phase of the program (2010-2014), which is 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 m2 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 researchers at the NIH, while simultaneously 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. We aim to establish an effective system that would (i) provide information to prevent the emergence of emerging and re-emerging infections, and (ii) promptly activate various countermeasures, should such a disease emerge, including developing therapeutics or vaccines. Finally, we wish to begin collaborations with laboratories from nations neighboring Thailand so that we can be at the frontline with the capacity to respond quickly 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 Associate Professor	Yumi Kumagai, Ph.D.
SA Assistant Professor	Kazuhisa Okada, Ph.D.
SA Researcher	Chetsada Boonthimat, M.Sc.
SA Researcher	Wirongrong Natakuathung, M.Sc.

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

Pneumonia, tuberculosis, and acute diarrheal diseases are associated with high morbidity and mortality rates in Thailand. Consequently, during the 2010–2014 fiscal years, the Program for the Promotion of Research Networks for Emerging and Re-emerging Infectious Diseases prioritized research on enteric infectious diseases in Thailand that are caused by *Vibirio cholerae*.

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. These diseases have mainly been observed in Asian countries, including Northern Thailand. We will examine this emerging zoonotic infection closely to elucidate the molecular pathogenesis of *S. suis* infections.

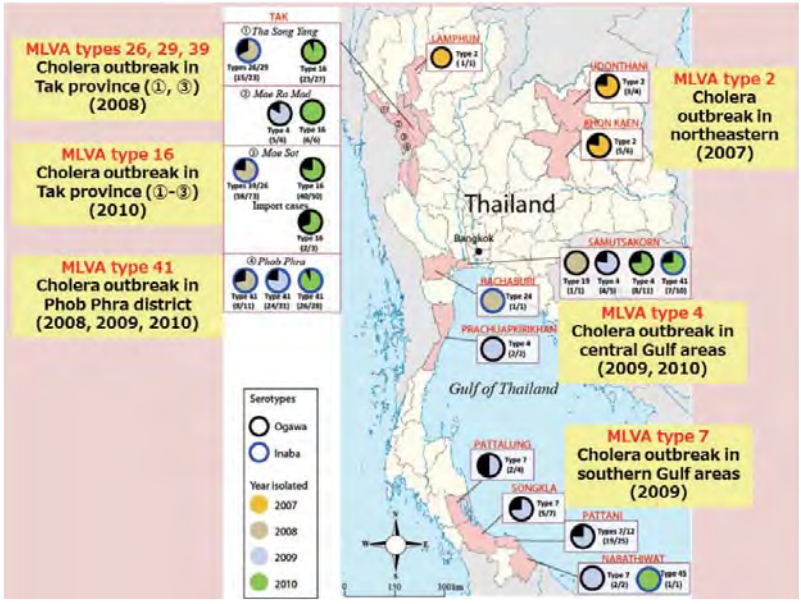


Figure 1. Geographical spread and temporal changes of *Vibrio cholerae* O1 during the 2007–2010 cholera outbreaks in Thailand. Yellow squares indicate the major MLVA type(s) in each outbreak site shown in the map.

Recent Publications

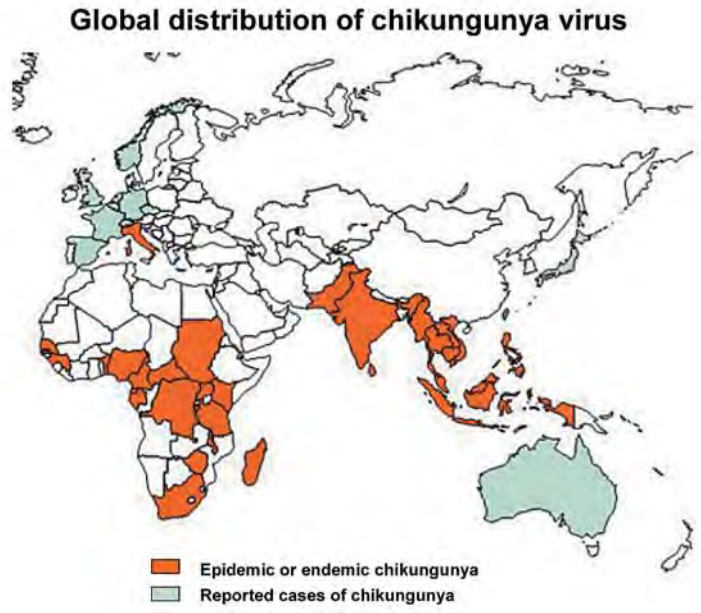
1. Okada K, Roobthaisong A, Nakagawa I, Hamada S, Chantaroj S. Genotypic and PFGE/MLVA analyses of Vibrio cholerae O1: geographical spread and temporal changes of isolates during the 2007-2010 cholera outbreaks in Thailand. ***PLoS One***. 2012 7(1):e30863.
2. Takeuchi D, Kerdsin A, Pienpringam A, Loetthong 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.
3. Okada K, Chantaroj S, Roobthaisong A, Hamada S, Sawanpanyalert P. A Cholera Outbreak of the *Vibrio cholerae* O1 El Tor Variant Carrying Classical CtxB in Northeastern Thailand in 2007. ***Am J Trop Med Hyg***. 2010 82 (5):875-878.
4. Puiprom O, Chantaroj S, Gangnonngiw W, Okada K, Honda T, Taniguchi T, Sawanpanyalert P. Identification of colonization factors of enterotoxigenic *Escherichia coli* with PCR-based technique. ***Epidemiol Infect***. 2010 138 (4):519-524.
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):135-139.

Section of Viral Infections

Research Group

SA Professor	Naokazu Takeda, Ph.D.
Postdoctoral Fellow	Natsuko Kishishita, Ph.D.
Postdoctoral Fellow	Uamporn Siripanyaphinyo, Ph.D.
Postdoctoral Fellow	Sompong Sapsutthipas, Ph.D.
Postdoctoral Fellow	Nitchakarn Noranate, Ph.D.
Research Fellow	Piraporn Utachee, M.Sc.
Research Fellow	Chris Verathamjamras, M.Sc.
Research Fellow	Uranan Tumkosit, M.Sc.

The Section of Viral Infections focuses on two emerging and re-emerging viral diseases that are prevalent in Asian countries, including Thailand, in collaboration with the National Institute of Health, Department of Medical Sciences of the Ministry of Public Health of Thailand. One subset of these diseases includes mosquito-borne infectious diseases and chikungunya fever, which we study from their epidemiological, molecular biological, and immunological aspects. The other subset of diseases includes blood-borne infectious diseases (HIV diseases/AIDS). We perform virological and immunological characterizations of the HIV-1 CRF01_AE strains prevalent in Southeast Asian countries, including Thailand. In addition, studies to elucidate the mechanism of anti-retroviral drug resistance are in progress.



Recent Publications

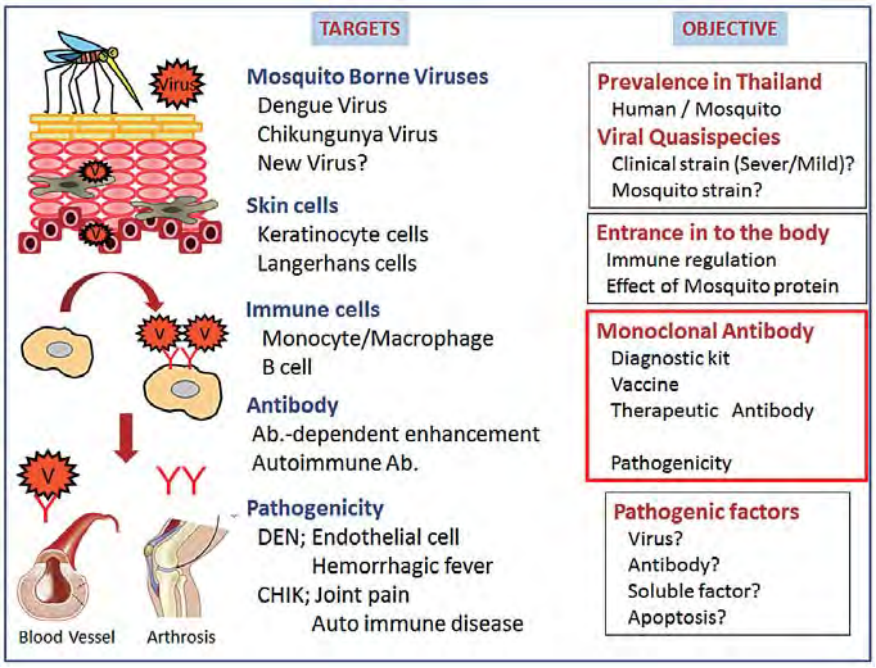
1. Boonchawalit S, Jullaksorn D, Uttiyoung J, Yowang A, Krathong N, Chautrakul S, Yamashita A, Ikuta K, Roobsoong A, Kanitvittaya S, Sawanpanyalert P, Kameoka M. Molecular evolution of HIV-1 CRF01_AE Env in Thai patients. ***PLoS One***.2011 6: e27098.
2. Utachee P, Nakamura S, Isarangkura-Na-Ayuthaya P, Tokunaga K, Sawanpanyalert P, Ikuta K, Auwanit W, Kameoka M. 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. ***J Virol***. 2010 84: 4311-20.
3. Soonthornsata B, Tian YS, Utachee P, Sapsutthipas S, Isarangkura-na-Ayuthaya P, Auwanit W, Takagi T, Ikuta K, Sawanpanyalert P, Kawashita N, Kameoka M. Design and evaluation of antiretroviral peptides corresponding to the C-terminal heptad repeat region (C-HR) of human immunodeficiency virus type 1 envelope glycoprotein gp41. ***Virology*** 2010 405: 157-64.
4. Li YG, Chittaganpitch M, Waicharoen S, Kanai Y, Bai GR, Kameoka M, Takeda N, Ikuta K, Sawanpanyalert P. Characterization of H5N1 influenza viruses isolated from humans in vitro. ***Virol J***. 2010 7: 112.
5. Kanai Y, Boonsathorn N, Chittaganpitch M, Bai G, Li Y, Kase T, Takahashi K, Okuno Y, Jampangern W, Ikuta K, Sawanpanyalert P. The impact of antigenic drift of influenza A virus on human herd immunity: Sero-epidemiological study of H1N1 in healthy Thai population in 2009. ***Vaccine*** 2010 28: 5437-44.

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	Mikiko Sasayama, Ph.D.
Postdoctoral Fellow	Promsin Masrinoul, Ph.D.
Research Fellow	Orapim Puiprom, M.Sc.
Research Fellow	Panjaporn Chaichana, M.Sc.

The interest of MOCID focuses on several tropical infectious diseases that are of human health importance in Thailand, particularly 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 viral diseases. We also study the prevalence of viral infections in human and mosquitoes and the pathogeneses of the viruses. We would like to raise young scientists' interests and research skills on infectious diseases through clinical collaborations with Mahidol University.



Our main research projects include:
1) Epidemiology of mosquito-borne viral infections in humans and mosquitoes in Thailand;
2) Generation and characterization of human and mouse monoclonal antibodies against dengue virus and chikungunya virus; and
3) Development of diagnosis kits for viral diseases.

Recent Publications

1. Co-existence of major and minor viral populations from two different origins in patients secondarily infected with dengue virus serotype 2 in Bangkok. Puiprom O, Yamashita A, Sasayama M, Limkittikul K, Boonha K, Jittmitraphap A, Leaungwutiwong P, Kurosu T, Ramasoota P, Ikuta K. ***Biochem Biopys Res Commun***. 2011, 143: 136-142.
2. Fab MAbs specific to HA of influenza virus with H5N1 neutralizing activity selected from immunized chicken phage library. Pitaksajjakul P, Lekcharoensuk P, Uparagarin N, Barbas C, Ibrahim MS, Ikuta K, and Ramasoota P. ***Biochem Biopys Res Commun***. 2010, 395: 496-501.

BIKEN Endowed Department of Dengue Vaccine Development

Research Group

Endowed Chair Professor Ph.D.	Eiji Konishi
Endowed Assistant Professor Ph.D.	Atsushi Yamanaka
Specialized Research Affiliate Ph.D.	Miwa Kuwahara

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.

Recent publications

Recent Publications

1. Eiji Konishi, Yuko Miyagawa: Balance of infection-enhancing and neutralizing antibodies induced by a dengue tetravalent DNA vaccine in a mouse model. ***Microbes and Infection***. 13(12-13):1091-8, 2011
2. Eiji Konishi, Mayu Konishi: Nonstructural Protein 1 Antibody-Based Epitope-Blocking Enzyme-Linked Immunosorbent Assay to Differentiate Japanese Encephalitis Virus from Dengue Virus Infections in Humans. ***Jpn J Infect Dis***. 64(4):284-91, 2011
3. Atsushi Yamanaka, Kris C. Mulyatno, Helen Susilowati, Eryk Hendrianto, Amor P. Ginting, Dian D. Sary, Fedik A. Rantam, Soegeng Soegijanto, Eiji Konishi: Displacement of the Predominant Dengue Virus from Type 2 to Type 1 with a Subsequent Genotype Shift from IV to I in Surabaya, Indonesia 2008-2010. ***PLoS One***. 2011;6(11):e27322. Epub 2011 Nov 7.
4. Kris C. Mulyatno, Atsushi Yamanaka, Ngadino, Eiji Konishi: Resistence of Aedes aegypti laevae to temephos insecticide in Surabaya, Indonesia. ***Southeast Asian J Trop Med Public Health***. 43:29-33. (2012).
5. Kris C. Mulyatno, Helen Susilowati, Atsushi Yamanaka, Soegeng Soegijanto, Eiji Konishi: First isolation and phylogeny of Chikungunya virus from Surabaya, Indonesia. ***Jpn J Infect Dis***. 65, 92-94 (2012).

Frontier Biomedical Science Underlying Organelle Network Biology

Research program

Our main goal is to create an interdisciplinary research center, which will coordinate work in cell biology, microbiology/immunology, and glycobiology in order to converge on a greater knowledge of the organellar network.
The subjects under study will range widely:

- modes of communication between organelles
- interactions of pathogens with the organelle network
- the roles of glycosylation in determining organelle function
- the effects of abnormal glycosylation on disease

By combining these fundamental studies with clinical research, we will drive the creation and development of the new field of organelle network medicine. To this end, we will conduct research focused on achieving an integrated understanding of human disease, and on developing technological solutions to clinical problems.
On the road to these goals, we will ask clinically relevant questions, such as:

- What is the mechanism leading from invasion by a pathogen to establishment of a full-fledged infection?
- How might we interfere in the interactions between pathogens and the organelle network?
- How does disruption of organelle network result in neurodegenerative diseases? To what extent does ER quality control play a role?
- Can we exploit changes in protein and sugar chain modifications to develop novel diagnostic tools?

Diseases are not merely the consequences of single causes or single gene mutations. We recognize that diseases are multifactorial conditions, in which many genes and environmental factors intertwine and interact in a complex way. Based on this recognition, our Center will encourage biological and clinical research that is committed to understanding disease at the level of both the molecular and organelle networks.
Ultimately, our greater understanding of the organelle network, and of clinically important issues in organelle biology, will allow us to develop novel therapeutic strategies that accelerate the medicine of the 21st century.

Education program

Young scientists will someday become the biologists and clinical researchers of the future. Therefore, we are committed to establishing a world-class training environment in which junior scientists are fully supported and actively trained to take global leadership roles in 21st century science.
Our training program has many aspects, all focused on training the future leaders of the new field of organelle network medicine:

- **Researcher development program** - Through a series of courses taught by domestic and international academics and industrialists, young scientists will improve their management capabilities, technical writing, English proficiency and grant application skills.
- **Interdisciplinary graduate curriculum** - Investigators will have the opportunity to study across multiple university schools, including the Schools of Medicine, Pharmaceutical Sciences, Dental Sciences, Science and Frontier Biosciences, and the Research Institute for Microbial Disease.
- **Support for interdisciplinary projects** - We will support research that breaks through barriers between departments and fields, and encourage young researchers to be creative and flexible in their work.
- **Meetings for international young investigators** - Our young researchers will themselves organize meetings, held in a "training camp" format, to facilitate bottom-up international exchanges.
- **Construction of an international network** - We will have a special staff devoted to the task of expanding cooperation with overseas centers
- **Securing career paths** - We will provide Ph.D.-level researchers with space and funding, in order to help move them along the road to independence. After completion of graduate training, we will cooperate with other research centers to help our alumni achieve tenured positions.
- **Research assistants (RAs)** - RA positions will be available in order to provide financial assistance to graduate students. Our students will also be actively encouraged and well supported to attend international meetings.
- **Overseas practical training program** - In order to advance clinical training, we will create practical courses using overseas centers.
- **Graduate student exchange** - Providing opportunities to study abroad.

We hope to create an environment where traditional barriers between departments and fields essentially don't exist - where young scholars can quickly and efficiently obtain advice from researchers in different fields.
Major changes like this don't happen by themselves - which is why our faculty will include a specially appointed associate professor whose primary responsibility is the cultivation of our collaborative networks with other institutions.

Members

Name Affiliated department, Position title, Specialized field, Academic degree	Division of roles
Yoshihiro Yoneda Graduate School of Frontier Biosciences (Department of Frontier Biosciences), Professor, Cell Biology, M.D., Ph.D.	Coordination of a center establishment and elucidation of the organelle network
Tatsushi Toda Graduate School of Medicine (Division of Medicine), Visiting Professor, Mol.Genetics & Neurology, M.D., Ph.D.	Sugar chain modifications and neurodegeneration diseases
Yoshihide Tsujimoto Graduate School of Medicine (Division of Medicine), Professor, Medical Genetics, Ph.D.	Mechanism of cell death and organelle
Kiyoshi Takeda Graduate School of Medicine (Division of Medicine), Professor, Immunology, M.D., Ph.D.	Analysis of activity regulation mechanism of the natural immunity system
Kazunori Tomono Graduate School of Medicine (Division of Medicine), Professor, Clinical Microbiology, M.D., Ph.D.	Analysis of antibacterial activity and development of new antimicrobial agents
Taiichi Katayama United Graduate School of Child Development (Department of Child Development), Professor, Neurochemistry & Cell Biology Ph.D.	Neurological function abnormalities and organella
Toshikazu Nakamura Office for University-Industry Collaboration, SA Professor, Biochemistry, Molecular Biology, Ph.D.	Treatment strategies against neurodegenerative and renal diseases with HGF
Tetsuo Takehara Graduate School of Medicine (Division of Medicine), Professor, Gastroenterology & Hepatology, M.D., Ph.D.	Hepatitis onset mechanism and treatment strategies
Eiji Miyoshi Graduate School of Medicine (Division of Health Sciences), Professor, Clinical and Laboratory Medicine, M.D., Ph.D.	Development of biomarkers using sugar chain technologies
Yoshinao Wada Graduate School of Medicine (Division of Medicine), Visiting Professor, Mass Spectrometry, M.D., Ph.D.	Development of sugar chain analysis methods
Yukari Fujimoto Graduate School of Science (Department of Chemistry), Associate Professor, Organic Chemistry, Ph.D.	Recognition system of bacteria with its glycoconjugate
Naoyuki Taniguchi RIKENCSystems Glycobiology Research Group), Group Director, Biochemistry, M.D., Ph.D.	Functional analyses of sugar chains/proteins
Tamotsu Yoshimori Graduate School of Frontier Biosciences (Department of Frontier Biosciences), Professor, Cell Biology, Ph.D.	Analyses of roles of membrane traffic in infection/immunity
Hitoshi Kikutani Research Institute for Microbial Diseases, Professor, Immunology, M.D., Ph.D.	Study on the dynamics of the acquired immunity
Eisuke Mekada Research Institute for Microbial Diseases, Professor, Cell Biology, Ph.D.	Analyses of factors related to toxicity manifestation of the diphtheria toxin
Yoshiharu Matsuura Research Institute for Microbial Diseases, Professor, Virology, Ph.D.	Infection mechanism of Hepatitis C virus and studies on the control methods
Tatsuo Shioda Research Institute for Microbial Diseases, Professor, Virology, Ph.D.	Studies on host factors related to HIV infection
Yasuhiko Horiguchi Research Institute for Microbial Diseases, Professor, Bacteriology, Ph.D.	Analyses of functions and structures of bacterial virulence factors
Kazuyoshi Ikuta Research Institute for Microbial Diseases, Professor, Virology, Ph.D.	Studying emerging viral infections and their pathogenesis
Toshihiro Horii Research Institute for Microbial Diseases, Professor, Parasitology, Ph.D.	Development of malaria vaccines and analyses of the host-parasite interactions
Shizuo Akira WPI Immunology Frontier Research Center, Professor, Immunology, M.D., Ph.D.	Studies on innate immunity
Taroh Kinoshita WPI Immunology Frontier Research Center, Professor, Immunology, Ph.D.	Analysis of significance of GPI anchor in the host-pathogen interactions
Hisashi Arase WPI Immunology Frontier Research Center, Professor, Immunology, M.D., Ph.D.	Studies on mechanism of immunoregulation by pathogens
Atsushi Kumanogoh Graduate School of Medicine (Division of Medicine), Professor, Immunology, M.D., Ph.D.	Studies of immunoregulation/regulatory molecules of cell migration
Tadashi Suzuki RIKEN (Systems Glycobiology Research Group), Team Leader, Biochemistry, D. Sc.	Quality controls of free sugar chains and glycoproteins
Yoshiki Yamaguchi RIKEN (Syetems Glycobiology Research Group), Team Leader, Structural Biology, D. Pharm.	Structural analyses of glycoconjugates by NMR

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, which occur within a distinct genomic region in germ cells.

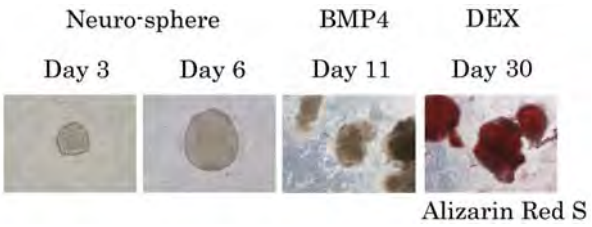


Figure legend. Neural crest cells derived from ES cells differentiated into osteoblasts.

(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 and their application to 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.

Animal Resource Center for Infectious Diseases

Research Group

Head, Professor	Masaru OKABE, Ph.D.
Associate Professor	Masahito IKAWA, Ph.D.
Assistant Professor	Ayako ISOTANI, Ph.D.
Assistant Professor (SUP)	Hidetoshi HASUWA, Ph.D.
Assistant Professor (SUP)	Naokazu INOUE, Ph.D.
SA Assistant Professor (SUP)	Yuhkoh SATOUH, 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. 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.

The Animal Resource Center for Infectious Diseases is a unique facility that was established in 1967 to meet these requirements. The center is separated into two major areas: one for animal experiments for microbial disease models (Biosafety Level 2 and 3: BSL2 and BSL3), and another is for SPF (Specific Pathogen Free) area. The section for animal experimentation for the disease models is completely air-conditioned and maintained at a negative air pressure to minimize the risk of contamination. Each subarea 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 available at our facility include the generation of gene-manipulated animals, in vitro fertilization, and cryopreservation of mouse strains (Table 1).

Figure 1: Biosafety level 3 room (Building A).
The room for disease model animals and experimentation at biosafety level 3. Hemorrhageic fever with renal syndrome-causing virus (HFRSV) was isolated in this area. Animal experiments for Creutzfeldt-Jakob Disease (CJD), severe acute respiratory syndrome (SARS), and Acquired Immune Deficiency Syndrome (AIDS) also 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
total	428	329	1471

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. Okuzaki D, Kimura S, Yabuta N, Oomine T, Nojima H. LeukoCatch, a quick and efficient tool for the preparation of leukocyte extracts from blood. ***BMC Clin Pathol***. 2011 Aug 17;11(1):9.
2. Okuzaki D, Fukushima T, Tougan T, Ishii T., Kobayashi S, Yoshizaki K, Akita T, and Nojima H. Genopal™: a novel hollow fiber array for focused microarray analysis. ***DNA Res***., 2010 Dec;17(6):369-79.
3. 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.
4. Kobayashi S, Ito A, Okuzaki D, Onda H, Yabuta N, Nagamori I, Suzuki K, Hashimoto H, Nojima H. Expression profiling of PBMC-based diagnostic gene markers isolated from vasculitis patients. ***DNA Res***. 2008 Aug;15 (4):253-65.
5. Tougan T, Onda H, Okuzaki D, Kobayashi S, Hashimoto H, Nojima H. Focused microarray analysis of peripheral mononuclear blood cells from Churg-Strauss syndrome patients. ***DNA Res***. 2008 Apr 30;15(2):103-14.

Center for genetic analysis of biological responses

Research Group

<Production laboratory for genetically manipulated animals>

Head, Professor	Masaru Okabe, Ph.D.
SA Associate Professor	Kazuo Yamagata, Ph.D.
Assistant Professor (SUP)	Hidetoshi Hasuwa, Ph.D.
SA Assistant Professor	Jun Ueda, Ph.D.

<Resource laboratory for genetically manipulated animals>

Visiting Professor	Kenichi Yamamura, M.D., Ph.D.
Associate Professor (SUP)	Masahito Ikawa, Ph.D.
Assistant Professor (SUP)	Ayako Isotani, Ph.D.

<Laboratory for promotion of collaborative research>

Visiting Professor	Yoichiro Iwakura, Ph.D.
Visiting Professor	Nobuaki Yoshida, M.D., Ph.D.
Assistant Professor (SUP)	Naohisa Goto, Ph.D.
SA Assistant Professor (SUP)	Yuhkoh Satouh, Ph.D.

<Laboratory for analysis of genetically manipulated animals>

Professor (SUP)	Shizuo Akira, M.D., Ph.D.
Professor (SUP)	Taroh Kinoshita, Ph.D.
Professor (SUP)	Hisashi Arase, M.D., Ph.D.
Professor (SUP)	Hitoshi Kikutani, M.D., Ph.D.
Professor (SUP)	Masato Okada, Ph.D.
Professor (SUP)	Nobuyuki Takakura, M.D., Ph.D.
Professor (SUP)	Hiroshi Nojima, Ph.D.
Assistant Professor (SUP)	Naokazu Inoue, Ph.D.

Our bodies are kept homeostatically stable through functions of proteins produced by many genes. In other words, our health is basically maintained in accordance with the balance of our gene products. Many diseases can be traced to a defect or malfunction in specific genes. To identify and develop new drugs or therapies, 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 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 and to screen new drugs. With this in mind, the preparation of gene-disrupted mouse lines of entire genes is planned and is progressing in the form of research projects on a national level in many countries. Gene-manipulated animals can also be valuable resources that are patent-protected and offer potential for the development of new drugs and therapeutic methods. It is extremely important for Japan to make a strong 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; and The Center for Animal Resources and Development, Kumamoto University), with headquarters at Kumamoto University. Within this collaborative consortium, we aim to produce many gene-manipulated animals that are closely focused on human diseases. At our center, we are mainly focused on genes related to reproduction, infection, and allergy, taking advantage of an existing disease-screening system in our university, including features such as fluorescent sperm and eggs (Figure 1). Through these gene-manipulated animals, we will perform translational research for the establishment of new therapeutic systems and pharmaceuticals.

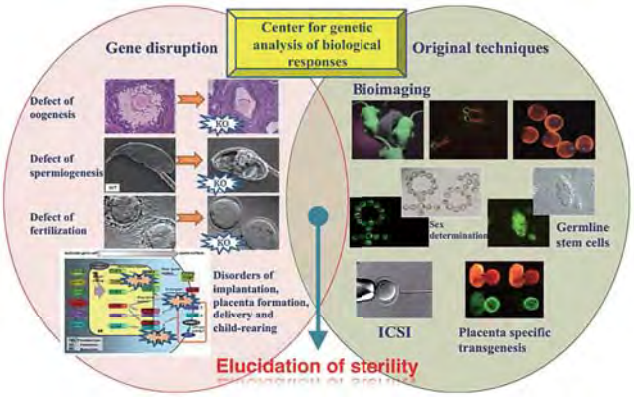


Figure 1. Strategy for elucidating sterility

Recent Publications

1. Tokuhiro K, Ikawa M, Benham AM, Okabe M. Testis specific PDILT is required for quality control of sperm membrane protein ADAM3 and male infertility. ***Proc Natl Acad Sci U S A***. 2012 March 6; 109 (10): 3850-3855.
2. Inoue N, Satouh Y, Ikawa M, Okabe M, Yanagimachi R. Acrosome-reacted mouse spermatozoa recovered from the perivitelline space can fertilize other eggs. ***Proc Natl Acad Sci U S A***. 2011 Dec 13;108(50):20008-11.
3. Kumasawa K, Ikawa M, Kidoya H, Hasuwa H, Saito-Fujita T, Morioka Y, Takakura N, Kimura T, Okabe M. Pravastatin induces placental growth factor (PGF) and ameliorates preeclampsia in a mouse model. ***Proc Natl Acad Sci U S A***. 2011 Jan 25;108(4):1451-5.
4. Isotani A, Hatayama H, Kaseda K, Ikawa M, Okabe M. Formation of a thymus from rat ES cells in xenogeneic nude mouse↔rat ES chimeras. ***Genes Cells.*** 2011 Apr;16(4): 397-405.
5. Ikawa M, Tokuhiro K, Yamaguchi R, Benham AM, Tamura T, Wada I, Satouh Y, Inoue N, Okabe M. Calsperin is a testis-specific chaperone required for sperm fertility. ***J Biol Chem***. 2011 Feb 18;286(7):5639-46.

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 Masato Okada, Ph.D.
Assistant Professor Shinji Higashiyama, Ph.D.

Since our laboratory was established in the late 1950s, it has grown to possess various high-performance instruments, including a laser microdissection system and mass spectrum analyzer, as well as ultracentrifuges, electron microscopes, cell sorters, automatic plasmid purification systems, and DNA sequencing machines. Our laboratory is installed with large liquid nitrogen tanks for the preservation of living materials, such as cells and viruses, and contains a room for specified chemical substances. Several technicians are employed to keep the instruments in proper working condition and to provide advice to beginners and ongoing support for researchers. In addition, the technicians execute cell sorting, nucleotide sequencing, electron microscope observations, and mass spectrometric analyses on samples upon request from Institute researchers. We anticipate that technical services will become increasingly more important in the future because many new instruments are precise, complicated, and require extensive training to use. Plans to accommodate such changes are currently in progress.

Radioisotope Laboratory

Head

Professor Masato Okada, Ph.D.
Assistant Professor Shinji Higashiyama, Ph.D.

The Radioisotope (RI) Laboratory was built adjacent to the main building of the Institute in 1967. It was extended by branch laboratories, for a combined space of about 600 m2, 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 the RI Laboratory in 2007. The Main RI Laboratory, North Building RI Laboratory, and Genome Information Research Center Radioisotope Laboratory were closed in 2010 and 2011, and the new RI Laboratory was established in the Immunology Frontier Research Center building in 2011.

The RI Laboratory is designed for biomedical experiments utilizing radioisotopes and it plays an important role in the Institute. Its facilities include an RI stockroom, distribution room, tissue culture room, and 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 m2 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 2010 and 2011, 50 and 52 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 Toshihiro Horii, Ph.D.

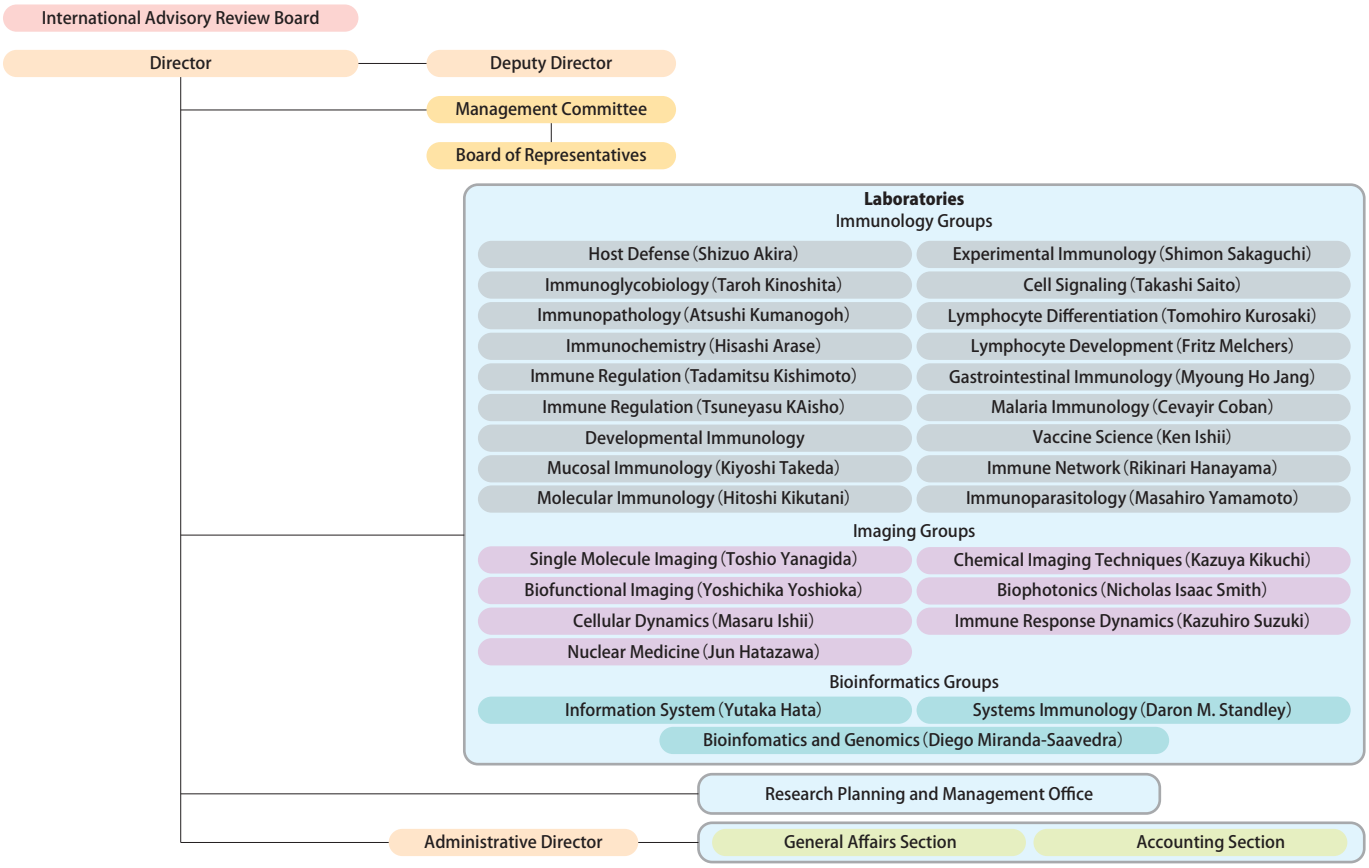
The RIMD library collects academic books and journals on microbiology and immunology, as well as work on related scientific fields such as cell biology, genetics, histology, developmental biology, biochemistry, pharmacology pathology, microbiology and oncology. In particular, we have collected rare books 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 13,000 books. We now purchase 34 and 25 journals published in English and Japanese, respectively. Most of the books are kept in the stock room, but textbooks and newly arrived journals 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 Inter Library Loan (ILL) system. One librarian handles the RIMD library together with two professors, two associate professors and one assistant professor who act as members of RIMD library committee. These members also take care of the publication of the “Annual Reports of the Research Institute for Microbial Diseases Osaka University” (online only from 2003).

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.

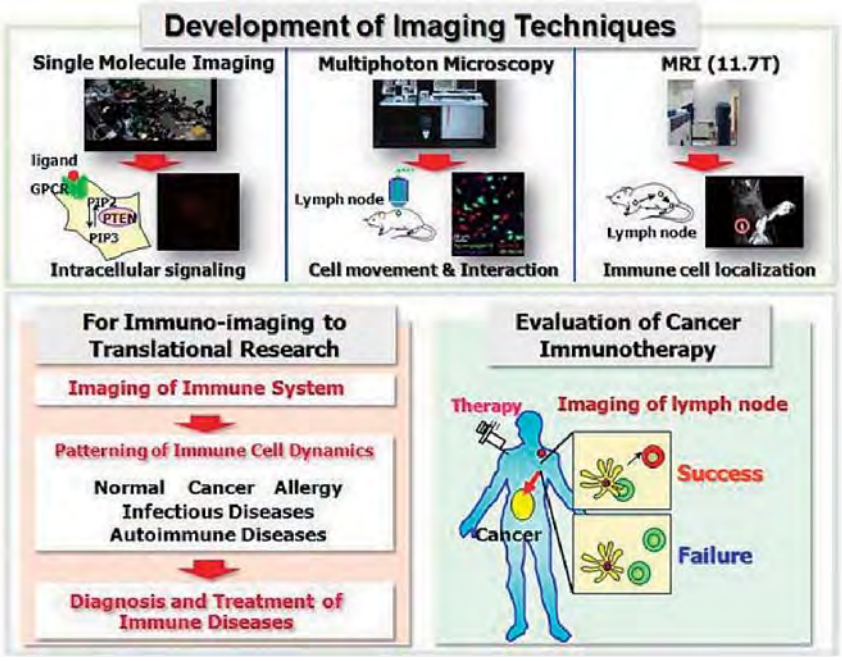


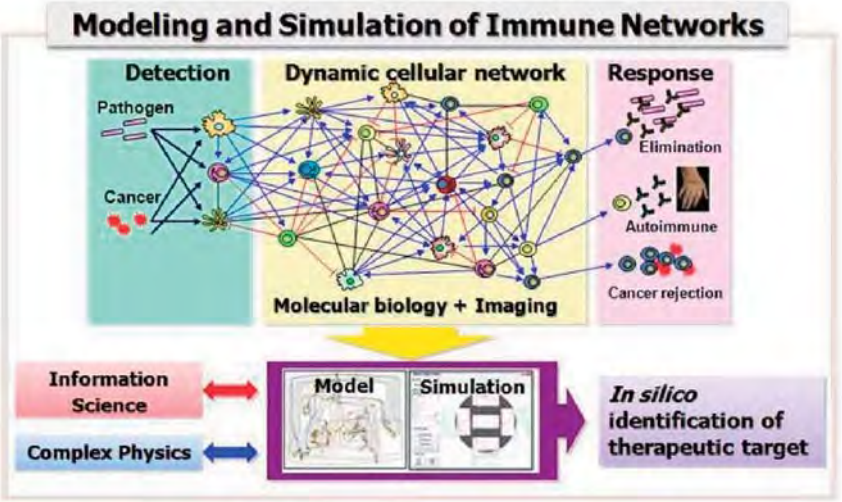
●Research fields / Expected Achievements

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	2007	2008	2009	2010	2011
Personnel	917,415	905,437	859,673	887,150	863,168
Non-Personnel	495,488	513,073	548,947	704,408	567,143
Total	1,412,903	1,418,510	1,408,620	1,591,558	1,430,311

Other Grants

(unit : thousand yen)

Classification	2007	2008	2009	2010	2011
Contract Research	1,175,396	1,022,353	1,040,180	908,861	711,772
Donations for Research	1,215,677	187,969	343,772	689,654	765,777
Miscellaneous	4,591	3,406	2,090	4,506	4,082
Total	2,395,664	1,213,728	1,386,042	1,603,021	1,481,631

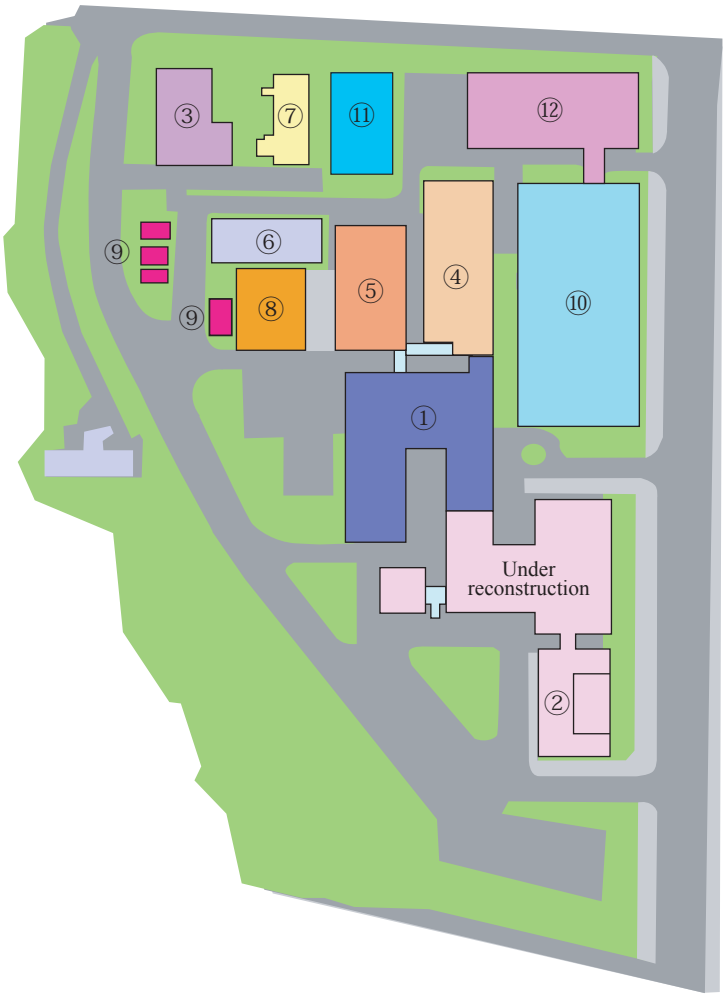
Grants-in-Aid for Scientific Research

(unit : thousand yen)

Classification	2007	2008	2009	2010	2011
MEXT Research Grants	613,870	863.592	688,999	453,744	466,212
Health and Labor Sciences Research Grants	237,575	163,278	118,789	107,632	87,913
Health and Labor other Research Grants	-	18,000	13,988	0	0
21st Century COE Program Grants	196,900	0	0	0	0
Global COE Program Grants	-	149,599	120,037	85,441	74,992
Total	1,048,345	1,194,469	941,813	646,817	629,117

Building Area

Site Area ····· **36,036**㎡ | Building Area ····· **6,061**㎡ Gross Floor Area ····· **23,120**㎡



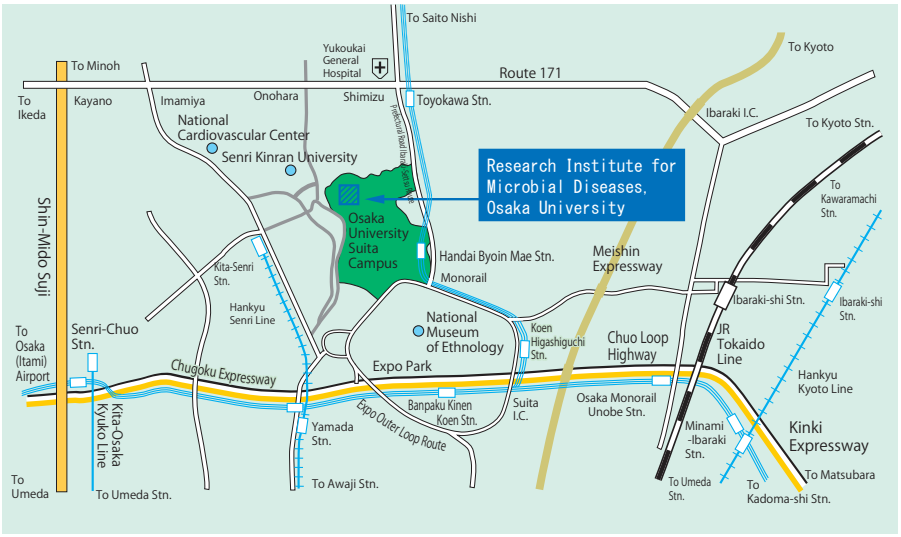
① Main building (left) and
⑩ Integrated Life Science building (right)

② South building Ⅱ

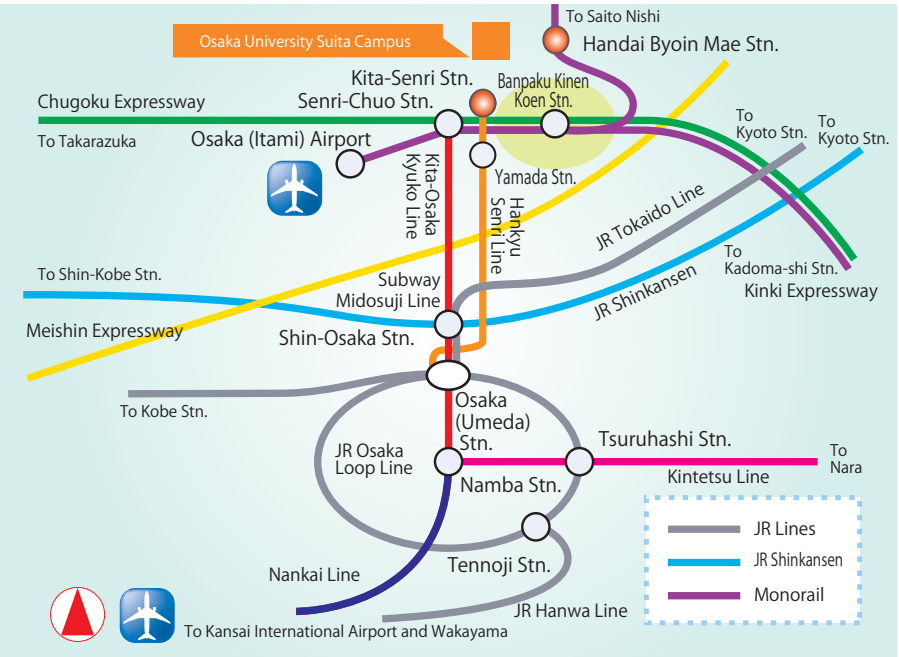
⑦ Central Laboratory for Biological Hazardous Microbes
and
⑤⑥ Animal Resource Center

Building name	Total floor numbers	Building area (㎡)	Total floor area (㎡)
①Main building	7	1,546	6,087
②South building Ⅱ	3 (1basement)	409	945
	DNA-chip Developmet Center and Genome Information Research Center are included		
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
⑪Animal Resource Center C (belonging to IFReC)	3 (1 basement)	738	2,482
⑫New IFReC building (tentative name)	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

Campus Map

1 Administration Bureau
2 Graduate School/School of Human Sciences
3 Graduate School/Faculty of Medicine
4 Faculty of Medicine (Dept. of Allied Health Sciences)
5 Osaka University Hospital
6 OSaka University Dental Hospital
7 Graduate School/School of Pharmaceutical Sciences
8 Graduate School/ School of Engineering
9 Graduate School of Frontier Biosciences
10 Graduate School of Information Science and Technology
11 United Graduate School of Child Development
12 Research Institute for Microbial Diseases
13 Institute for Protein Research
14 Low Temperature Center
15 Radioisotope Research Center
16 Research Center for Environmental Preservation
17 International Student Center
18 International Center for Biotechnology
19 Center for Advanced Science and Innovation
20 The Center for Advanced Medical Engineering and Informatics
21 Global Collaboration Center
22 Sustainability Design Center
23 Institute of Laser Engineering
24 Immunology Frontier Research Center
25 Institute of Scientific and Industrial Research
26 Institute of Social and Economic Reseach
27 Joining and Welding Research Institute
28 Research Center for Ultra-high Voltage
29 Cybermedia Center
30 Research Center for Nuclear Physics

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