# Osaka University

# Research Institute for Microbial Diseases

2010

## 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 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.

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.

# 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 Pathology
- 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

## Endowed Chair

- Laboratory of Molecular Biology (Life Technologies Co.- Endowed Chair)

## Common Research Facilities

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

## Administration

- General Affairs Section
- Accounting Section
- Research Cooperation Section

## Related Institution

- World Premier International Research Center
- Immunology Frontier Research Center

## Former Directors

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

## Former Professors

Yashiro Kotake, M.D., Professor
Sadao Yoshida, M.D., Professor
Arao Imamura, M.D., Professor
Yukichi Satani, M.D., Professor
Tenji Taniguchi, M.D., Professor
Kota Sera, M.D., Professor
Tatsunori Masayama, M.D., Professor
Shohei Otani, M.D., Professor
Teishiro Seki, M.D., Professor
Masami Suda, M.D., Professor
Kaoru Morishita, M.D., Professor
Hisashi Yamaguchi, M.D., Professor
Tsunesaburo Fujino, M.D., Professor
Masakazu Ito, M.D., Professor
Juntaro Kamahora, M.D., Professor
Shinji Nishimura, M.D., Professor
Mitsuhiko Kato, M.D., Professor
Masahiko 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
Michiaki Takahashi, M.D., Professor
Hajime Fujio, M.D., Professor
Tetsuo Taguchi, M.D., Professor
Aizo Matsushiro, D.Sc., Professor
Atsuo Nakata, D.Sc., Professor
Hiroto Okayama, M.D., Professor
Kumao Toyoshima, M.D., Professor
Teruo Kitani, M.D., Professor
Shin-ichiro Takai, M.D., Professor
Morihiro Matsuda, M.D., Professor
Takashi Kurimura, M.D., Professor
Koichi Yamanishi, M.D., Professor
Akira Hakura, D.Sc., Professor
Tetsu Akiyama, D.Sc., Professor
Takeshi Kurata, M.D.,D.Med.Sci., Professor
Shigeharu Ueda, M.D.,D.Med.Sci., Professor
Kazunori Shimada, M.D.,D.Med.Sci., Professor
Chihiro Sasakawa, M.D., Professor
Akio Sugino, D.Sci., Professor
Hiroshi Kiyono, D.D.S.,Ph.D., Professor
Yoshitake Nishimune, M.D., Professor
Toru Nakano, M.D., D.Med. SC., Professor
Hideo Shinagawa, D.Sc., Professor
Shin-ichi Tamura, Ph.D., Professor
Michiyuki Matsuda, M.D., D.Med. SC., Professor
Takeshi Honda, M.D., Ph. D.
Naoyuki Taniguchi, M.D., Ph. D.
Tamotsu Yoshimori, M.D., Ph. D.
Kazuyuki Tanabe, M.D., Ph. D.

# Department Heads

2010,04,01

| | |
|---|---|
| Director | Professor Hitoshi Kikutani M. D., Ph.D. |
| Vice Director | Professor Eisuke Mekada 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 Pathology | Professor Atsushi Kumanogoh M. D., Ph.D. |
| Division of Host Defense | |
| Department of Molecular Immunology | Professor Hitoshi Kikutani M. D., Ph.D. |
| Department of Immunoregulation | Professor Taroh Kinoshita Ph.D. |
| Department of Host Defense | Professor Shizuo Akira M. D., Ph.D. |
| Department of Cell Biology | Professor Eisuke Mekada Ph.D. |
| Department of Immunochemistry | Professor Hisashi Arase M. D., Ph.D. |
| Division of Cellular and Molecular Biology | |
| Department of Molecular Microbiology | |
| Department of Molecular Genetics | Professor Hiroshi Nojima Ph.D. |
| Department of Oncogene Research | Professor Masato Okada Ph.D. |
| Department of Signal Transduction | Professor Nobuyuki Takakura M. D., Ph.D. |
| Department of Cellular Regulation | |
| 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 | SA Professor Kazunori Oishi M. D., Ph.D. |
| Laboratory of Infection Cell Biology | SA Associate Professor Yukako Fujinaga Ph.D. |
| Laboratory of Viral Pathogenesis and Immunity | SA Associate Professor Takaaki Nakaya M. D., 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 | SA 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 Hitoshi Kikutani M. D., Ph.D. |
| Research Promotion Group | Associate Professsor Yoshiko Murakami M. D., Ph.D. |
| Education Promotion Group | Associate Professsor Hodaka Fujii M. D., Ph.D. |
| Research Collaboration Center in Overseas | Head, SA Professor Shigeyuki Hamada, D.D.S., Ph.D. |
| Section of Bacterial Infections | SA Professor Shigeyuki Hamada, D.D.S., Ph.D. |
| Section of Viral Infections | SA Professor Naokazu Takeda, Ph.D. |
| Laboratory of Molecular Biology (Life Technologies Co.- Endowed Chair) | Professor Fumio Imamoto 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 Hisashi Arase M. D., Ph.D. |
| Administration | Head, Keiichi Kitomi |

*SA : Specially Appointed

## Staff

2010.04.01

| | |
|---|---|
| Professor | 12 |
| Endowed Chair Professor | 1 |
| SA Professor | 5 |
| Associate Professor | 16 |
| Endowed Chair Associate Professor | 0 |
| SA Associate Professor | 4 |
| SA Lecturer | 2 |
| Assistant Professor | 27 |
| Endowed Chair Assistant Professor | 1 |
| SA Assistant Professor | 5 |
| Educational Support Staff | 3 |
| Technical Staff | 3 |
| Administrative Staff | 17 |
| SA Researcher | 54 |
| Research Collaborator | 5 |
| Part-time General & Technical staff | 43 |
| Total | 198 |

SA : Specially Appointed

## Graduate Students

2010.04.01

| | Doctor Course | Master Course |
|---|---|---|
| Graduate School of Medicine | 57 | 5 |
| Graduate School of Science | 4 | 7 |
| Graduate School of Pharmaceutical Science | 2 | 6 |
| Graduate School of Dentistry | 0 | 0 |
| Graduate School of Frontier Biosciences | 2 | 7 |
| Total | 65 | 25 |

## Research Fellows & Research Students

2010.04.01

| | |
|---|---|
| Special research students | 1 |
| Research Students | 6 |
| Visiting Research Scholars | 1 |
| JSPS Research Fellows | 4 |
| Total | 12 |

# 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. |
| Assistant Professor | Aya Fukui, Ph. D. |
| SA Researcher | Hirono Toshima, Ph. D. |


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

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

(2) Analysis of whooping cough pathogenesis.
*Bordetella pertussis*, a pathogenic bacteria, infects the human respiratory tract and causes whooping cough, which is characterized by paroxysmal coughing. There are two significant questions about the pathogenesis of *B. pertussis* infection. First, why does *B. pertussis* infect humans but no other mammals? Second, how does this bacterium induce the paroxysmal coughing? We are currently examining the pathology of the disease and the function of *B. pertussis* virulence factors by using an animal model of the infection.

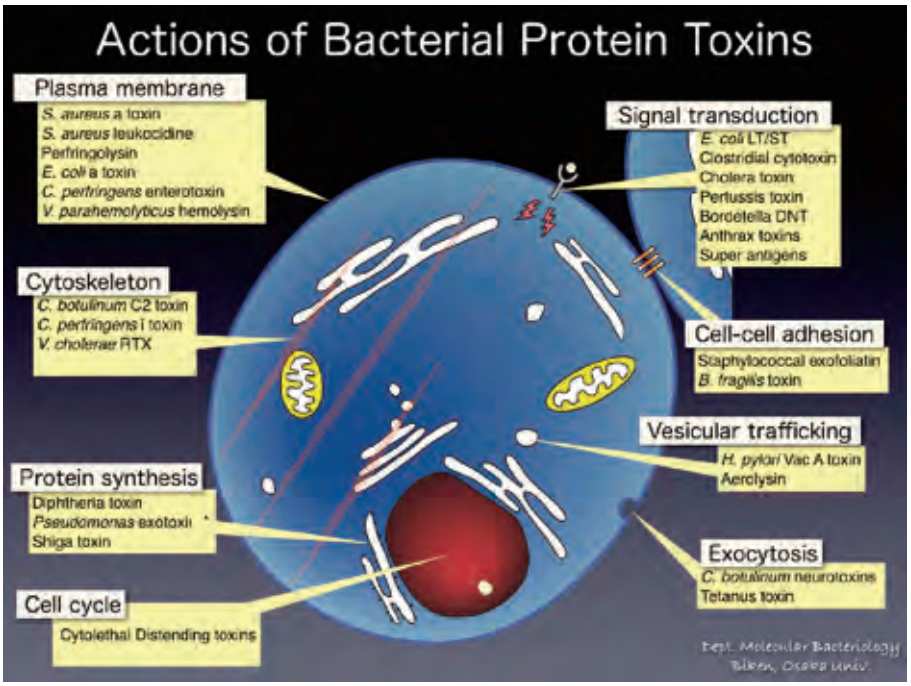



Fig. 1: Bacterial protein toxins with various activities that influence particular cellular functions.
Many bacterial protein toxins exert their toxic effects by modifying important functions of the host cells. Significantly, the relevant physiological functions of the cells can also be determined by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

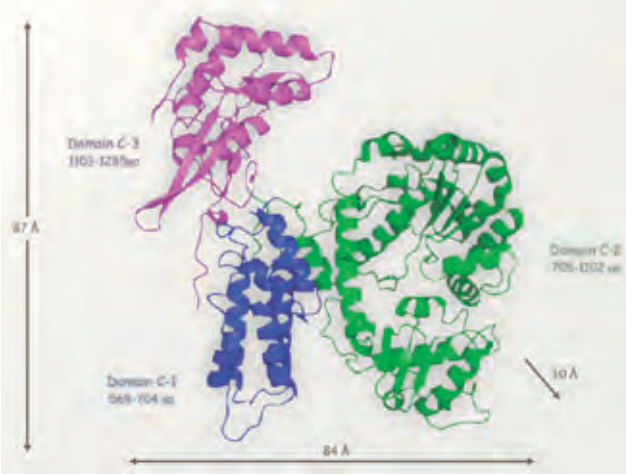



Fig. 2: Overall structure of the intracellular active region of *Pasteurella multocida* toxin, which is composed of three domains and has a Trojan horse-like shape.

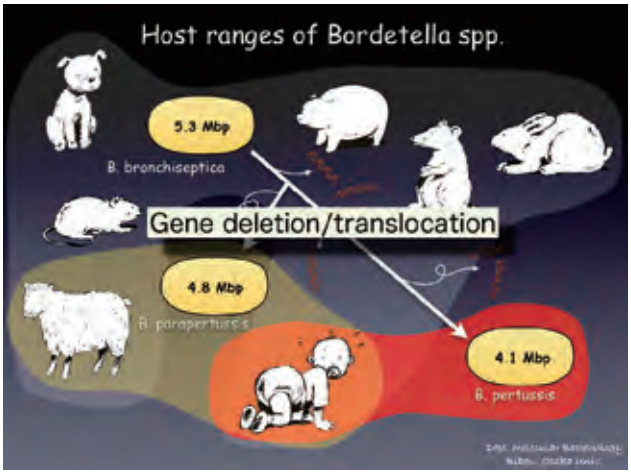



Fig. 3: *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica* are closely-related pathogenic bacteria. *B. bronchiseptica* has the largest genome and the broadest host range, whereas *B. pertussis* has the smallest genome and the narrowest host range. It is believed that *B. pertussis*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

## Recent publications


1. Kimura J, Abe H, Kamitani S, Toshima H, Fukui A, Miyake M, Kamata Y, Sugita-Konishi Y, Yamamoto S, and Horiguchi Y. *Clostridium perfringens* enterotoxin interacts with claudins via electrostatic attraction. J Biol Chem. 2010 Jan 1;285(1):401-8.
2. Miyake M, Sakane S, Kobayashi C, Hanajima-Ozawa M, Fukui A, Kamitani S, and Horiguchi Y. A colorimetric assay for studying effector secretion through the bacterial type III secretion system. FEMS Microbiol Lett. 2008 Jan;278(1):36-42.
3. Ohnishi H, Miyake M, Kamitani S, and Horiguchi Y. FEMS Microbiol Lett. 2008 Feb;279(2):174-9. The morphological changes in cultured cells caused by *Bordetella pertussis* adenylate cyclase toxin. FEMS Microbiol Lett. 2008 Feb;279(2):174-9.
4. Kitadokoro K, Kamitani S, Miyazawa M, Hanajima-Ozawa M, Fukui A, Miyake M, and Horiguchi Y. Crystal structures reveal a thiol protease-like catalytic triad in the C-terminal region of *Pasteurella multocida* toxin. Proc Natl Acad Sci U S A. 2007 Mar 20;104(12):5139-44.
5. Hanajima-Ozawa M, Matsuzawa T, Fukui A, Kamitani S, Ohnishi H, Abe A, Horiguchi Y, and Miyake M. Enteropathogenic *Escherichia coli, Shigella flexneri,* and *Listeria monocytogenes* recruit a junctional protein, zonula occludens-1, to actin tails and pedestals. Infect Immun. 2007 Feb;75(2):565-73.

# Department of Viral Infections

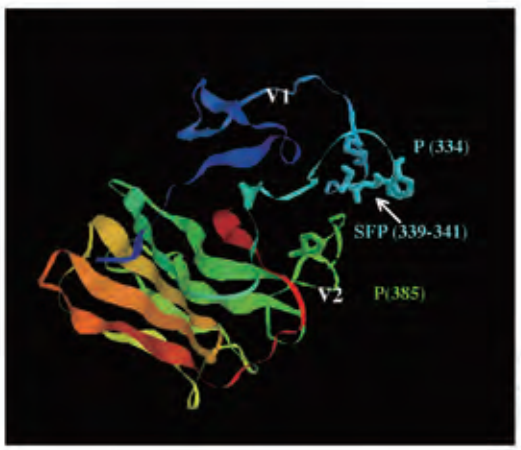
Research Group

| | |
|---|---|
| Professor | Tatsuo Shioda, D.Med.Sc. |
| Assistant Professor | Jun-ichi Sakuragi, D.Med.Sc. |
| Assistant Professor | Emi E. Nakayama, M.D., D.Med.Sc. |
| SA Researcher | Sayuri Sakuragi, D.Med.Sc. |
| Postdoctoral Fellow | Ken Kono, D.Med.Sc. |
| Postdoctoral Fellow | Ayumu Kuroishi, D.Med.Sc. |

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

(1) Anti-retroviral factors

HIV does not establish a productive infection in any other monkey except for the chimpanzee; this is thought to be due to inhibitors in simian lymphocytes that act at the early stage (reverse transcription) of viral infection. To date, TRIM5$\alpha$ and cyclophilin A have been identified as such restriction factors. We had shown that differences in the amino acid sequences in the C-terminal domain of TRIM5$\alpha$ of different monkey species affect the species-specific restriction of retrovirus infection (Fig.1, left). We also found that sequence variations in the N-terminal half of the viral capsid protein (Fig. 1, right) determine viral sensitivity to TRIM5$\alpha$-mediated restriction, which indicates that there is an interaction between TRIM5$\alpha$ and the virus capsid. In addition, we showed that HIV-2 replication levels in infected individuals are associated with capsid variations, and we suggested that viral sequence analysis can predict AIDS progression. Furthermore, we have succeeded in improving the simian-tropic HIV-1 virus and the methods of monkey genome analysis. These new developments 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 also seeking to identify the binding surface between the viral capsid protein and TRIM5$\alpha$, as this may be useful for the development of new anti-retroviral drugs.




Structure of C-terminal SPRY domain of TRIM5$\alpha$.The amino acids that are important for viral restriction are located in the surface of SPRY domain. V1 and V2 denote the regions that vary between the different monkey species.

The 3D structure model of viral capsid protein. A single amino acid change from P to A or Q radically affected the configuration of the loop.

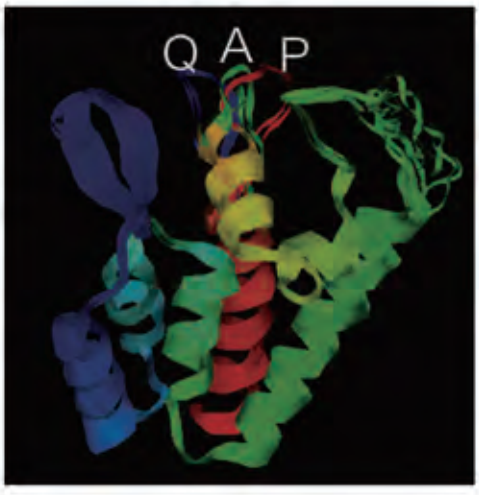



Fig. 1 Structural models of TRIM5$\alpha$ (left) and the viral capsid (right).

(2) Host factors that participate in HIV pathogenesis and anti-retroviral drug side-effects.
As an animal model of AIDS has not yet been established, we are utilizing epidemiological procedures to understand the mechanisms of AIDS pathogenesis. There are cases who are not infected despite repeated exposure to HIV. There are also HIV-positive patients who do not develop symptoms of AIDS despite not receiving any anti-retroviral treatment. These cases are suspected to bear a resistance-inducing factor (RIF) against HIV. To characterize these RIFs, we have compared the genome sequences of the cases described above to those of HIV-infected patients and uninfected individuals. We found (a) the deletion mutant CCR5-893 (-), which fails to produce a co-receptor that is needed for HIV entry, (b) a polymorphism in the promoter of the chemokine RANTES, and (c) a polymorphism in the promoter of IL4, which regulates the expression of the co-receptor. We then demonstrated that these mutations affect susceptibility to HIV infection and the rate with which the disease progresses to AIDS.
At present, in collaboration with Thai groups, we are also focusing on the relationship between human genomic variation and anti-retroviral therapy side-effects, with the aim of establishing "tailor-made therapies" that will improve the quality of life of HIV-infected patients.

(3) Molecular mechanisms of HIV particle formation.
The HIV genomic RNA always forms dimers in the mature virion. It was suggested previously that the presence of the dimerized genome in the virion is advantageous for survival, as it provides an extra template that can be used when one RNA molecule is damaged; it may also endow the progeny with genetic variety. We were able to identify the minimal HIV genome region that is sufficient for genome dimerization. Our data suggest that RNA dimerization is part of RNA packaging. We also found that HIV genome dimerization affects the early stage of HIV replication after its entry into cells.

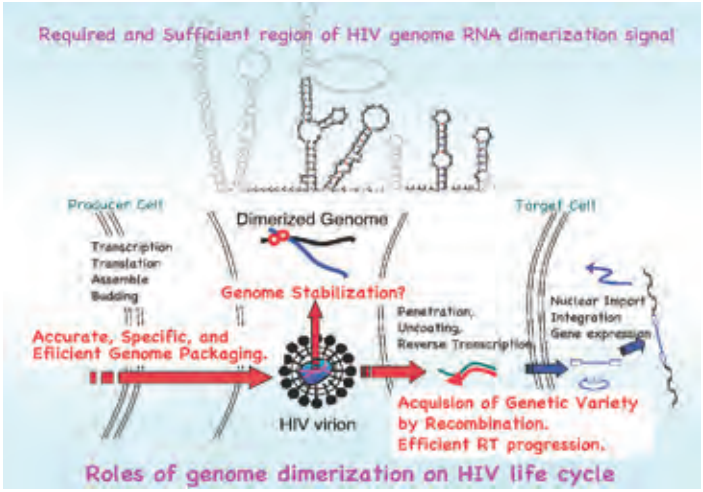



Fig. 2 HIV-1 genome RNA dimeriation.

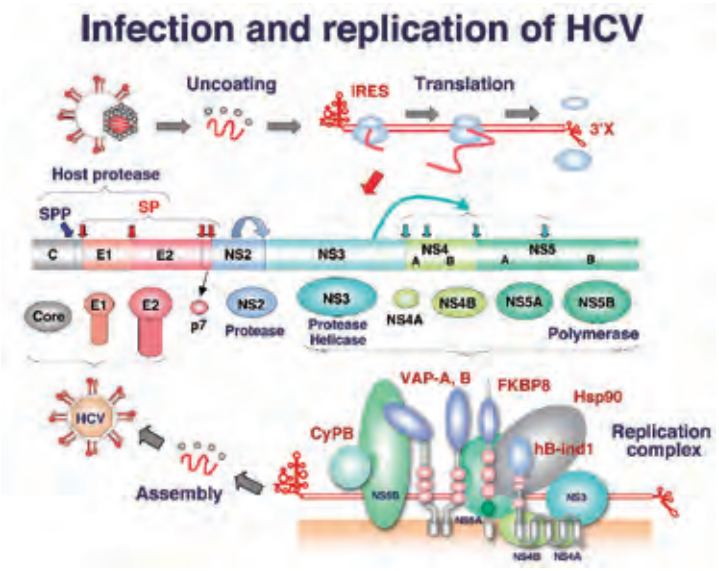
## Recent publications


1. Nakayama EE, ShiodaT. Anti-retroviral activity of TRIM5alpha. Rev Med Virol. 2010 Mar;20(2):77-92.
2. Likanonsakul S, Rattanatham T, Feangvad S, Uttayamakul S, Prosithsirikul W, Tunthanathip P, Nakayama EE, Shioda T.HLA-Cw*04 allele associated with nevirapine-indued rash in HIV-infected Thai patients. AIDS Res Ther. 2009 Oct 21;6:22.
3. Nakajima T, Nakayama EE, Kaur G, Terunuma H, Mimaya JI, Ohtani H, Mehra N, Shioda T, Kimura A. Impact of novel TRIM5alpha variants, Gly110Arg and G176del, on the anti-HIV-1 activity and the susceptibility to HIV-1 infection. AIDS. 2009 Oct 23;23(16):2091-100.
4. Kuroishi A, Saito A, Shingai Y, Shioda T, Nomagushi M, Adachi A, Akari H, Nakayama EE. Modification of a loop sequence between alpha-helices 6 and 7 of virus capsid (CA) protein in a human immunodeficiency virus type 1 (HIV-1) derivative that has simian immunodeficiency virus (SIVmac239) vif and CA alpha-helices 4 and 5 loop improves replication in cynomolgus monkey cells. Retrovirology. 2009 Aug 3;6:70.
5. Sakuragi J, Sakuragi S, ShiodaT. Minimal region sufficient for genome dimerization in the human immunodeficiency virus type 1 virion and its potential role in the early stages of viral replication. J Virol. 2007 Aug;81(15):7985-92.

# Department of Molecular Virology


| Research Group | | |
|---|---|---|
| | Professor | Yoshiharu Matsuura, D.V.M., Ph.D. |
| | Associate Professor | Kohji Moriishi, D.V.M., Ph.D. |
| | SA Associate Professor | Wataru Kamiya, Ph.D. |
| | Assistant Professor | Takayuki Abe, Ph.D. |
| | SA Assistant Professor | Hideki Tani, Ph.D. |
| | Postdoctoral Fellow | Yuuki Kaname, Ph.D. |
| | Postdoctoral Fellow | Takasuke Fukuhara, M.D., Ph.D. |
| | Postdoctoral Fellow | Hiroshi Kato, D.V.M., Ph.D. |


We are seeking to understand the molecular mechanisms by which hepatitis C virus (HCV) enters host cells, replicates, escapes the immune system, and induces disease. This department is also currently developing a novel virus vector for gene delivery.

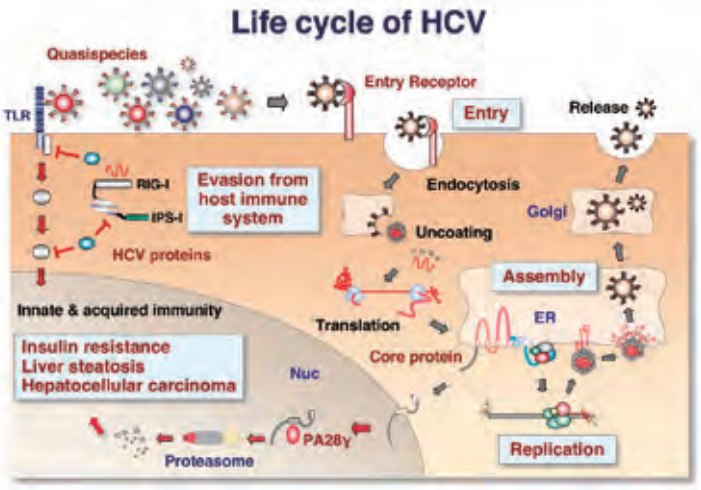



1. Studies on the molecular biology of HCV replication and pathogenesis

HCV has infected more than 3% of the world's population, 80% of whom will be persistently infected. Persistent HCV infection often leads to chronic hepatitis, cirrhosis and hepatocellular carcinoma. The incidence of hepatitis C in Japan has decreased significantly since a screening system for anti-HCV antibodies was introduced in 1999. However, more than two million people in Japan are already infected with HCV. The proportion of patients who achieve a sustained virological response to therapy has been increased by the use of pegylated-IFN$\alpha$ and ribavirin combination therapy. However, half of the patients with HCV genotype 1 fail to mount a response to this combination therapy.

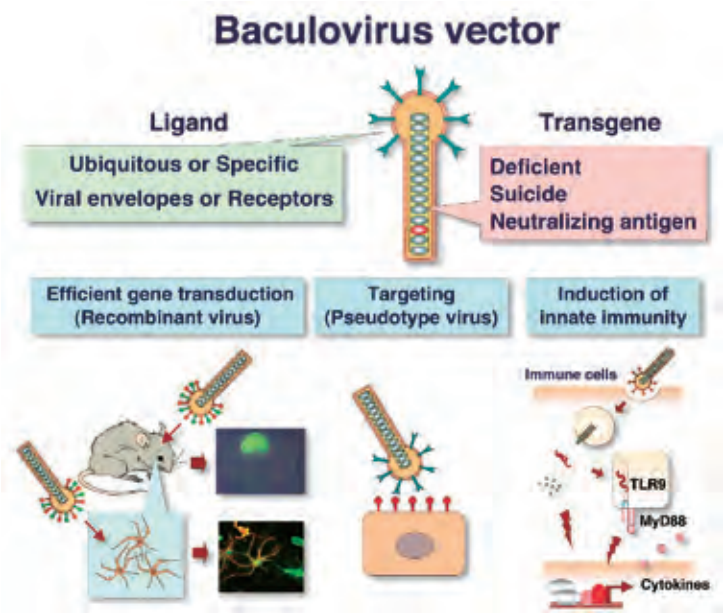
Since HCV exhibits quasispecies heterogeneity, it is difficult to determine the mechanisms by which HCV infects the human host by using surrogate systems such as pseudotype and recombinant viruses derived from a single HCV clone. The in vitro replication of genotype 2a HCV (HCVcc) was established recently and surrogate viruses and HCVcc were used to identify several receptor candidates for HCV entry, including hCD81, SR-BI, and Claudins. However, the sera of persistently infected patients contain high levels of neutralization antibodies that recognize these artificial viruses, which suggests that these antibodies do not play a crucial role in the clearance of HCV. Our studies also showed that HCV NS3/4A protease cleaves adaptor molecules involved in the TLR- and RIG-I–dependent signaling pathways, that HCV particles are internalized into cells through endocytosis, and that after uncoating, a viral RNA is translated into a large precursor polyprotein composed of 3,000 amino acids. With regard to the latter viral polyprotein, it is then cleaved by signal peptidase (SP), signal peptide peptidase (SPP) and virus-encoded proteases to generate at least 10 viral proteins. The open reading frame of the polyprotein is flanked at both ends by highly conserved untranslated regions (UTRs), which are required for viral RNA replication. The 5'-UTR harbors an internal ribosome entry site (IRES) that is essential for Cap-independent translation of viral RNA.

Although there has been considerable successful innovation in the drugs used in the clinic to eliminate the virus in patients with HCV, drug-resistant viruses emerge easily. Therefore, the most ideal targets for the development of new chronic hepatitis C therapies that have a broad spectrum and are unlikely to generate breakthrough viruses are host proteins that are indispensable for HCV replication. To search for such molecules, we have taken advantage of the fact that HCV belongs to the family of Flaviviridae, which includes the flavivirus Japanese encephalitis virus (JEV). JEV research is favored by the existence of a robust cell culture system and a small animal model. We are thus investigating the replication and pathogenesis of JEV as a surrogate model of HCV.



2. Development of baculoviral vectors

Viral vectors are essential tools for studies on replication-deficient viral infectious diseases such as HCV. Furthermore, the development of novel viral vectors is essential for future gene therapy. We are seeking to convert the baculovirus Autographa californica nucleopolyhedrovirus (AcNPV) into a versatile viral vector that will mediate gene delivery both in vitro and in vivo. AcNPV is an insect virus that has a 134-kb double-stranded circular DNA genome. Due to their strong promoters, baculoviruses are commonly used for the large-scale production of recombinant protein in insect cells. Baculoviruses are also capable of entering a variety of mammalian cells and, without replicating the viral genome, facilitating the expression of foreign genes under the control of mammalian promoters. Therefore, baculoviruses are useful viral vectors, not only because they can be used to induce the abundant expression of foreign genes in insect cells, but also because they can efficiently deliver genes 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, compared to other viral vectors that are currently in use, AcNPV is much less likely to generate replication-competent revertants that express baculoviral gene products and can induce harmful immune responses against mammalian cells. Furthermore, we have shown that intranasal inoculation with AcNPV induces a strong innate immune response that protects mice from lethal challenges with influenza viruses. In addition, we have demonstrated that AcNPV induces this innate immune response via a TLR9/MyD88-dependent pathway, and that this requires the internalization of viral DNA via membrane fusion with the envelope glycoprotein in the endosome. This finding raises the possibility that AcNPV can be harnessed therapeutically to induce host immune responses against various infectious diseases, especially those that are caused by pathogens that invade from the respiratory tract.

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5. Abe T., Kaname Y., Wen X., Tani H., Moriishi K., Uematsu S., Takeuchi O., Ishii K.J., Kawai T., Akira S., and Matsuura Y. Baculovirus induces type I IFN production through TLR-dependent and -independent pathways in a cell type-specific manner. J. Virol., **83**, 7629-7640 (2009).

# Department of Immunopathology

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## Regulation of immune responses by "Immune Semaphorins"

The semaphorin family contains soluble and membrane-bound proteins that were first identified as axonal guidance cues that function during neuronal development. However, cumulative findings suggest that semaphorins also play diverse roles in processes that are unrelated to axon guidance, including organogenesis, vascularization, angiogenesis, apoptosis and neoplastic transformation. After we discovered that CD100/Sema4D participates in the immune system, we have found that a subset of semaphorins called the 'Immune Semaphorins' also function in the immune system. These include Sema4A, CD100/Sema4D, Sema6D, and Sema7A. In addition, we found recently that Sema3A plays an essential role in the migration of dendritic cells (Fig. 2). We are currently studying the molecular mechanisms by which semaphorins and their receptors regulate immune responses.

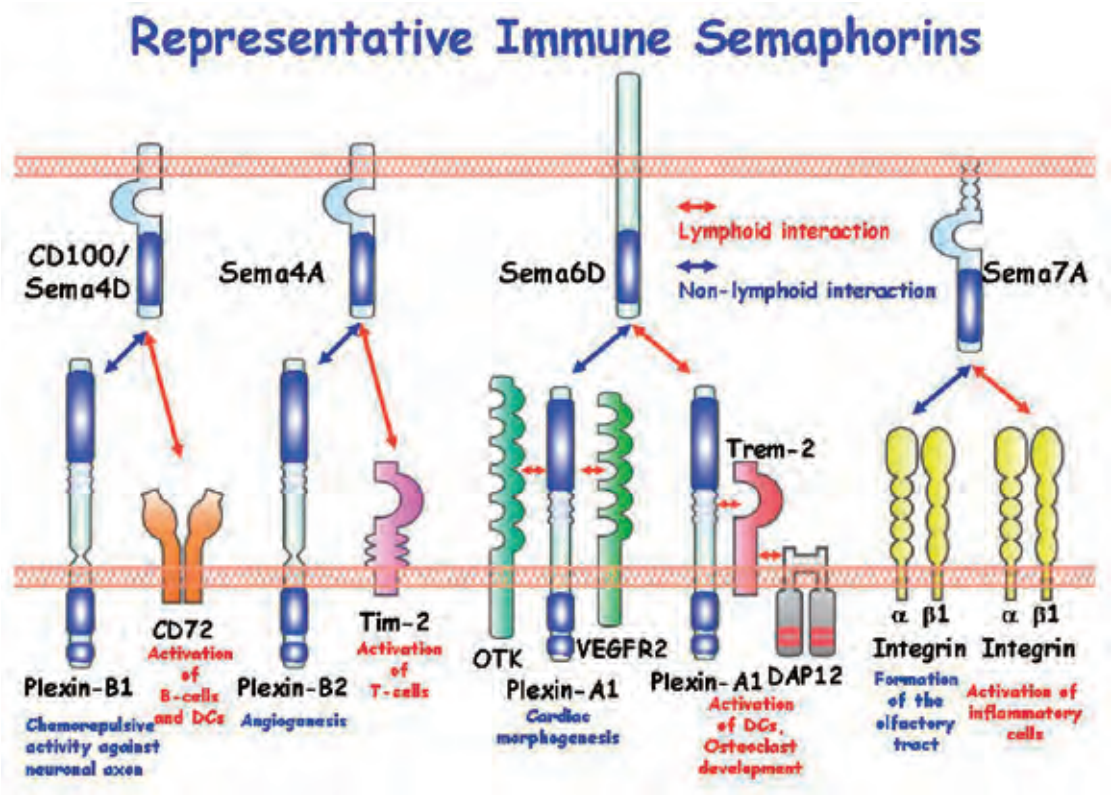


Figure 1. Representative immune semaphorins. The involvement of these semaphorins in immune responses has been demonstrated by using gene-targeted mice.

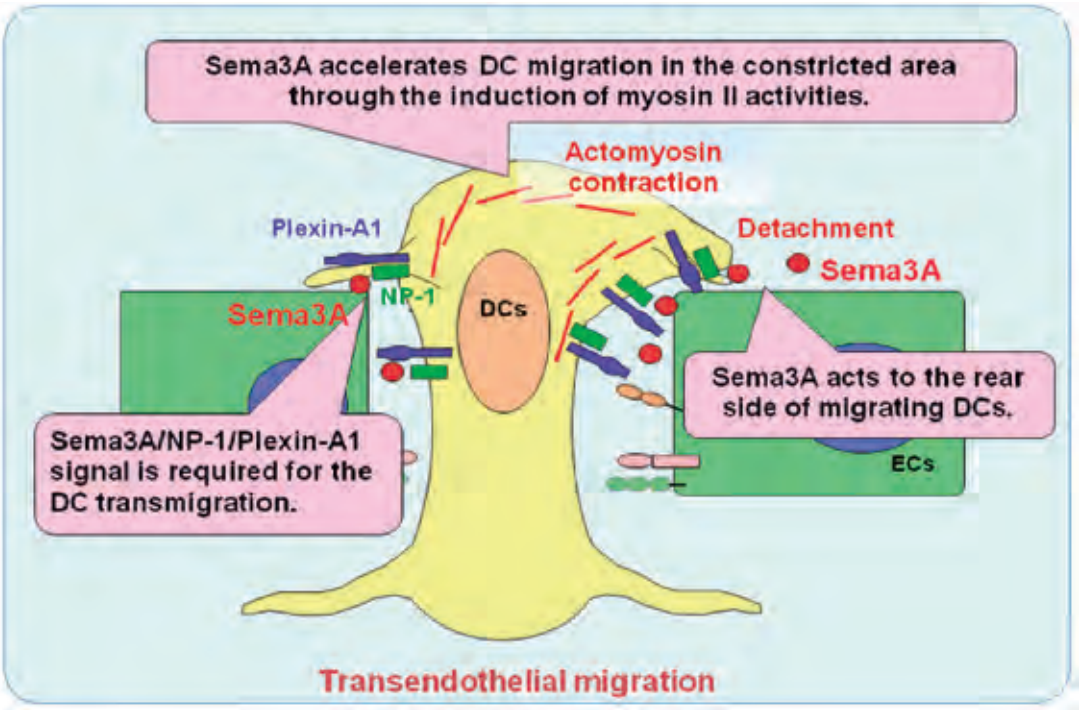


Figure 2. Sema3A is important in the migration of DCs.

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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.

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| | Postdoctoral fellow | Kentaro Morita, Ph.D. |

Molecular mechanisms involved in the regulation of immune responses.
T cells are activated by MHC-bound antigenic peptides on antigen-presenting cells. Once activated, the T cells differentiate into functional, helper, or effector T cells. In contrast, antigen-stimulated B cells differentiate into antibody-forming or memory B cells with the help of antigen-specific T cells. Thus, T- and B-cell differentiation requires physiological interactions between T cells and antigen-presenting cells, and between T cells and B cells, respectively. Such cell-cell interactions are mediated by a variety of costimulatory molecules, including CD40, CD40 ligand, B-7 and CD28. In addition, it was revealed recently that several members of the semaphorin family play crucial roles in immune cell interactions. We are currently studying how these molecules function in the regulation of immune responses.

A) Mechanisms by which semaphorin molecules regulate immune responses:
The semaphorin family molecules were first identified as axonal guidance factors that function during neuronal development. However, a series of studies by our laboratory has 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, while Sema4A participates in both T-cell priming and Th1 differentiation. The interaction between Sema6D and its receptor Plexin-A1 was also shown to participate in cellular immune responses since it activates dendritic cells and promotes bone homeostasis by inducing osteoclastgenesis. Furthermore, we demonstrated recently that Sema7A on activated T cells stimulates macrophages, which then produce inflammatory cytokines; it also triggers inflammatory responses through $\alpha 1\beta 1$ integrin (Figure 2).

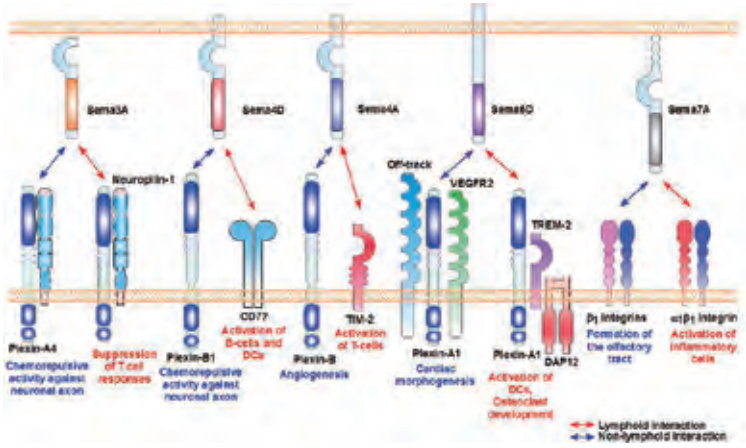


Fig. 1 Representative immune semaphorins
Semaphorins and their receptors have been shown by our research group to participate in immune regulation.

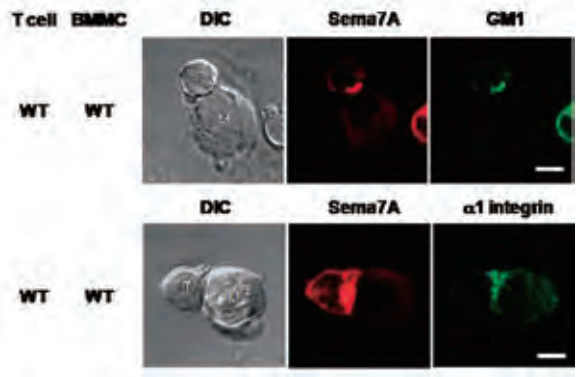


Fig. 2. Accumulation of Sema7A and $\alpha 1$ integrin in the immunological synapse between T cells and macrophages.

B) Elucidation of the molecular mechanism by which B cells survive and differentiate into effector cells:
Effective responses to the invasion of non-self antigen-bearing entities require that B cells differentiate into antibody-secreting cells and memory B cells. B cell survival and differentiation are driven by B cell-antigen receptor (BCR) signaling along with the signals of members of the TNF receptor family, such as CD40 and BAFF-R, on the B cell surface. To date, our group has demonstrated the immunological significance of the molecules that are involved in the signaling pathways downstream of CD40. In particular, we found that TRAF3, which interacts with the cytoplasmic region of both CD40 and BAFF-R, plays a crucial role in B cell survival and differentiation. Furthermore, we identified a PKC family member, PKN1, which is associated with the TRAF family and serves as a negative regulator of Akt in BCR signaling. Our observations together suggest that PKN1 may be responsible for the immunological tolerance that eliminates autoreactive B cells.

Molecular mechanism by which Epstein-Barr virus (EBV) induces immunological disorders.
EBV is a human herpes virus that causes infectious mononucleosis in healthy donors and proliferative disorders in patients who are immunosuppressed because of aging, immunosuppressive therapy, or HIV infection. It appears that EBV infection may be associated with B cell malignancies such as Burkitt's lymphomas and Hodgkin's lymphomas. It may also be linked to autoimmune diseases such as systemic lupus erythematosus (SLE) and multiple sclerosis (MS). EBV infects B cells in a latent fashion and is prevalent worldwide. We are currently studying EBV biology to determine how EBV leads to human carcinogenesis. The outcomes of this study may also reveal attractive therapeutic strategies for EBV-associated immune disorders (Figure 3).

A) The molecular mechanism by which EBV infects the human host:
EBV infection induces B cell growth transformation and immortalization. The mechanism by which EBV invades B cells involves multiple steps, namely virus entry, latency, and lytic infection. We are currently seeking to establish a system that will allow us to trace in vitro the infection dynamics of EBV and the frequency of cell growth transformation. This valuable system involves the production of recombinant EBV particles that carry the gene for GFP, which facilitates the visualization of EBV as it infects human peripheral B cells (Figure 4).

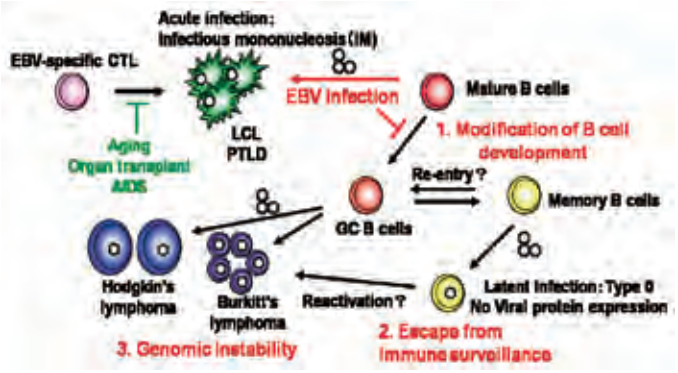


Fig.3. EBV and the host immune system.
The mechanism by which EBV induces human B cell growth transformation is associated closely with the vulnerability of the host immune system.

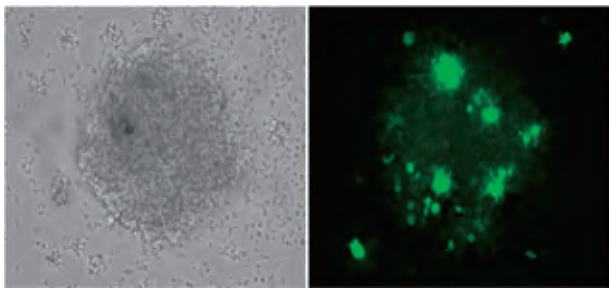

Fig.4. Immortalization of human peripheral blood B lymphocytes by recombinant EBV particles that carry the GFP gene.

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4. Mizui M, Kikutani H.Neuropilin-1: the glue between regulatory T cells and dendritic cells? Immunity. 2008 Mar;28(3):302-3.
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| | Postdoctoral Fellow | Noriyuki Kanzawa, D. Med. Sc. |
| | Postdoctoral Fellow (SUP) | Matthew Stokes, Ph. D. |
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1) Biogenesis, transport and remodeling of GPI-anchored proteins (GPI-APs).
Glycosylphosphatidylinositol (GPI) is a glycolipid that consists of phosphatidylinositol, glucosamine, mannoses and phosphoethanolamines, and acts as a lipid anchor for various plasma-membrane proteins. GPI-APs play important roles in host self-defense, intercellular signal transduction, and other important processes. In addition, some GPI-APs function as receptors for certain viruses and toxins. The GPI-anchor is widely distributed and conserved in various eukaryotes and is essential for the development of higher animals, as well as for the growth of yeasts and protozoan parasites. The modification of proteins due to the attachment of the GPI-anchor functions as a protein localization and sorting signal. Our current project is to identify and clarify the functions of all the genes involved in the biosynthesis of the GPI-anchor in the ER (PIG genes; PhosphatidylInositol glycan) and in the sorting and localization of GPI-APs after their anchorage with GPI (PGAP genes; Post GPI-Attachment to Proteins). We expect that these studies will reveal why many proteins are modified with the GPI-anchor.

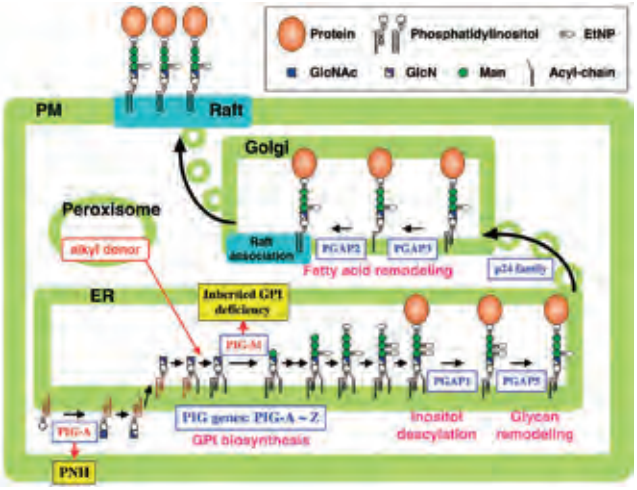


Fig. 1 GPI-anchor biosynthesis and the transport/remodeling of GPI-APs.
PIG genes are involved in the biosynthesis of the GPI-anchor in the ER. Thereafter, GPI-APs are transported to the plasma membrane and enriched in rafts. PGAP genes are involved in these later processes. PGAP1 and PGAP5, which localize in the ER, and PGAP2 and PGAP3, which localize in the Golgi, are involved in the lipid or glycan remodeling of the GPI-anchor. We found that the 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, and their absence on erythrocytes makes these cells very sensitive to complement and lysis during infections and other events. We are proposing 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, where a subclone bearing the growth phenotype is generated (Fig. 2). We identified HMGA2 as the candidate gene for Step 3.
Along with our colleagues in England, we have also identified a novel disease that is characterized by venous thrombosis and seizures, and is caused by a GPI deficiency that has been inherited in an autosomal recessive manner. The patients have a point mutation in the promoter of PIG-M, a mannosyltransferase-encoding gene that plays an essential role in GPI biosynthesis. The point mutation severely reduces PIG-M expression and leads to partial GPI deficiency. While complete GPI deficiency is lethal, partial GPI deficiency could be caused by a partial defect in one of the GPI biosynthesis genes, and the symptoms may vary depending on the defect.

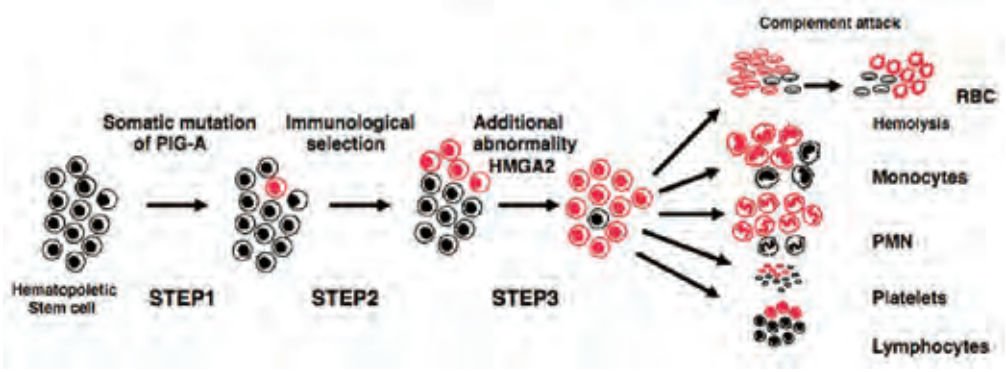


Fig. 2 Pathogenesis of PNH
Step 1 involves the generation of GPI-deficient hematopoietic stem cells due to the somatic mutation of the PIG-A gene. Step 2 involves the immunological selection of GPI-deficient hematopoietic stem cells. In this step, GPI-deficient cells survive and proliferate much more 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.

3) Glycolipid biosynthesis in pathogens and its use in drug development.

Our research focuses on elucidating the biosynthesis of GPIs in mycobacteria and *Trypanosoma brucei*. *T. brucei* is the causative agent of African sleeping sickness while mycobacteria cause a number of diseases, including tuberculosis. GPIs are located on the cell surface of these pathogens and appear to play key roles in their evasion of host immune attack. In particular, GPI-like molecules found in mycobacteria have anti-inflammatory activities and are thought to be important for the establishment of the infection. We aim first to identify the genes that are involved in these GPI biosynthetic pathways, after which we can create and characterize gene deletion/overexpression mutants. This research will help us to understand the roles these GPI molecules play at the molecular level in cell surface structure maintenance and host immune response modulation. We are also seeking to determine the key enzymes of the biosynthetic pathways and develop a high-throughput screening system that will help us to identify lead compounds in drug libraries.

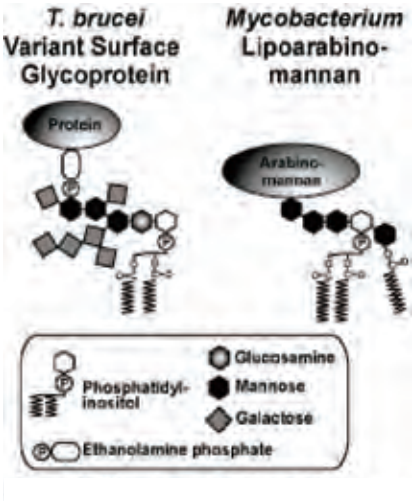


Fig. 3 Structure of the GPIs of pathogens

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Our laboratory studies pathogen recognition by the innate immune system and the mechanisms that regulate innate immune responses. 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 (PAMPs), which are conserved molecular features of microbial pathogens. We are seeking to clarify the complex regulatory mechanisms of the innate immune system.

1) Characterization of the pathogen recognition by Toll-like receptors (TLRs) and their signaling pathways
TLR family members play essential roles in the recognition of pathogens by the innate immune system. Their signaling pathways also play an important role in the gene induction involved in inflammation and immune responses. We have identified many TLR family members and their signaling molecules, and have characterized their functions by generating knockout mice. As a result, we have identified most of the ligands for these TLR family members and their signaling pathways (Figure 1). We also found that the stimulation of TLRs induces not only proinflammatory cytokine genes, but also type I interferon genes. For example, the TRIF-TBK1/IKK-i-IRF-3 pathway plays an important role in the TLR3- and TLR4-mediated induction of IFN-$\beta$ (Figure 2). Moreover, TLR7 and TLR9 are preferentially expressed in plasmacytoid dendritic cells (pDCs), which produce large amounts of IFN-$\alpha$ upon viral infection. We identified a specific signaling pathway in pDCs that is stimulated by TLR7 and TLR9 ligands and induces IFN-$\alpha$ expression (Figure 2). In summary, TLR signaling is regulated by distinct and complex mechanisms that operate in a ligand- and cell-type specific manner. We are currently expanding our understanding of the in vivo functions of TLRs and their signaling pathways.

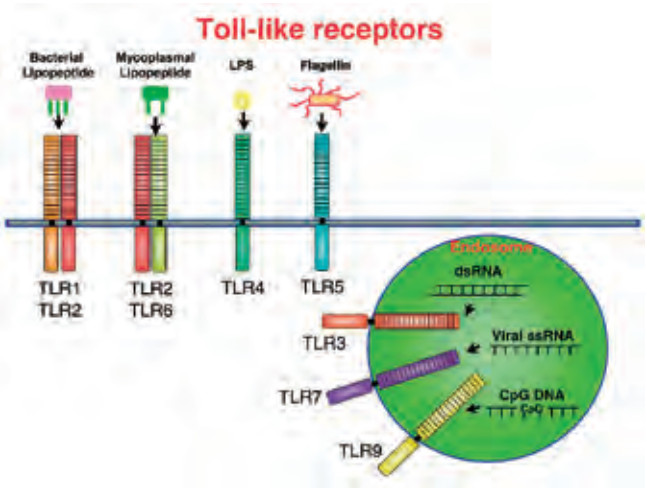


Figure 1: Pathogen recognition by TLRs. TLRs recognize molecular patterns associated with a broad range of pathogens, including bacteria, fungi, protozoa and viruses.

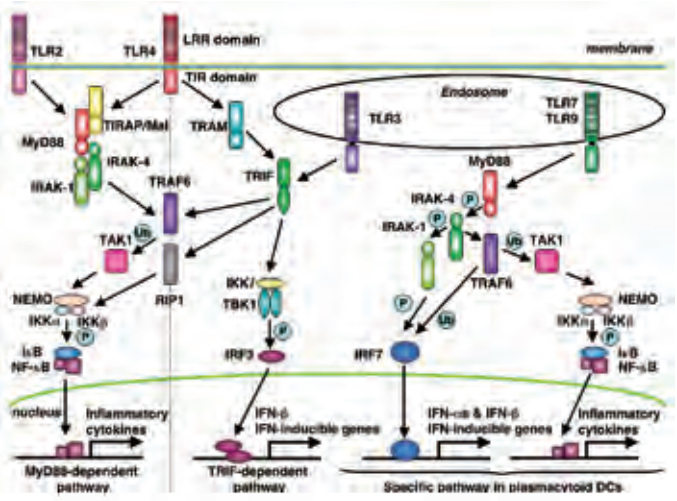


Figure 2: TLR signaling pathways. All TLR family members apart from TLR3 share a common pathway called the MyD88-dependent pathway that induces inflammatory cytokine production. Each TLR family member also has its own specific signaling pathway. Thus, TLR3 and TLR4 operate via a TRIF-dependent pathway while TLR7 and TLR9 act in pDCs via a unique pathway to induce IFN-$\alpha$ expression.

2) Therapeutic applications of TLR agonists and antagonists
Appropriate agonist-induced stimulation of TLRs could stimulate an innate immune response that boosts host resistance to cancer, allergy, and infectious diseases. This approach could also be used to promote the development of an adaptive immune response to a co-administered vaccine. TLR antagonists may also have therapeutic potential, as they could prevent or ameliorate the inappropriate or exaggerated TLR stimulation that leads to deleterious outcomes such as autoimmune diseases, sepsis or atherosclerosis.

3) Investigation of pathogen recognition mechanisms by cytoplasmic receptors.
Infection with pathogens such as viruses induces type I IFNs in both a TLR-dependent and a TLR-independent manner. RIG-I and MAD5, which are RNA helicases that recognize viral RNAs, recognize different viruses and are important for host antiviral responses (Figure 3). We also identified a new adaptor molecule, IPS-1, which plays an essential role in RIG-I- and MDA5-mediated antiviral responses (Figure 3). We are currently exploring these TLR-independent mechanisms further by generating knockout mice.

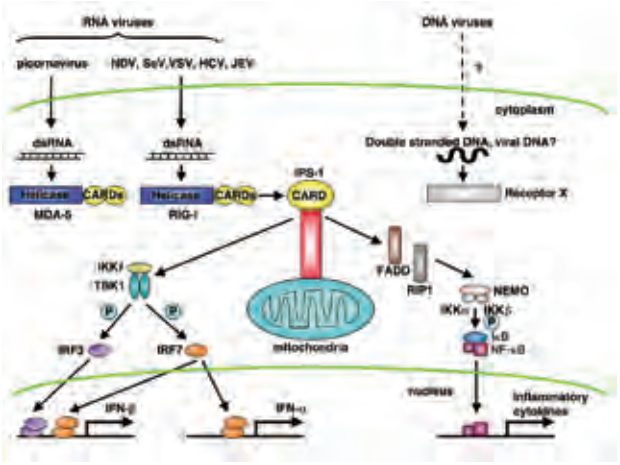


Figure 3: Signaling pathways employed by anti-viral RNA helicases. Viruses produce dsRNA during their replication in the host cell cytoplasm. RIG-I and Mda5 recognize this viral RNA and initiate antiviral signaling. In this signaling pathway, IPS-1 interacts with RIG-I and Mda5 via the CARD-like domain, and this leads to the TBK1- and IKKi-dependent phosphorylation and activation of IRF3 and IRF7. IPS-1 also activates NF-kB via FADD/RIP1-dependent pathways. In addition, synthetic dsDNA activates type I IFN promoters, although the receptor responsible for the dsDNA recognition has not yet been identified.

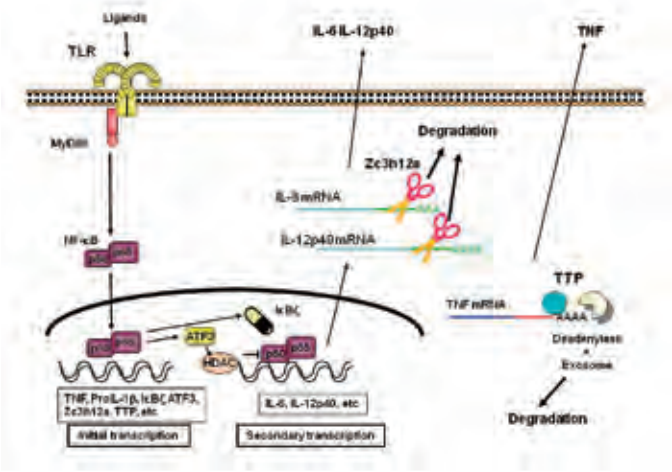


Figure 4: Mechanisms that regulate the inflammatory responses generated by TLR-inducible genes. TLR-inducible IkBz induces the transcription of genes such as Il6 via NFkBp50. In contrast, Zc3h12a, another TLR-inducible gene, functions as an Rnase that degrades the mRNAs for Il6 and Il12, among others, and negatively regulates inflammatory responses. TTP is also known to be involved in the degradation of TNF mRNA.

4) Investigation of the mechanisms that regulate inflammatory responses.
The inflammatory responses that are elicited by the activation of innate immunity are properly regulated by various mechanisms. Our recent studies revealed that TLR signal-inducible molecules further positively and negatively regulate inflammatory responses in response to infection. For example, the TLR-inducible nuclear factor IkBz functions as a transcriptional modulator that is responsible for inflammatory cytokine production (Figure 4). In contrast, TLR-inducible Zc3h12a, an RNase, destabilizes a set of mRNAs such as Il6 and negatively regulates inflammation. Mice that lack Zc3h12a spontaneously develop severe autoimmune inflammatory diseases. Currently, we are examining the posttranscriptional regulation of inflammatory responses.

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# Department of Cell Biology

Research Group

| | |
|---|---|
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| Assistant Professor | Hiroto Mizushima, Ph.D. |
| SA Researcher | Hiroki Moribe, Ph.D. |
| SA Researcher | Takashi Nakamura, Ph.D. |

We are studying cell growth and differentiation mechanisms that involve growth factors and adhesion molecules presented at cell-cell contact sites. In particular, we are focusing on the mode of action of HB-EGF, a membrane-anchored EGF family of growth factors, and the molecules to which they bind, 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 the activity of this growth factor, and therefore this process is tightly regulated. HB-EGF is secreted by various tissues and cells and functions in several physiological processes. For example, it maintains heart muscle function, suppresses the cell proliferation involved in heart valve and lung alveolar development, promotes the cell migration that participates in wound healing and eyelid closure, supports blastocyst attachment to the uterus during implantation, and promotes the cell proliferation involved in skin hyperplasia. ProHB-EGF is not only a precursor of the soluble form, it is also a biologically active molecule that regulates the growth of neighboring cells in a juxtacrine fashion. How is the conversion of the membrane-anchored form into the soluble form regulated? How does HB-EGF function in the manifold physiological processes that are dependent on this molecule? What roles do sHB-EGF and proHB-EGF play in such physiological processes? Do they participate in pathological processes? These questions are currently being analyzed at the molecular level.

2) Development of anti-cancer drugs targeting HB-EGF
HB-EGF is involved in the growth, invasion and metastasis of various cancers. We are developing new anti-cancer drugs that target HB-EGF, and pre-clinical and clinical studies assessing an anti-HB-EGF monoclonal antibody and a non-toxic mutant protein of diphtheria toxin CRM197 are currently in progress.

3) 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 C. elegans worms that lack CD9 or other tetraspanins.

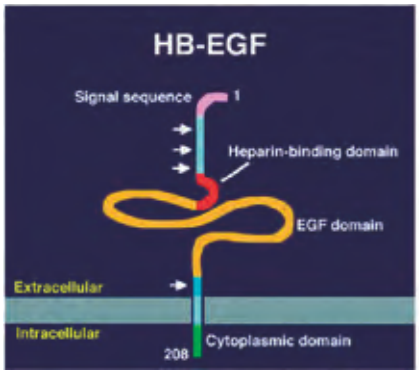


Fig. 1. Structure of proHB-EGF.

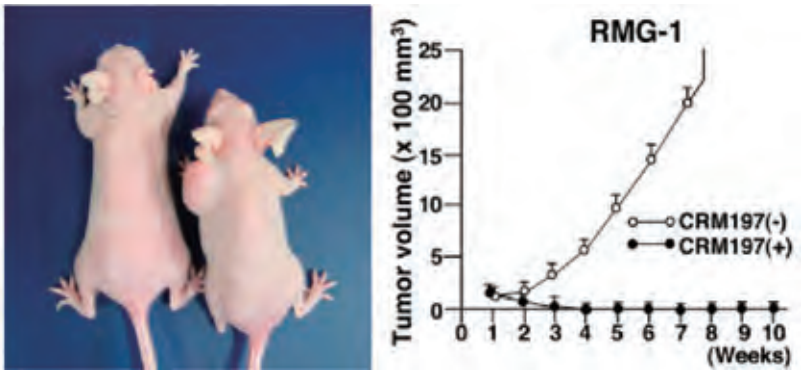


Fig. 2. Tumorigenesis in nude mice explanted with ovarian cancer cells (left) and inhibition of this tumorigenesis by CRM197, a non-toxic mutant of diphtheria toxin (right).

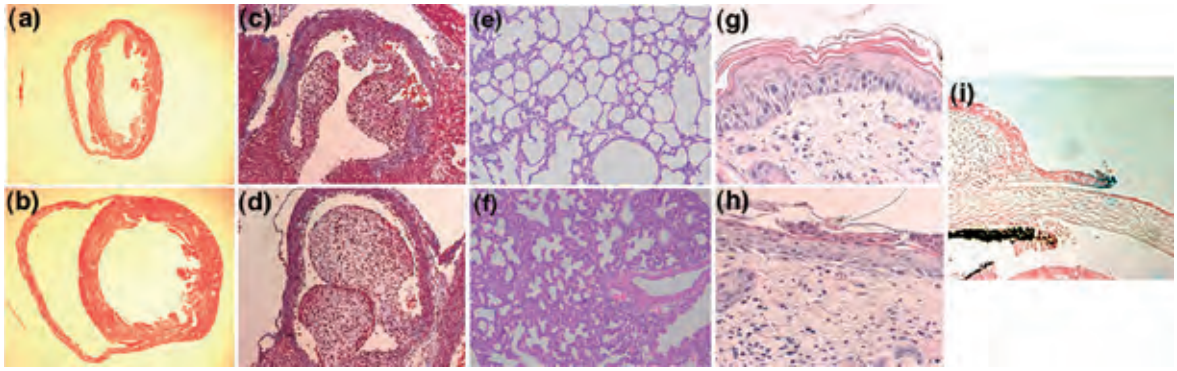


Fig. 3. HB-EGF KO mice exhibit several tissue abnormalities. Unlike WT mice (a, c, e, g), KO mice (b, d, f, h) show abnormal phenotypes in the heart (a, b), heart valves (c, d), and lung alveoli (e, f), as well as retinoid-induced skin hyperplasia (g, h). HB-EGF KO embryos also show defects in eyelid closure because HB-EGF is normally expressed at the tip of the leading edge of migrating epithelium (i).

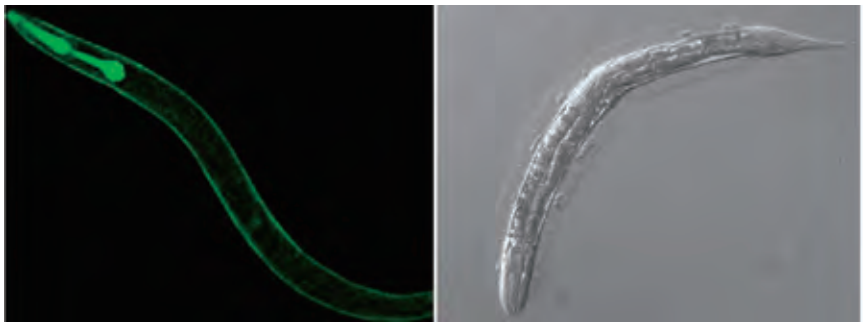

Fig. 4. Expression of TSP-15 in C. elegans (left) and reduction of tsp-15 function in C. elegans by RNA interference (right), which induces abnormalities of the hypodermis, including dissociation of the cuticle and degeneration of the hypodermis.

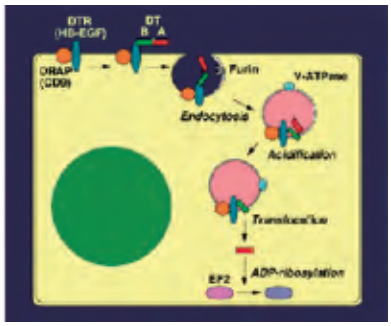


Fig. 5. Entry mechanism of diphtheria toxin.

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1. Koshikawa N, Mizushima H, Minegishi T, Iwamoto R, Mekada E, Seiki M. MT1-MMP cleaves off the NH2-terminal portion of HB-EGF and converts it into a heparin-independent growth factor. Cancer Res. 2010 in press.
2. 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 in press.
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# Department of Immunochemistry

Research Group

| | |
|---|---|
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| Assistant Professor | Tadahiro Suenaga, M.D., Ph.D. |
| Assistant Professor | Junji Uehori, Ph.D. |
| Postdoctoral Fellow | Fumiji Saito, Ph.D. |
| Postdoctoral Fellow | Kouyuki Hirayasu,Ph.D. |


Our department analyzes how pathogens such as viruses acquire the ability to evade the immune system and how host immune systems have acquired resistance to various pathogens. In particular, we are studying a fundamental host defense mechanism that acts against various pathogens by employing various immune regulatory receptors. Of particular interest are the 'paired receptors' that are expressed on various immune cells and consist of activating and inhibitory receptors (Figure 1). Our identification of the host ligands and viral ligands that these paired receptors recognize has led us to propose that these paired receptors have evolved in tandem with pathogens. In addition, we have found that these receptors are also involved in viral entry into cells. These studies will help to elucidate the fundamental mechanisms by which pathogens evade the host immune system and the host factors that shape resistance to various infections. This research will help to build the foundation that is required for the development of new vaccines and therapies for infectious diseases.

(1) Analysis of the ligands recognized by 'paired receptors' and the consequences of recognition.
Immune cells express various receptor pairs that consist of activating and inhibitory receptors that are highly homologous to each other. The inhibitory receptors recognize self-antigens such as MHC molecules. In contrast, the activating receptors generally do not recognize self-antigens and their ligands remain unclear at present. We have found that one of these paired receptors recognizes cytomegalovirus protein and we 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 to elucidate the interactions between pathogens and the host immune system.

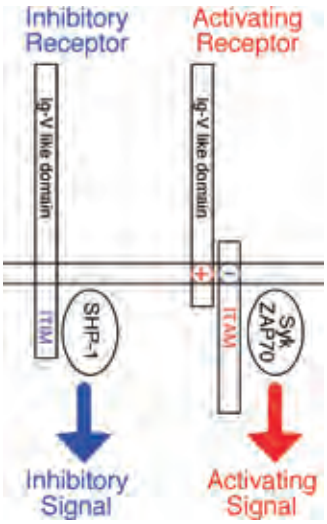


Figure 1. Paired receptors
Paired receptors consist of inhibitory and activating receptors that are highly homologous to each other. The inhibitory receptors transduce inhibitory signals via ITIM in their cytoplasmic domain, whereas the activating receptors transduce activating signals by associating with ITAM-bearing adaptor molecules.

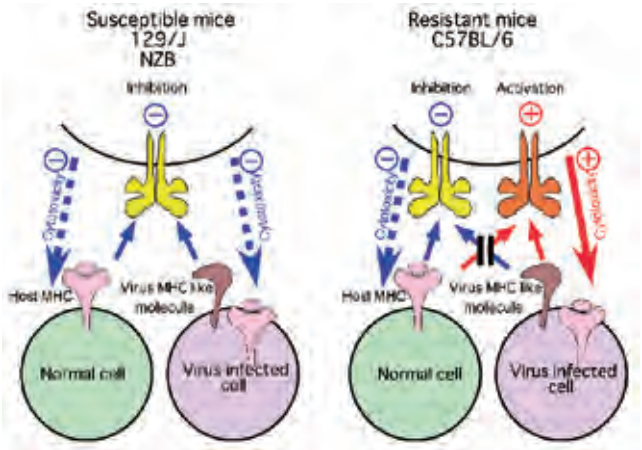


Figure 2. Recognition of cytomegalovirus-infected cells by inhibitory and activating paired receptors
Viruses have acquired MHC-like molecules that serve as ligands for inhibitory receptors expressed on the NK cells of susceptible mice strains. As a result, virus-infected cells are not killed by NK cells, even though they express MHC at low levels (left). In contrast, NK cells from resistant mouse strains do not express inhibitory receptors that recognize virus MHC-like molecules. Instead, they express activating receptors that do recognize virus MHC-like molecules; therefore, these cells can efficiently eliminate virus-infected cells (right) (Arase et al. Science 2002).

(2) Mechanisms by which viruses enter cells.

Several viruses that show persistent infection downregulate immune responses by expressing ligands that are recognized by inhibitory receptors. Interestingly, we have found that some viruses also exploit these inhibitory receptors to enter the cell. For example, such an interaction between immune receptors and viral proteins is involved in the entry mechanism of herpes simplex virus (HSV) and varicella-zoster virus (VZV) (Figure 3). Since other viruses may also use similar receptors to enter cells, we are continuing to investigate the molecular mechanisms by which viruses enter cells.

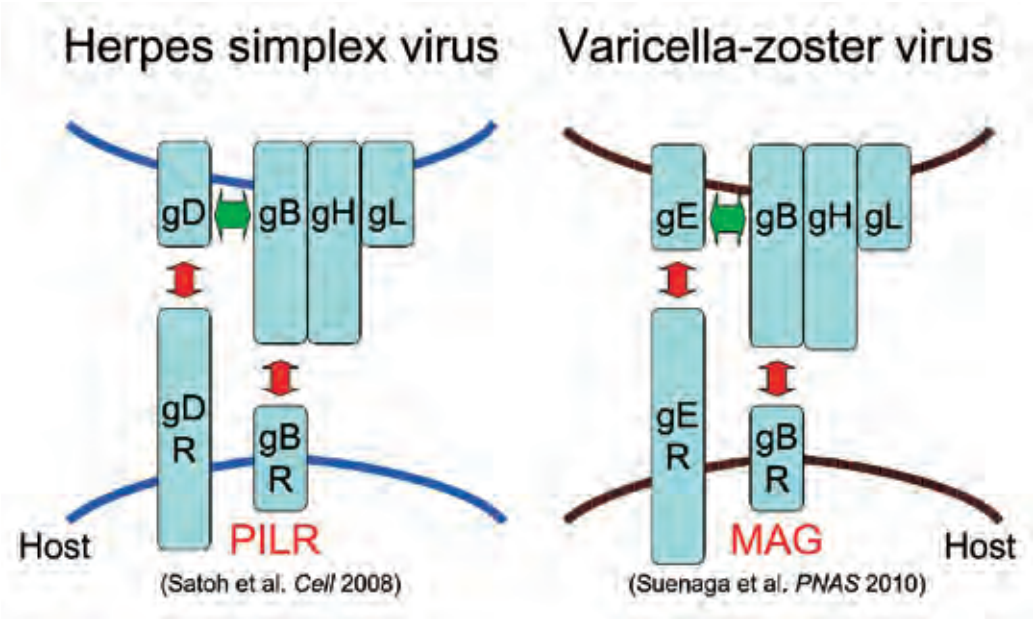


Figure 3. Mechanism by which viruses enter cells

Some viruses express ligands for inhibitory receptors and downregulate the immune response. We found that PILR$\alpha$, the inhibitory member of a receptor pair, recognizes herpes simplex virus (HSV)-infected cells. Molecular cloning of the ligands for PILR$\alpha$ revealed that PILR$\alpha$ associates with HSV glycoprotein B, which plays an essential role in HSV infection. Furthermore, the interaction between PILR$\alpha$ and glycoprotein B was found to be involved in the entry of the virus into cells. We also found that glycoprotein B of varicella-zoster virus (VZV) associates with myelin-associated glycoprotein (MAG, Siglec-4), one of paired receptors, and that this association mediates the cell entry of VZV. Thus, paired receptors play important roles in both immune regulation and the host cell entry of viruses.

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# Department of Molecular Genetics

Research Group

| | |
|---|---|
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| Assistant Professor (SUP) | Daisuke Okuzaki, Ph.D. |
| Postdoctoral Fellow | Yoko Naito, Ph.D. |


We are studying the eukaryotic cell cycle to understand the mechanism that is responsible for the chromosome instability in cancer cells. Chromosome instability is observed in cancer cells, but not in normal cells. Indeed, many human cancer cells exhibit mitotic defects (such as centrosome aberrations, abnormal spindle formation, and chromosome missegregation), and the resulting chromosome instability has been shown to be a major cause of malignant tumor progression. We are focusing on functional analyses of the Ser/Thr kinases Lats (large tumor suppressor) and GAK (cyclin G-associated kinase). These kinases localize at 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 correlation in their function (Fig. 1).


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

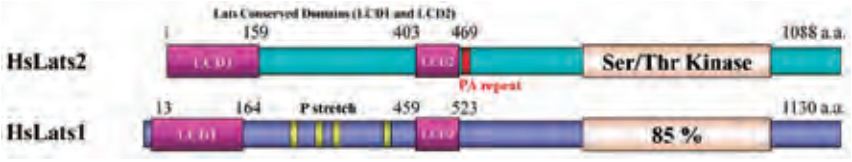


Fig. 2: The structures of the Lats1 and Lats2 proteins are similar.

In addition, to inhibit spontaneous metastasis and the growth of malignant tumors by inhibiting connexin 26, our laboratory has developed safe oleamide-derivative drugs that are associated with few side effects.

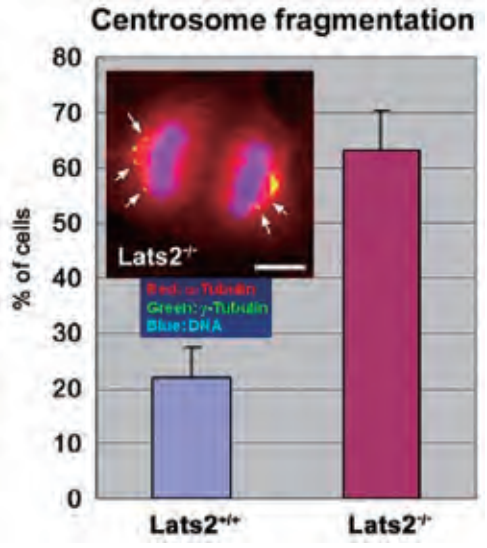


Fig. 3: Loss of Lats2 leads to centrosome fragmentation.

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 at the centrosome during the cell cycle (Fig. 2). Two miRNAs, miRNA-372 and -373, function as potential novel oncogenes in testicular germ cell tumors by inhibiting *LATS2* expression, which suggests that Lats2 is an important tumor suppressor (Voorhoeve *et al.*, Cell, 2006). Lats2 binds Mdm2, thereby inhibiting its E3 ligase activity and activating p53; in turn, p53 rapidly and selectively upregulates Lats2 expression in G2/M cells. This positive feedback loop constitutes a novel checkpoint pathway 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. 3), misalignment of the chromosome at M phase, abnormal chromosome segregation, and aberrant cytokinesis. These results indicate the essential role Lats2 plays in proper M phase progression. (C) Aurora-A phosphorylates Lats2 on three distinct serines during mitosis; Lats2 localizes at the centrosome, the mitotic spindle, or the spindle midzone during the cell cycle depending on which site is phosphorylated. (D) Down-regulation of Lats2 by siRNA causes the mislocalization of the chromosomal passenger complex (CPC) during the metaphase/anaphase transition, with the consequence that cytokinesis fails. These observations suggest that the Aurora-A-Lats2-CPC axis is a novel pathway that regulates proper cytokinesis. (E) *Lats1*/*Lats2* knockout mice show arrested development at a very early stage of embryogenesis.

(2) GAK Group:

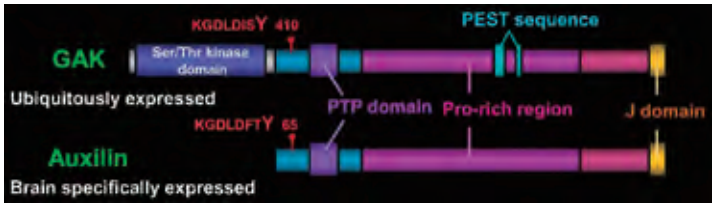


Fig. 4: GAK is similar to auxilin except for bearing a kinase domain.

GAK is an association partner of clathrin heavy chain (CHC) and is essential for clathrin-mediated membrane trafficking. Unlike neuron-specific auxilin, which plays a similar role in neural cells, GAK has a kinase domain (Fig. 4) whose function has remained unclear. We have discovered the following: (A) GAK forms the KBG(KBG1 and KBG2) complex with PP2A B' $\gamma^1$ and cyclin G (cyclin G1 and cyclin G2), which regulate the dephosphorylation activity of PP2A. (B) GAK localizes not only in the cytoplasm but also at the nucleus, where it has two additional functions, namely the maintenance of proper centrosome maturation and mitotic chromosome congression. (C) GAK knockdown by siRNA causes cell cycle arrest at the 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. 5) (D) CHC is involved in this regulatory process since GAK functions cooperatively with clathrin during mitotic progression as well as during endocytosis. (Fig. 6)

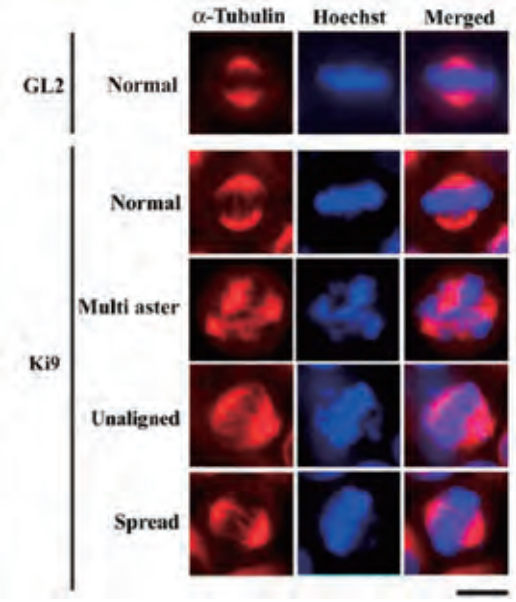


Fig. 5: GAK knockdown by siRNA (Ki9) generates abnormal chromosomes.

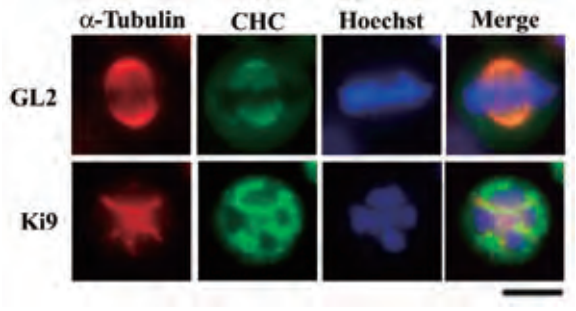


Fig. 6: GAK knockdown by siRNA (Ki9) causes the abnormal localization of CHC.

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# Department of Oncogene Research

Research Group

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|---|---|
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| Assistant Professor | Chitose Oneyama, D.Sc. |
| SA Researcher | Kentaro Kajiwara, D.Sc. |


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

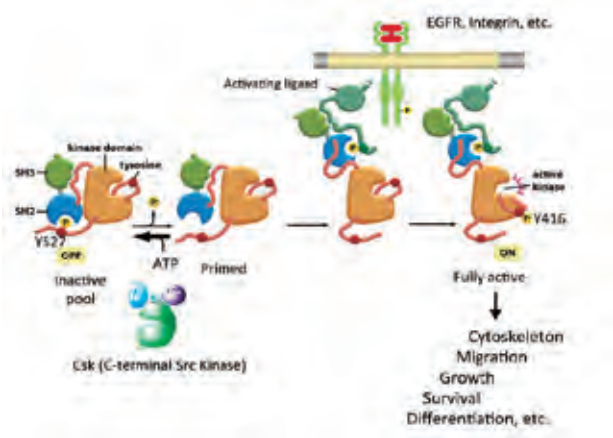


Fig. 1. Functions and regulation of c-Src.

I. Molecular mechanisms that suppress c-Src–mediated cell transformation.

In normal cells, c-Src is present in an inactive form that is phosphorylated by its negative regulatory kinase Csk. Extracellular stimuli transiently activate it (Fig. 1), after which it in turn activates downstream pathways such as the MAPK pathway, thereby inducing the gene expression that is required for cell growth and the phenotypic changes that are involved in cell transformation (Fig. 2). While the c-Src gene is rarely mutated in human cancers, its protein is frequently hyperactivated and overexpressed. This aberrant activation of c-Src is suggested to contribute to cancer malignancy (Fig. 2).

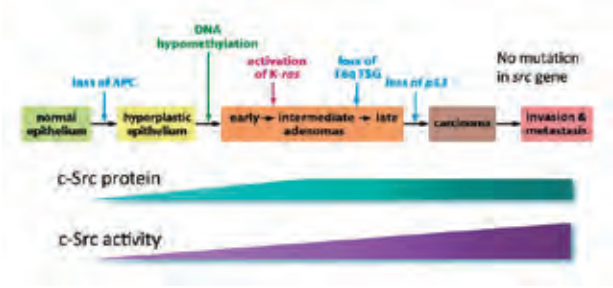


Fig. 2. c-Src and human cancer.

Recently, we found a new system by which the aberrant activation of c-Src could be suppressed. We previously showed that the inactivation of c-Src is facilitated when Csk is recruited to lipid rafts by the specific adaptor Cbp. Further analysis then revealed that Cbp can recruit activated c-Src to lipid rafts directly, and that this is sufficient for suppressing cell transformation. Furthermore, we found that the expression of Cbp is substantially downregulated in various human cancers, which suggests that the Cbp gene serves as a tumor suppressor gene (ref. 3, Fig. 3). Currently, we are analyzing the molecular mechanisms behind the downregulation of the Cbp gene in cancer cells.

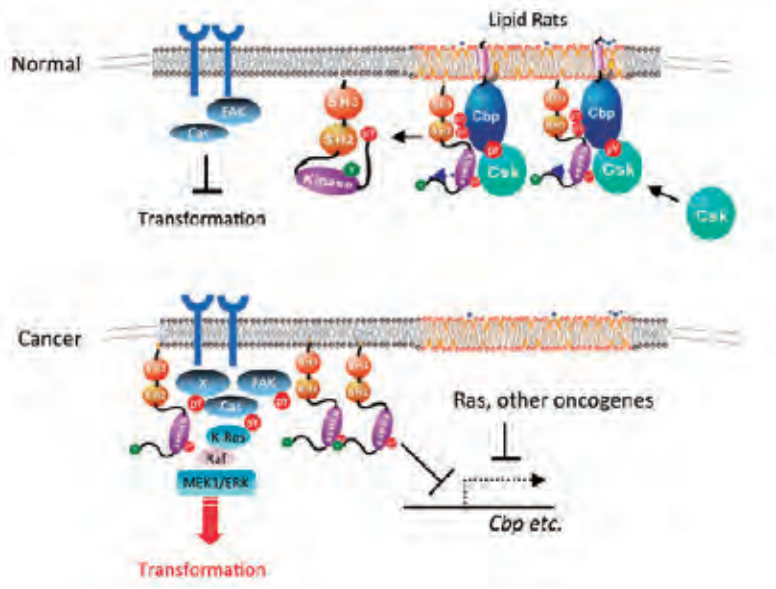


Fig. 3. Tumor-suppressing role of Cbp.

II. Cell signaling pathway of c-Src–mediated cell transformation.

To elucidate the main pathway by which c-Src induces malignant transformation, we searched for new targets of c-Src. Recently, one such potential c-Src target was found to be the novel adaptor protein p18, which is exclusively localized at the lipid rafts of late endosomes. p18 can recruit a branch of MAPK pathway to late endosomes by directly binding the p14/MP1 complex, which is known to be a specific scaffold of MEK1. Analyses of p18 KO mice (embryonic lethal), p18 KO cells, and epidermis-specific p18 KO mice revealed that p18 plays a pivotal role in endosome dynamics by regulating membrane fusion between endosomal vesicles. More recently, we also found that the p18-dependent MAPK pathway is essential for the cell transformation induced by Src, K-Ras and Pak1 (ref. 2, Fig. 4). We are currently analyzing the details of this pathway. We have also started a project that seeks to identify anti-cancer agents that target the p18-dependent pathway.

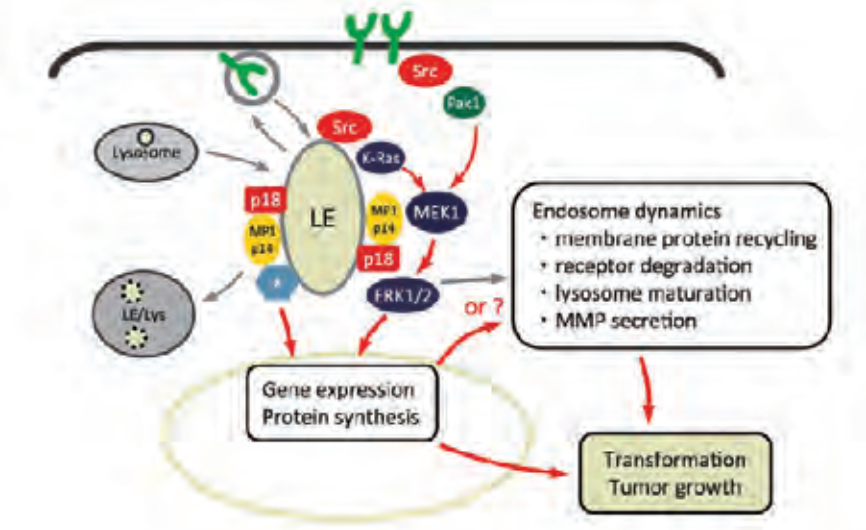


Fig. 4. Roles played by the p18-MAPK pathway in endosome dynamics and cancer growth.

## Recent publications


1. Oneyama C, Iino T, Saito K, Suzuki K, Ogawa A, Okada M. Transforming potential of Src family kinases is limited by the cholesterol-enriched membrane microdomain. Mol Cell Biol. 2009 Dec;29(24):6462-72.
2. Nada S, Hondo A, Kasai A, Koike M, Saito K, Uchiyama Y, Okada M. The novel lipid raft adaptor p18 controls endosome dynamics by anchoring the MEK-ERK pathway to late endosomes. EMBO J. 2009 Mar 4;28(5):477-89.
3. 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.
4. Oneyama C, Hikita T, Nada S, Okada M. Functional dissection of transformation by c-Src and v-Src. Genes Cells. 2008 Jan;13(1):1-12.
5. Yagi R, Waguri S, Sumikawa Y, Nada S, Oneyama C, Itami S, Schmedt C, Uchiyama Y, Okada M. C-terminal Src kinase controls development and maintenance of mouse squamous epithelia. EMBO J. 2007 Mar 7;26(5):1234-44.

# Department of Signal Transduction

Research Group

| | |
|---|---|
| Professor | Nobuyuki Takakura, M.D., Ph.D. |
| Research Associate | Hiroyasu Kidoya, Ph.D. |
| Postdoctoral Fellow | Yumi Kinugasa, Ph.D. |
| Postdoctoral Fellow | Hisamichi Naito, M.D., Ph.D. |
| Postdoctoral Fellow | Tomomi Mohri, Ph.D. |
| Postdoctoral Fellow | Yinglu Han, Ph.D. |

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

I. Analysis of the molecular mechanism of blood vessel formation
1) Molecular analysis of sprouting angiogenesis from preexisting vessels, with a particular focus on the Tie2 receptor.
2) Identification and characterization of adult endothelial stem cells (the CD31-positive side population cells).
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
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) Bioimaging of the niches that are inhabited by living cancer and normal stem cells.
4) Establishment of a strategy that can inhibit the formation of the vascular niche inhabited by cancer stem cells.

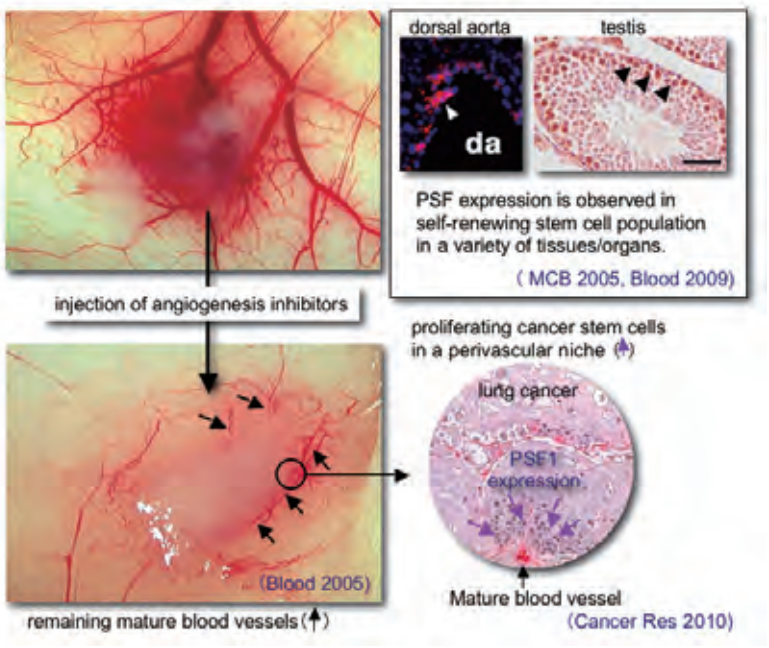


Figure 1. Vascular niche of cancer stem cells.
After treatment of a tumor with angiogenesis inhibitors, the mature blood vessels at the tumor edge persist (left panels). Cancer stem cells marked by PSF1 (a member of the GINS DNA replication factor family that is expressed by the self-renewing normal stem cell population in a variety of tissues and organ) are present and proliferate in the vascular niche represented by these mature blood vessels (right panels). Molecular analysis of vascular niche formation may be highly useful for the development of a new therapy for cancer stem cells.

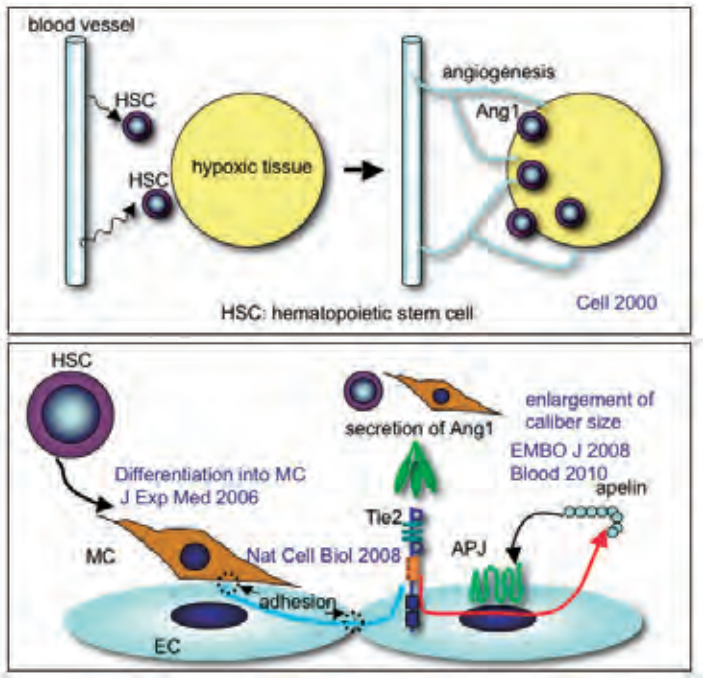


Figure 2. Maturation of blood vessels at the tumor edge.
Hematopoietic stem cells (HSCs) migrate into hypoxic tissues, produce angiopoietin-1 (Ang1), and stimulate Tie2 on endothelial cells (ECs), which results in EC migration and proliferation (upper panel). Many HSCs accumulate at the edge of the tumor during the early stage of tumorigenesis and a portion of HSCs may differentiate into mural cells (MCs) and stabilize blood vessels. Ang1 from HSCs and MCs 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.

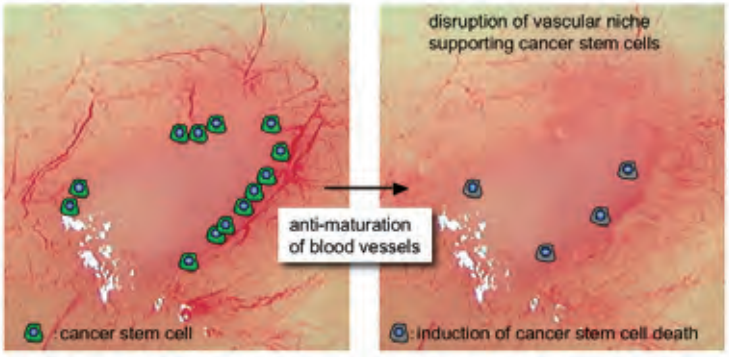


Figure 3. Disruption of the vascular niche in the tumor limb.
Our goal is to completely inhibit tumor growth by destroying mature blood vessels in the tumor environment. For this purpose, we need a drug delivery system that will deliver drugs that specifically block the blood vessel maturation functions of ECs in the tumor.

## Recent publications


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2. 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.
3. 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.
4. 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.
5. Yamada, Y., and Takakura, N. (2006). Physiological pathway of differentiation of hematopoietic stem cell population into mural cells. J Exp Med. 2006 Apr 17;203(4):1055-65.

# Department of Molecular Protozoology

Research Group

| | |
|---|---|
| Professor | Toshihiro Horii, Ph.D. |
| Assistant Professor | Nobuko Arisue, Ph.D. |
| Assistant Professor | Takahiro Tougan, Ph.D. |
| Postdoctoral Fellow | Masanori Yagi, Ph.D |
| Postdoctoral Fellow | Kohhei Tetsutani, M.D., Ph.D. |

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 (Fig. 1). Controlling malaria has become more challenging since the emergence of drug-resistant malaria parasites. This has intensified the need for novel drug target strategies and an effective malaria vaccine. Our department is focused on the development of both anti-malarial vaccines and drugs. We are also seeking to understand the mechanisms that the malaria parasite uses to survive in the host.

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

We are developing a malaria vaccine that is based on SE36, which is a recombinant protein that spans an amino acid sequence in the serine repeat antigen (SERA) of malaria parasites (Fig. 2). We and co-researchers in malaria-endemic areas have demonstrated that naturally acquired immunity against malaria correlates exclusively with the development of anti-SERA IgG3 antibodies. We have also shown that, after vaccination with SE36, many types of animals, including chimpanzees, develop antibodies that inhibit the growth of malaria parasites (Fig. 3). Together with the Kanonji Institute of the Research Foundation of Osaka University, we have constructed a system by which the SE36 malaria vaccine can be mass-produced (Fig. 4). In 2005, we conducted a phase I clinical trial in Japan with SE36 to assess its safety and immunogenicity. All vaccine-administered volunteers were sero-converted and showed no serious adverse events. We are currently in the process of conducting additional phase Ib clinical trials in an endemic region in Uganda. This project is under taken 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, in collaboration with colleagues in Uganda, Thailand, Indonesia and the Solomon Islands, we have started a new research project that aims to develop a *Plasmodium vivax* vaccine.

Fig. 1: Patients waiting at the Out-patient Department of Apac Hospital in Northern Uganda: major victims of malaria are children under the age of 5 years.

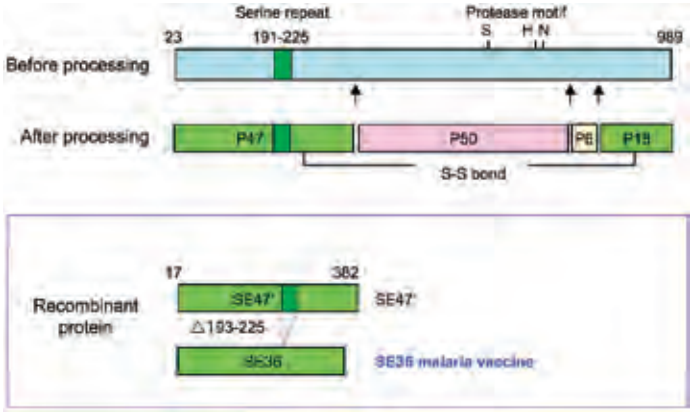


Fig. 2: Processed fragments of *P. falciparum* SERA and the structure of the recombinant SE36.

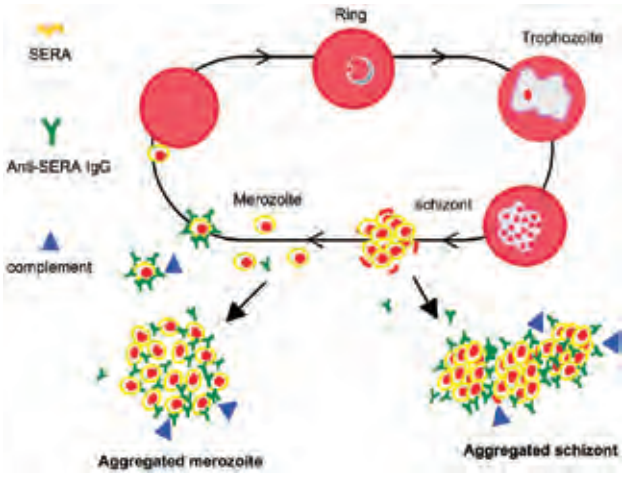


Fig. 3: Model that explains how anti-SERA IgG inhibits erythrocytic P. falciparum growth.

Fig. 4: The SE36 malaria vaccine for clinical trials is produced under Good Manufacturing Practices (GMP) at the Kanonji Institute of the Research Foundation for Microbial Diseases of Osaka University.

(2) Identification of SERA genes from several Plasmodium species.

The Plasmodium SERA gene family consists of several gene members. To trace the evolution of the SERA genes, we identified the SERA genes of several Plasmodium species and constructed the SERA gene family tree (Fig. 5). Transcription and polymorphic analyses are being used to search for functional or vaccine target molecules in the Plasmodium SERA genes.

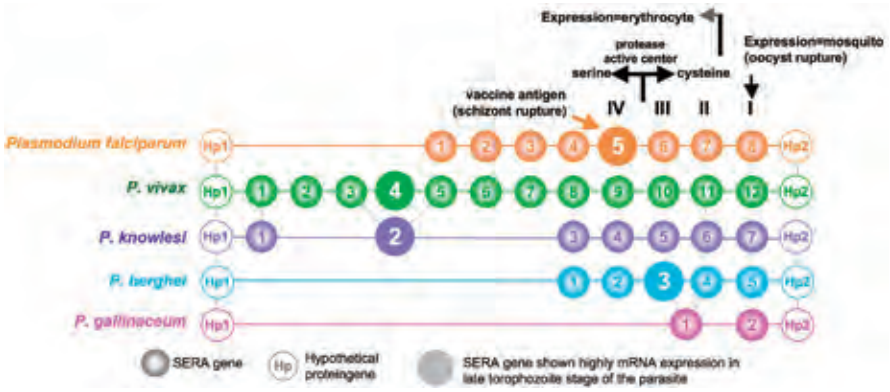


Fig. 5: An overview of the Plasmodium SERA gene family.

## Recent publications


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2. Kimata-Ariga Y, Saitoh T, Ikegami T, Horii T, Hase T. Molecular interaction of ferredoxin and ferredoxin-NADP+ reductase from human malaria parasite. J Biochem. 2007 Dec;142(6):715-20.
3. Arisue N, Hirai M, Arai M, Matsuoka H, Horii T. Phylogeny and evolution of the SERA multigene family in the genus Plasmodium. J Mol Evol. 2007 Jul;65(1):82-91.
4. Palacpac NM, Leung BW, Arisue N, Tanabe K, Sattabongkot J, Tsuboi T, Torii M, Udomsangpetch R, Horii T. *Plasmodium vivax* serine repeat antigen (SERA) multigene family exhibits similar expression patterns in independent infections. Mol Biochem Parasitol. 2006 Dec;150(2):353-8.
5. Krungkrai SR, Tokuoka K, Kusakari Y, Inoue T, Adachi H, Matsumura H, Takano K, Murakami S, Mori Y, Kai Y, Krungkrai J, Horii T. Crystallization and preliminary crystallographic analysis of orotidine 5'-monophosphate decarboxylase from the human malaria parasite *Plasmodium falciparum*. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2006 Jun 1;62(Pt 6):542-5.

# Department of Virology

Research Group

| | |
|---|---|
| Professor | Kazuyoshi Ikuta, Ph.D. |
| Associate Professor | Keizo Tomonaga, D.V.M., Ph.D. |
| Assistant Professor | Takeshi Kurosu, D.V.M., Ph.D. |
| Postdoctoral Fellow | Yohei Watanabe, D.V.M., Ph.D. |
| Postdoctoral Fellow | Yuji Inoue, Ph.D. |
| Postdoctoral Fellow | Masahiro Sasaki, Ph.D. |
| Postdoctoral Fellow | Mayo Yasugi-Ueda, D.V.M., Ph.D. |
| Postdoctoral Fellow | Juan Fernando Arias, MD, Ph.D. |

The research in this department focuses on several viruses and prions that target the immune, respiratory, and central nervous systems, with the aim of understanding the mechanisms by which they replicate and induce disease. Our ultimate objective is to devise ways to control these agents, remove them from blood products, and diagnose them rapidly.

(1) Infections of the immune system

We are working on the mechanism(s) by which HIV establishes a persistent/latent infection of the immune system. In particular, we are currently characterizing Thai and Indian patient-derived HIV subtypes, which are the most prevalent subtypes in the world. In addition, we are characterizing the mechanism by which dengue viruses derived from South Asian countries induce hemorrhagic fever.

(2) Infections of the respiratory system

We recently succeeded in preparing human neutralizing monoclonal antibodies against an influenza virus that induces typical acute infectious disease in the respiratory region. Since the epitope that is recognized by the monoclonal antibodies is highly conserved and has a conformational structure, we are working in collaboration with several companies to develop a new type of vaccine bearing this conformational epitope.

(3) Infection of the nervous system

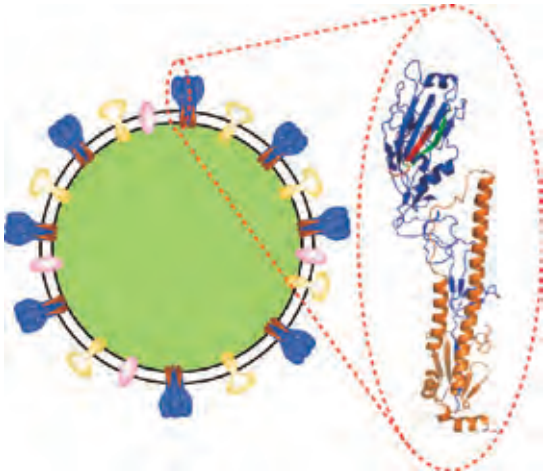
Borna disease virus (BDV) has several unique infection features, namely it exhibits highly neurotropic, noncytopathic replication and a long-lasting persistent infection. Epidemiological studies have shown that a wide variety of vertebrate species can be naturally infected with BDV. Interestingly, BDV has been suggested to be associated with human neuropsychiatric disorders. Our focus regarding this virus involves: 1) its epidemiology, 2) its replication, and 3) the mechanisms that mediate its persistence and neuropathogenesis.

(4) Blood-borne infections

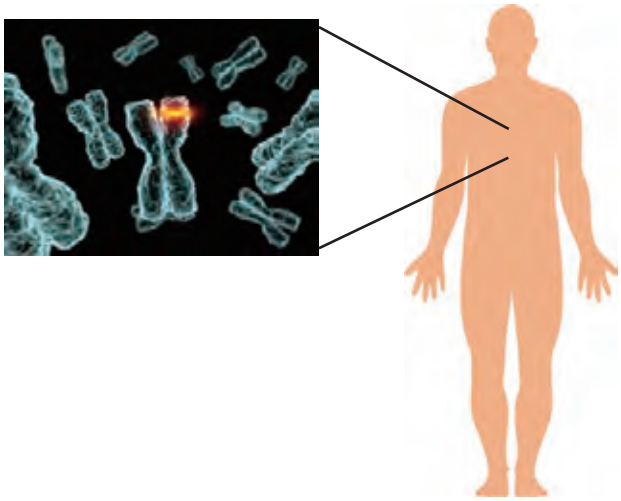
In collaboration with a company, we are working on methods to remove infectious agents such as parvovirus B19, SARS-corona virus, hepatitis E virus, and prions from blood products.

(5) Rapid diagnosis kits

There are many techniques that are used to diagnose virus infections, namely immunofluorescence, ELISA, Western blot, and PCR assays. We are currently working in collaboration with several companies to develop rapid diagnosis kits against several infectious diseases.



Human monoclonal antibodies (MAbs) that show neutralizing activity against a broad range of strains within the H3N2 subtype recognize a highly conserved conformational epitope (red and green) within the HA protein. Since similar corresponding regions are also detected in other subtypes, we are currently working on the development of a vaccine that can induce neutralizing antibodies against influenza virus.



We found bornavirus N gene-related endogenous elements in the genomes of several mammals, including humans. Such elements, known as EBLN, may encode functional proteins in some primate species.

## Recent publications


1. Horie M, Honda T, Suzuki Y, Kobayashi Y, Daito T, Oshida T, Ikuta K, Jem P, Gojobori T, Coffin JM, Tomonaga K. Endogenous non-retroviral RNA virus elements in mammalian genomes. Nature. 2010 Jan 7;463(7277):84-7.
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.
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4. Yunoki M, Kubota-Koketsu R, Urayama T, Sasaki T, Du A, Konoshima Y, Ideno S, Fukunaga Y, Morikawa S, Hiroi S, Takahashi K, Okuno Y, Hagiwara K, Ikuta K. Significant neutralizing activity of human immunoglobulin preparations against pandemic 2009 H1N1. Br J Haematol. 2009 Nov 24;143:953-955.
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# 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 were used to elucidate the mechanisms of various diseases. In the "post-genome" project era, however, genetically manipulated animals that can serve as animal models for human diseases play a key role in such investigations. Our laboratory assists other research facilities in generating such genetically manipulated animals, as shown by our web page (http://kumikae01.gen-info.osaka-u.ac.jp/EGR/index.cfm). This objective is undertaken in collaboration with the Animal Resource Center for Infectious Diseases.

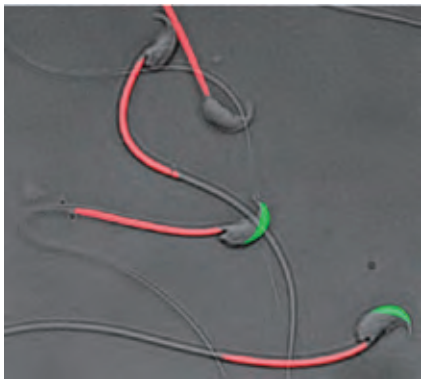


Fig. 1. A transgenic mouse line whose sperm express green fluorescent protein (GFP) in their acrosome and red fluorescent protein (RFP) in their mitochondria. This development makes it possible to obtain live images of sperm in vivo (3).

Research Projects

We were the first in the world to produce a genetically altered "green mouse" that glows in the dark. These mice are highly useful for many types of research, including stem cell transplantation and regeneration. By utilizing these animals, we showed that the sex of murine embryos can be determined at the preimplantation stage. These mice have been used to study the fertilization process (Fig. 1) (3) and the sex determination mechanism in germ cells.

We are also interested in the fertilization process in terms of self-nonself recognition. By utilizing homologous recombination technology, we showed protein IZUMO1 as the first sperm factor that plays an essential role in the fusion of sperm with eggs; we also found recently that the sperm protein SPESP1 is needed for the production of fully fusion-competent sperm (Figs. 2 and 3) (1, 2).

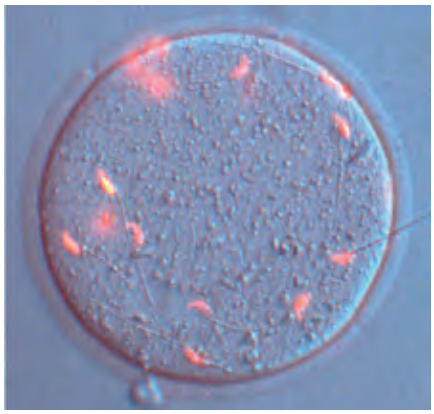


Fig. 2. Izumo1 KO sperm accumulate in the perivitelline space of the egg because they cannot fuse with the egg (1).

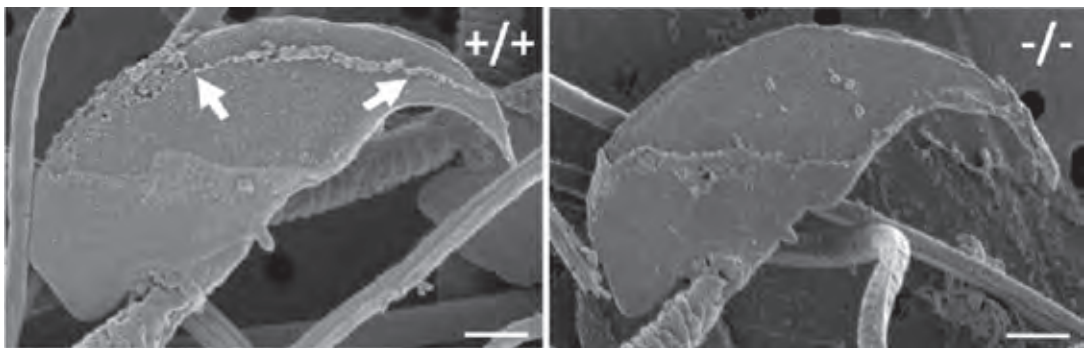



Fig. 3. The membrane of the entire equatorial segment area is detached in almost all acrosome-reacted Spesp1-deficient sperm (right) (2).

In addition to our studies on the sperm-egg interaction, we are studying the roles the placenta plays in feto-maternal immune tolerance. Since we believe gene functions are best observed in live animals, we sought a method that would permit genetic manipulation of the placenta. We were eventually successful in developing a Lentiviral vector-based method that mediates the genetic manipulation of the placenta without affecting the embryos (Fig. 4) (4).

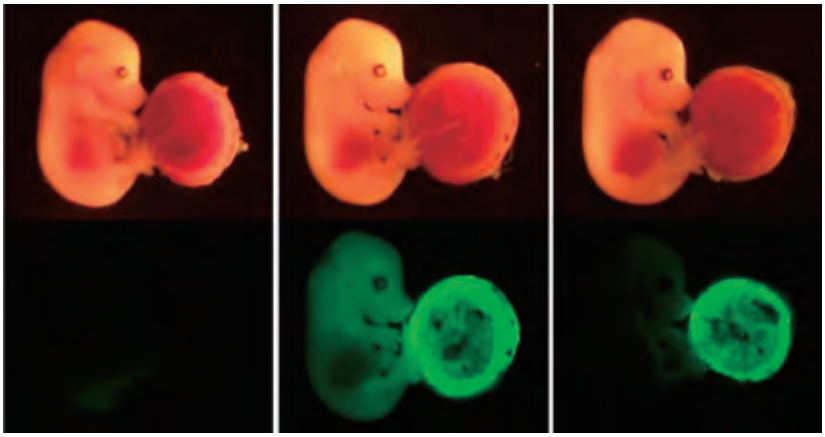


Fig. 4. Placenta-specific gene manipulation. GFP-transgene expression at E14.5 after gene manipulation. Shown are embryos in mice with (left) an untransduced placenta, (middle) a placenta that had been subjected to the normal transgenic procedure, and (right) a placenta that had been altered by our newly developed method for placenta-specific genetic manipulation (4).

We are also interested in understanding the biological function of non-coding RNA such as miRNA. The miRNA knock-out technique is being used to identify the roles miRNA plays in live animals.

Miwa's group is using genetically manipulated animals to investigate the molecular biological mechanisms that are involved in human diseases, especially cardiovascular diseases. To understand the cellular and molecular aspects of vascular smooth muscle (SM) cell growth in atherosclerotic plaques, we characterized the transcriptional mechanisms of one SM-specific gene, the SM alpha-actin (SmaA) gene. Since SmaA is also expressed in many tissues during acute inflammation, we are currently analyzing its gene expression system and its functional roles (Fig. 5) (5). We are also analyzing the molecular pathogenic mechanisms of diastolic heart failure by using a Dahl salt-sensitive rat model. Specifically, we are currently investigating how the endothelin and renin-angiotensin systems participate in the excessive hypertrophy and fibrosis that eventually leads to diastolic heart failure.

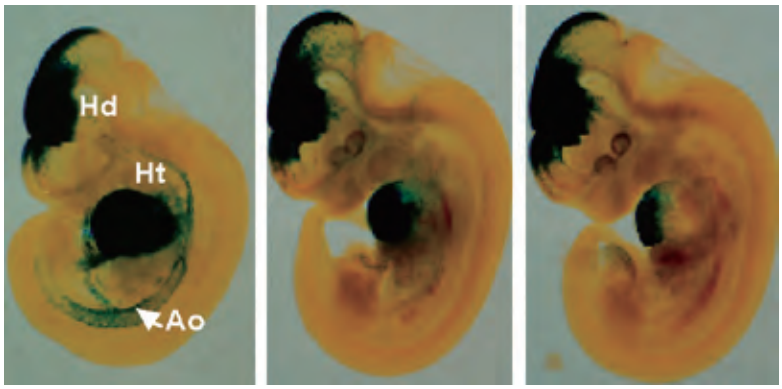



Fig. 5. The human SM alpha-actin promoter (left) expresses in the embryonic aorta. However, point mutations in the enhancer region of this promoter, including the -1M (center) and 4M (right) mutations, eliminate this specific expression pattern (5).

## Recent publications

1. Inoue N, Kasahara T, Ikawa M, Okabe M. Identification and disruption of sperm-specific angiotensin converting enzyme-3 (ACE3) in mouse. PLoS ONE. 2010. 5, e10301.
2. Fujihara Y, Murakami M, Inoue N, Satouh Y, Kaseda K, Ikawa M, Okabe M. Sperm equatorial segment protein 1, SPESP1, is required for fully fertile sperm in mouse. J Cell Sci. 2010. 123, 1531-1536.
3. Hasuwa H, Muro Y, Ikawa M, Kato N, Tsujimoto Y, Okabe M. Transgenic mouse sperm that have green acrosome and red mitochondria allow visualization of sperm and their acrosome reaction in vivo. Exp Anim. 2010. 59,105-107.
4. Okada Y, Ueshin Y, Isotani A, Saito-Fujita T, Nakashima H, Kimura K, Mizoguchi A, Oh-hora M, Mori Y, Oagata M, Oshima RG, Okabe M, Ikawa M. Complementation of placental defects and embryonic lethality by trophoblast-specific lentiviral gene transfer. Nat Biotechnol. 2007. 25, 233-237.
5. Takeji M, Moriyama T, Oseto S, Kawada N, Hori M, Imai E, Miwa T. Smooth muscle a-actin deficiency in myofibroblasts leads to enhanced renal tissue fibrosis. J Biol. Chem. 2006. 281,40193-40200.

# 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 (SUP) | Norihito Kawashita, Ph.D. |
| | SA Researcher | U. Chandimal de Silva, M.Sc. |

We use high performance computers to study the genome information of various organisms in an effort to identify new biological phenomena and to understand how organisms evolve. In addition, we develop software tools for bioinformatics and molecular biology. We also operate a computer system that can be used to analyze genome sequence data. This system is available to researchers in our university and we hold training courses in genome analysis at least once every year.

(1) Large-scale analysis of genomes

Today, the complete genome sequences of more than 1,000 organisms are available. We are currently analyzing this enormous body of genome data by using bioinformatics and molecular evolution techniques. We are also developing software and algorithms that facilitate large-scale genome analysis. These include CONSERV, a conserved sequence finder. When this algorithm was used to analyze the complete genome sequences of 266 organisms, it identified invariant sequences that may have been present in the last common ancestor of all extant life forms (Goto et al, 2007; Fig. 1). In addition, we are striving to understand the evolutionary pathways of the influenza virus by comprehensively analyzing its genome (Fig. 2).

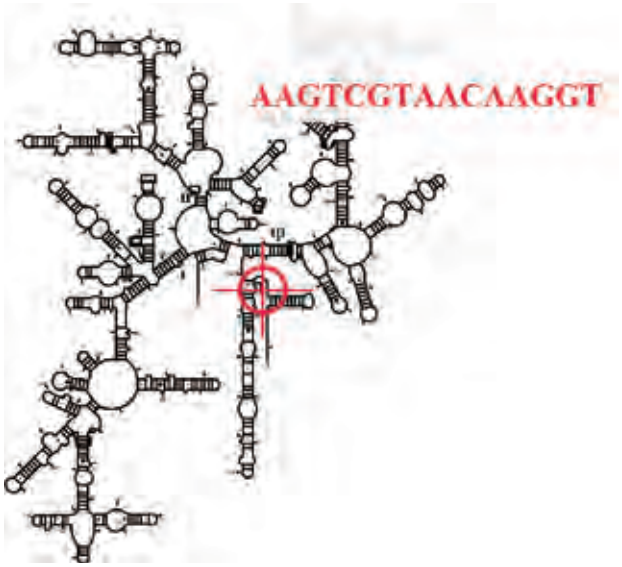



Figure 1. Large-scale genome analysis of 266 organisms revealed a sequence that is conserved in almost all known genomes.

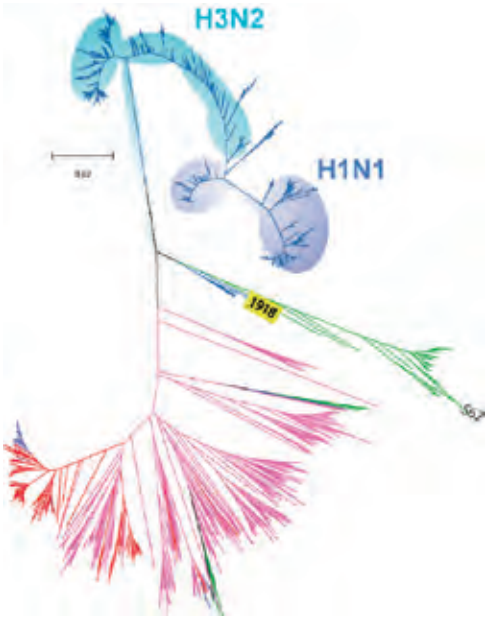



Figure 2. Comprehensive analysis of the influenza virus genome.

(2) Next generation sequencer data analysis

The recently developed "next generation sequencing" technology has made it possible to sequence the entire genome of any microorganism in one sequencer run. Thus, each run produces a massive amount of nucleotide sequence data. We are developing software that can handle this data and have set up an analysis system that is used for microorganism sequencing projects that are performed in collaboration with other laboratories (Fig. 3).

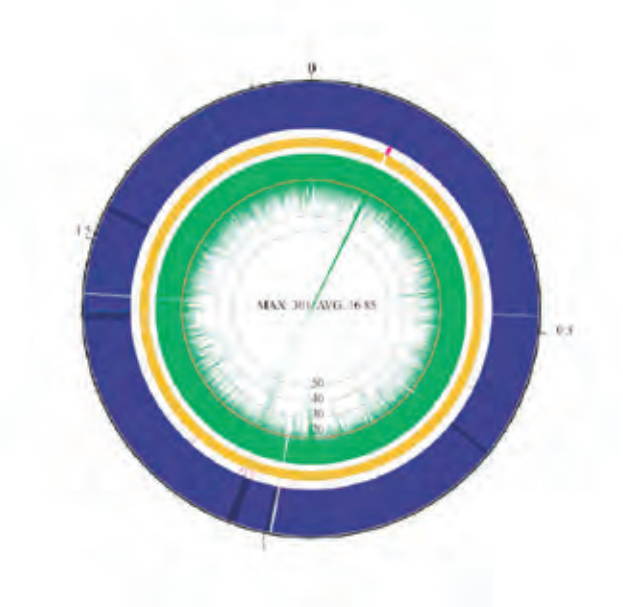


Figure 3. Next generation sequencing enables sequencing of an entire genome in one run.

Figure 4. Genome Information Research Center Computer System.

(3) Operation of a computer system that permits the analysis of genome information 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.

## Recent publications


1. 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.
2. 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.
3. 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.
4. Yoshida M, Yamashita A, Idoji Y, Nishiguchi S, Shimada K, Yasunaga T, Yamanishi H. In silico study of a novel gene evolved from an ancestral SVIP gene and highly expressed in the adult mouse testes. Int J Mol Med. 2008 Aug;22(2):143-8.
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. |
| | SA Associate Professor (SUP) | Takaaki Nakaya, Ph.D. |
| | Assistant Professor (SUP) | Naohisa Goto, Ph.D. |
| | SA Assistant Professor | Shota Nakamura, Ph.D. |

1. RAPID (Robotics Assisted Pathogen IDentification)
Under the aegis of the Program of Research Centers for Emerging and Re-emerging Infectious Diseases of MEXT, Japan, we are constructing, in collaboration with the Omics Science Center, RIKEN, a framework called "RAPID" that will facilitate the emergency diagnosis of infectious diseases. We are also cooperating with the research centers of eight countries in Asia and Africa in an effort to identify the causative agents in naturally occurring outbreaks.
2. Metagenomic Diagnosis of Infectious Diseases
Metagenomic analysis allows us to diagnose many of the major human infectious diseases (including respiratory tract infections, enteric infections, and blood-borne infections) by using a single common protocol. In addition, to pre-empt zoonotic disease outbreaks, we are seeking to identify new pathogenic microorganisms in animal-derived samples that may have zoonotic potential.
3. Metagenomic Analysis of the Intestinal Microbiome
The intestinal microbiome plays an important role in protecting the host from pathogen invasion. We are currently analyzing the intestinal microbiome of patients with diarrheal diseases to elucidate how the human host, the intestinal microbiome, and pathogenic microorganisms interact. This will help us to understand the changes that take place 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 that amplify the genome of pathogenic microorganisms and subtract the host genome.

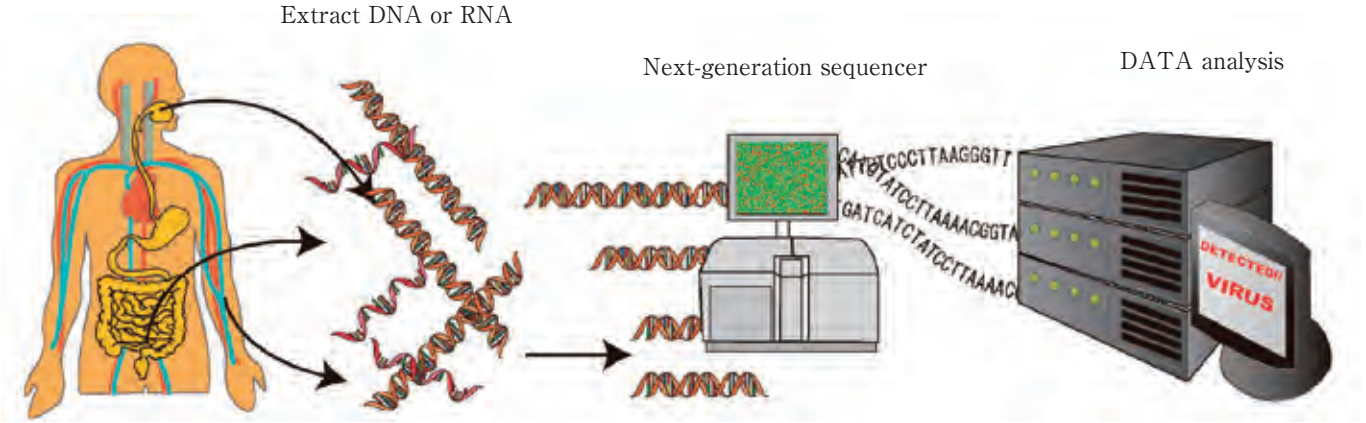



Fig. 1. Metagenomic diagnosis of infectious diseases using a next-generation sequencer.

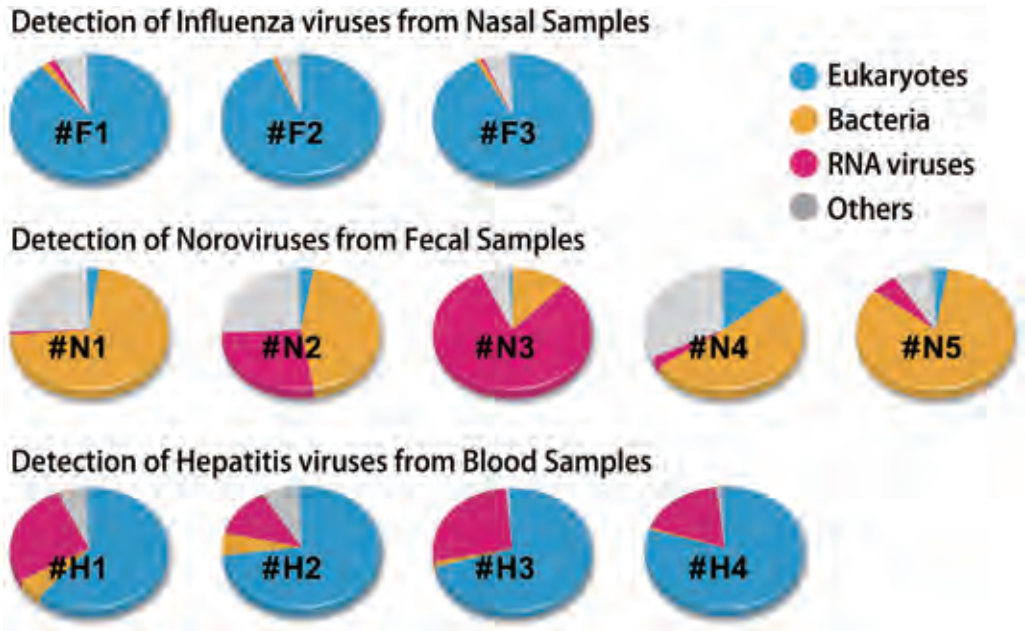



Fig. 2. Distribution of detected organisms in viral infection cases

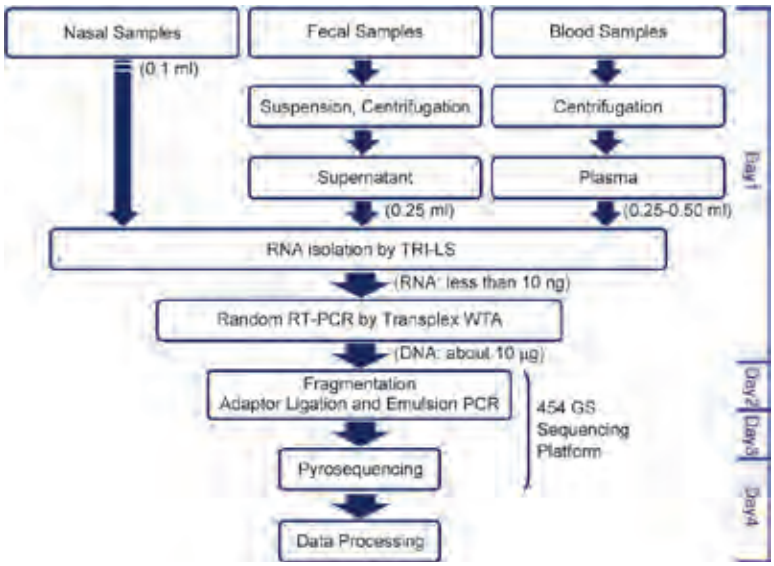



Fig. 3. Standard operating protocol for RAPID

Fig. 4. Next-generation sequencer, 454 GS Junior System

## Recent publications

1. Nakamura S, Yang CS, Sakon N, Ueda M, Tougan T, Yamashita A, Goto N, TakahashiK, 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.
2. Nakamura S, Maeda N, Miron IM, Yoh M, Izutsu K, Kataoka C, Honda T, YasunagaT, 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

| | |
|---|---|
| SA Professor | Kazunori Oishi M.D., Ph.D. |
| Assistant Professor | Yukihiro Akeda Ph.D. |
| Postdoctoral Fellow | Tatsuya Nakayama Ph.D. |
| Postdoctoral Fellow | Zhenyu Piao Ph.D. |
| Research Staff | Yumi Hattori |


The research activities in our department aim to 1) investigate the epidemiology, pathogenesis and prevention by vaccines of pneumonia and invasive bacterial infections, 2) elucidate the mechanisms by which dengue viruses induce disease, and 3) analyze the 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, when necessary, will join the outbreak response team in the global effort to control emerging and re-emerging infectious diseases.

1) Epidemiology, pathogenesis, and vaccine-mediated prevention of pneumonia and invasive bacterial infections

1. Study of pneumonia in Thailand
We have conducted a study project entitled "Surveillance of emerging respiratory infections and analysis of mechanism of secondary bacterial pneumonia in Thailand". This project allowed us to investigate how virus-host-bacteria interactions promote secondary bacterial infections in pediatric patients with pneumonia. In 2009, we examined the clinical features of 24 adult cases of pandemic H1N1 influenza-associated severe community-acquired pneumonia at Buddachinaraj Hospital, Phitsanulok.

2. Clinical applications of the 23-valent pneumococcal polysaccharide vaccine (PPV) and the development of new pneumococcal vaccines
a) Clinical application of 23-valent PPV
We found that the combined PPV and influenza vaccine (IV) vaccination program reduced the incidence of acute exacerbation in patients with chronic obstructive pulmonary diseases (Vaccine, 2008). An open-label, randomized study was conducted involving 786 Japanese subjects older than 65 years of age who were receiving a routine IV. Concomitant PPV vaccination significantly reduced the number of admissions and medical costs for all-cause pneumonia for subjects older than 75 years. We also started a project in 2008 that examines the effects of PPV in combination with IV on long-term–care residents. Our goal is the nationwide and routine vaccination of the elderly in Japan.
b) Development of a nasal mucosal pneumococcal vaccine
Pneumococcal surface protein A (PspA) is known to elicit protective antibodies in animals. We have demonstrated the effects of PspA plus TLR agonist on bacterial clearance in a mouse model of pneumococcal pneumonia (Vaccine, 2009). In addition, we have shown that the PspA nasal vaccine is effective in a mouse model of secondary pneumonia after influenza virus infection.

3. Research on *Streptococcus suis* infections, which are prevalent in Thailand
*Streptococcus suis* is an important zoonotic pathogen that causes invasive infections such as meningitis in humans who are in close contact with infected pigs or contaminated pork-derived products. The number of such human cases is rapidly increasing in Thailand because of a tradition of consuming raw pork or blood in the north. We showed that the clinical manifestations of serotype 2 infections are related to the genotypic profiles of the isolates; we also reported the clonal dissemination in humans of serotype 14, which has been a rarely occurring serotype up until now (Figure 1). We are currently developing an epidemiological study of S. suis infections in Phayao Province as a project of RCC-DMSc.

2) Mechanisms by which dengue virus infections lead to thrombocytopenia
Dengue illness has become a major public health concern, particularly in tropical countries. We have conducted a number of clinical studies in the Philippines and recently found in an ex vivo setting that patients with thrombocytopenia during acute phase secondary dengue virus infections showed increased phagocytosis of platelets (AJTMH, 2009). Since we found that IVIG treatment did not significantly hasten the recovery from thrombocytopenia in such patients (AJTMH, 2007), this suggests that the Fc$\gamma$ receptor is not involved in platelet phagocytosis by macrophages. We are currently elucidating the novel mechanism by which the platelets are phagocytosed in this disease.

3) Protein secretion systems of pathogenic bacteria
The development of bacterial infections requires many virulence factors. Since most are proteins that are secreted by the pathogenic bacteria, it is essential to study the mechanisms by which proteins are secreted by pathogenic bacteria during the course of infection. We are using the food-borne pathogen *Vibrio parahaemolyticus* and a causative agent of pneumonia, *Streptococcus pneumoniae*, to study such protein secretion systems and the secreted virulence factors.

4) Response to emerging and re-emerging infectious diseases
The objective of GOARN is to combat the international spread of infectious disease outbreaks by ensuring that appropriate technical assistance reaches the affected areas rapidly and by promoting long-term epidemic preparedness. Our group is registered as a member of GOARN and our team will join the WHO-organized response team when there is an outbreak of infectious diseases in developing countries.

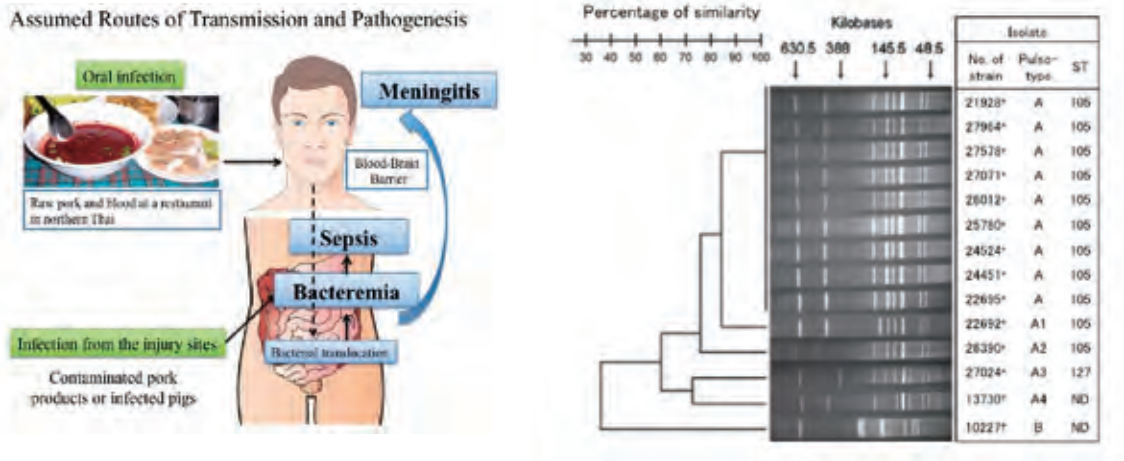



Figure 1. Assumed routes of transmission and pathogenesis of S. suis infection, and the clonal dissemination of serotype 14 infections in Thailand (J Med Microbiol, 2009).

## Recent publications


1. Kerdsin A, Oishi K. Sripakdee S, Boonkerd N, Polwichai P, Nakamura S, Uchida R, Sawanpanyalert P, Dejsirilert S. Clonal Dissemination of Streptococcus suis serotype 14 in Thailand. J Med Microbiol. 2009 Nov;58(Pt 11):1508-13.
2. Honda S, Saito M, EM Dimaano, Morales PA, Alonzo MTG, Suarez LC, Koike N, Inoue S, Kumatori A, Matias RR, Natividad FF, Oishi K. Increased platelet phagocytosis from patients with secondary dengue virus Infection by human macrophages. Am J Trop Med Hyg. 2009 May;80(5):841-5.
3. Akeda Y, Okayama K, Kimura T, Dryselius R, Kodama T, Oishi K, Iida T, Honda T. Identification and characterization of a type III secretion-associated chaperone in the type III secretion 1 of Vibrio parahaemolyticus. FEMS Microbiol Lett. 2009 Jul;296(1):18-25.
4. Oma K, Zhao J, Ezoe H, Akeda Y, Koyama S, Ishii KJ. Kataoka K, Oishi K. Intranasal immunization with a mixture of PspA and a Toll-like receptor agonist induces specific antibodies and enhances bacterial clearance in the airways of mice. Vaccine. 2009 May 21;27(24):3181-8.
5. Chen M, Ssali F, Mulungi M, Awio P, Yoshimine H, Kuroki R, Furumoto A. Tanimura S, Kityo C, Nagatake T, Mugyenyi P, Oishi K. Induction of opsonophagocytic killing activity with pneumococcal conjugate vaccine in human immunodeficiency virus-infected Ugandan adults. Vaccine. 2008 Sep 8;26(38):4962-8.
6. Furumoto A, Ohkusa Y, Chen M, Kawakami K, Masaki H, Sueyasu Y, Iwanaga T, Aizawa H, Nagatake T, Oishi K. Additive effect of pneumococcal vaccine and influenza vaccine on acute exacerbation in patients with chronic lung disease. Vaccine. 2008 Aug 5;26(33):4284-9.

# Laboratory for Infection Cell Biology

Research Group

| | |
|---|---|
| SA Associate Professor | Yukako Fujinaga, Ph.D. |
| SA Researcher | Yo Sugawara, Ph.D. |
| Postdoctoral Fellow | Takuhiro Matsumura, Ph.D. |


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

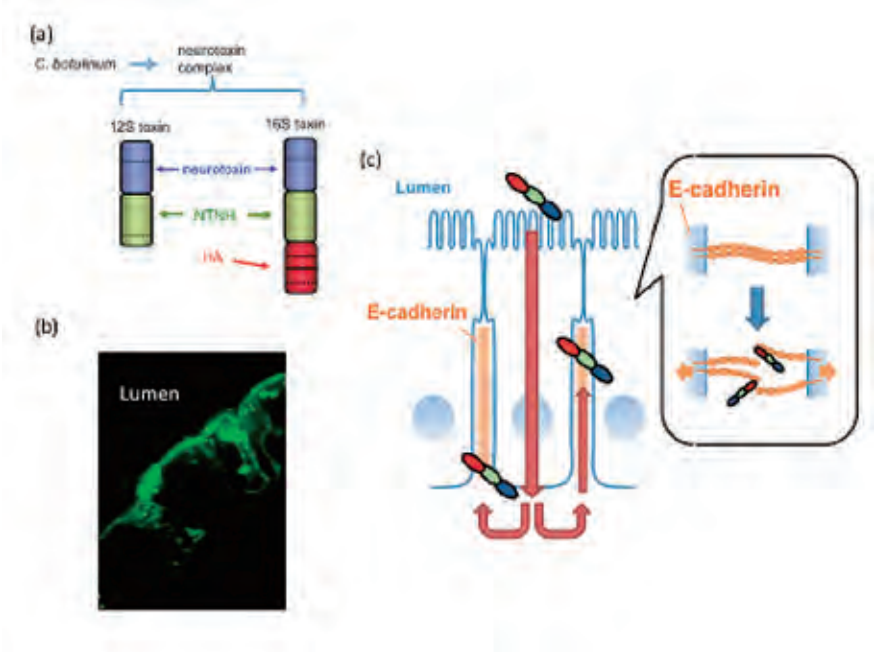



Figure (a) Schematic depiction of the botulinum neurotoxin complex (16S and 12S toxins). Orally ingested neurotoxin complexes cross the intestinal epithelial barrier to cause food-borne botulism. (b) 16S toxin (green) penetrates the intestinal epithelium. (c) Interaction of botulinum neurotoxin complexes with the intestinal epithelial barrier. The HA of the botulinum neurotoxin complex binds E-cadherin and disrupts E-cadherin–mediated cell-to-cell adhesion, thereby disrupting the epithelial paracellular barrier.

## Recent publications

1. 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., (in press)
2. 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 Jan; 155(Pt 1): 35-45. [1] These authors are contributed equally.
3. 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 Feb; 10(2): 355-364.
4. 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 Jan 26; 352(4), 867-872.
5. Fujinaga Y. Transport of bacterial toxins into target cells: Pathways followed by cholera toxin and botulinum progenitor toxin. J Biochem. 2006 Aug; 140(2), 155-160.

# Laboratory for Viral Pathogenesis and Immunity

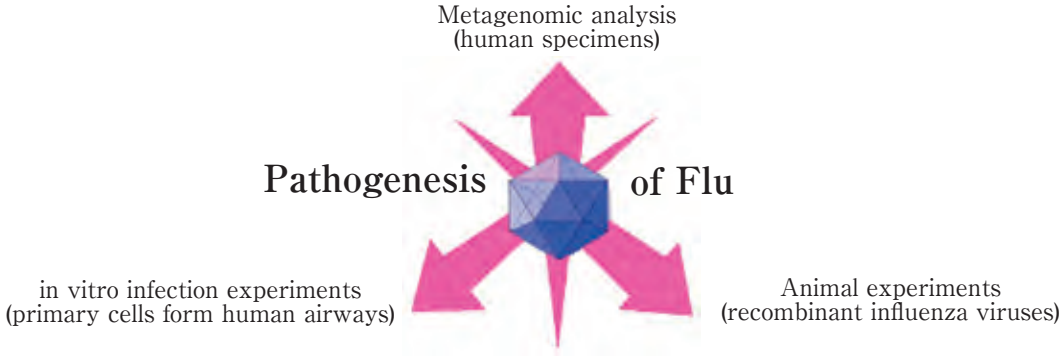
Research Group
SA Associate Professor Takaaki Nakaya, Ph.D.
Postdoctoral Fellow Tomo Daidoji, D.V.M., Ph.D.

◆ Molecular mechanism of H5N1 avian influenza virus pathogenesis

In recent years, the highly pathogenic avian influenza virus (AIV) H5N1 emerged from southeast Asia and raised serious worldwide concern about the risk of an influenza pandemic. However, how H5N1 induces disease remains poorly understood. We are using in vitro and animal experiments to study the role the viral glycoprotein hemagglutinin (HA) plays in viral growth and cell toxicity. Recent achievements are listed below:

By genetically changing H5 AIV by recombinant DNA techniques, H5N1-HA has been shown to be one of the major viral factors that determine lethality in mice. We confirmed that the pathogenicity of HA depends on its cleavage sequence, which is consistent with previous observations. However, our further experiments suggest that other region(s) and amino acids of H5N1-HA may also participate in the pathogenicity of H5N1.

That H5N1-HA can induce significant cellular toxicity was demonstrated by in vitro experiments. We showed that H5N1-HA–specific cell toxicity (apoptosis) was observed in porcine as well as human primary airway epithelial cells. Similar results were also observed in primary cells from a water fowl known to be a natural AIV host. In contrast, HA proteins from previously isolated AIVs, including the H5 subtype, did not induce this severe cell toxicity. Thus, the genotype of HA may be critical for the pathogenicity and/or cellular toxicity of H5N1 AIVs.




◆ Metagenomic analysis of viral pathogens in humans: Development of Pathogen Identification System by using a high-throughput "Next-Generation" DNA sequencer (RAPID system; Department of Infection Metagenomics)

We are establishing a protocol to rapidly obtain the whole genome information of viral pathogens. This is expected to significantly accelerate the speed with which pathogens can be identified. Using this protocol, we have successfully demonstrated the presence of pathogenic microbes in clinical human samples without resorting to conventional selective procedures for specific pathogens.

## Recent publications

1. Daidoji T, Kaihatsu K, Nakaya T. The role of apoptosis in influenza virus pathogenesis and the mechanisms involved in anti-influenza therapies. Curr Chem Biol [Review, in press]
2. Ueda M, Daidoji T, Du A, Yang C-S, Ibrahim M-S, Ikuta K, Nakaya T. Highly pathogenic H5N1 avian influenza virus induces extracellular Ca2+ influx, leading to apoptosis in avian cells. J Virol. 2010 84(6):3068-78.
3. Nakamura S, Yang C-S, 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. Du A, Daidoji T, Koma T, Ibrahim M-S, Nakamura S, de Silva U-C, Ueda M, Yang C-S, Yasunaga T, Ikuta K, Nakaya T. Detection of circulating Asian H5N1 viruses by a newly established monoclonal antibody. Biochem Biophys Res Commun. 2009 378(2):197-202.
5. Daidoji T, Koma T, Du A, Yang C-S, Ueda M, Ikuta K, Nakaya T. H5N1 avian influenza virus induces apoptotic cell death in mammalian airway epithelial cells. J Virol. 2008 82(22): 11294-307.

# Laboratory of Genomic Research on Pathogenic Bacteria

Research Group

| | |
|---|---|
| SA Professor | Tetsuya Iida, Ph.D. |
| Postdoctoral Fellow | Kaori Izutsu, Ph.D. |
| Postdoctoral Fellow | Shigeaki Matsuda, Ph.D. |
| Postdoctoral Fellow | Natsumi Okada, Ph.D. |
| Postdoctoral Fellow | Hirotaka Hiyoshi, Ph.D. |


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

Our main research targets are as follows:

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

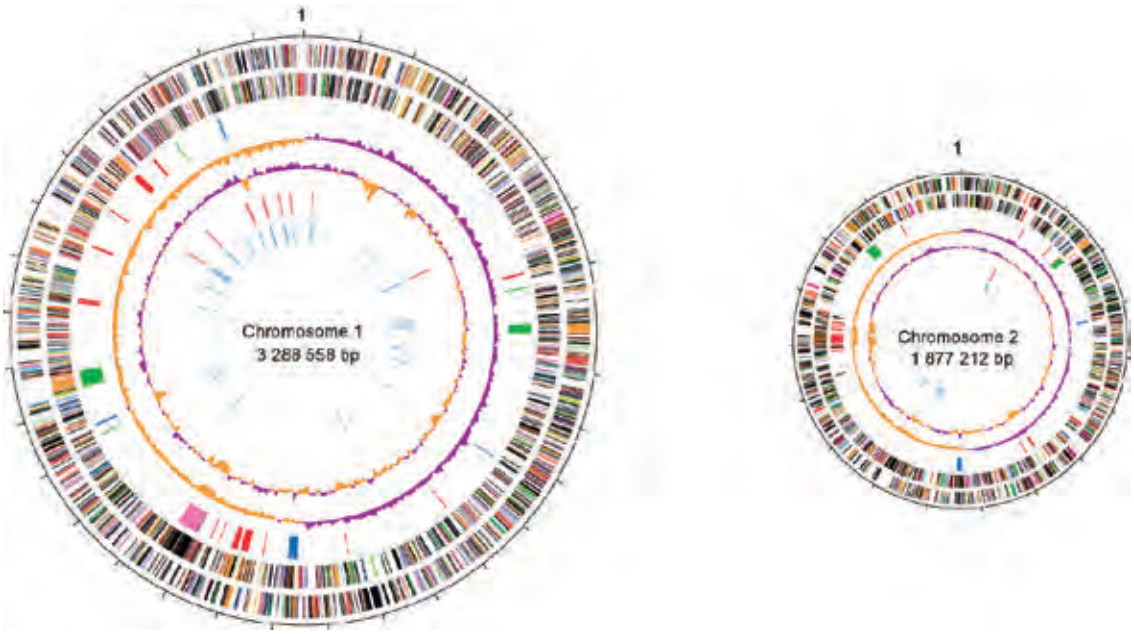



Figure 1. Whole genome sequence of *Vibrio parahaemolyticus*

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

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

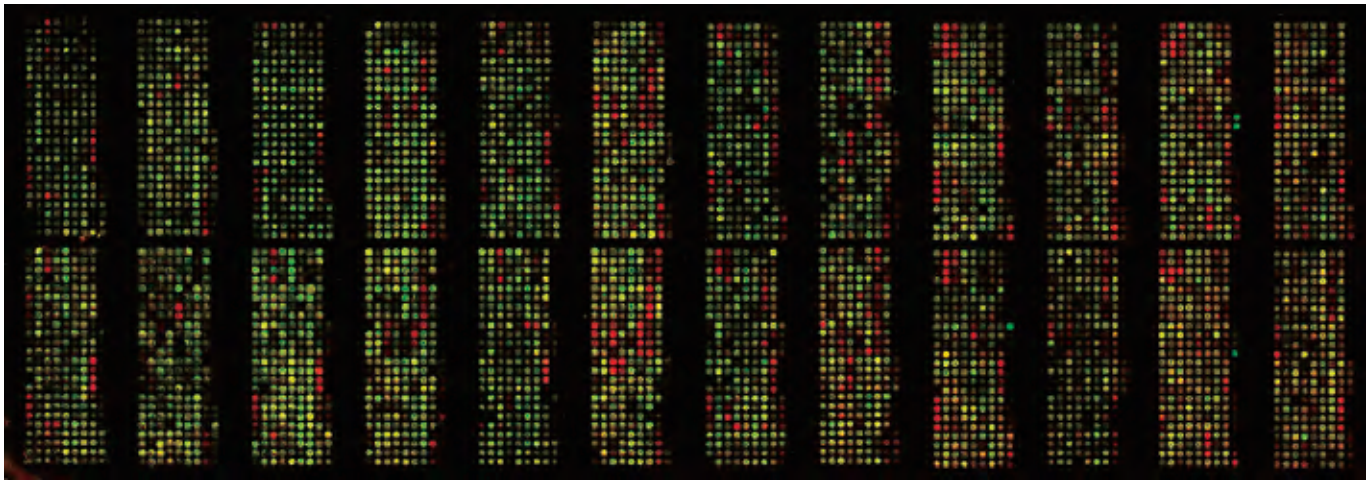


Figure 2. Characterization and comparison of the genomes of pathogenic bacteria by using DNA microarrays

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

## Recent publications


1. Kodama T, Gotoh K, Hiyoshi H, Morita M, Izutsu K, Akeda Y, Park KS, Cantarelli VV, Dryselius R, Iida T, Honda T. Two regulators of *Vibrio parahaemolyticus* play important roles in enterotoxicity by controlling the expression of genes in the Vp-PAI region. PLoS One. 2010 Jan 13;5(1):e8678.
2. Okada N, Iida T, Park KS, Goto N, Yasunaga T, Hiyoshi H, Matsuda S, Kodama T, Honda T. Identification and characterization of a novel type III secretion system in *trh*-positive *Vibrio parahaemolyticus* strain TH3996 reveal genetic lineage and diversity of pathogenic machinery beyond the species level. Infect Immun. 2009 Feb;77(2):904-13.
3. 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.
4. 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.
5. Kodama T, Rokuda M, Park KS, Cantarelli VV, Matsuda S, Iida T, Honda T. Identification and characterization of VopT, a novel ADP-ribosyltransferase effector protein secreted via the *Vibrio parahaemolyticus* type III secretion system 2. Cell Microbiol. 2007 Nov;9(11):2598-609.

# Laboratory of Malariology

| Research Group | | |
|---|---|---|
| | Invited Professor | Kazuyuki Tanabe, D. Sc., D. Med. Sc. |
| | Postdoctoral Fellow | Kenji Hikosaka, D. Agr. Si. |
| | Postdoctoral Fellow | Shin-ichiro Tachibana, D. Agr. Si. |
| | JSPS PD Fellow | Hajime Honma, D. Agr. Si. |

Malaria imposes a huge burden on human health. We are studying the genetic diversity of malaria parasites in order to elucidate their history and adaptive evolution mechanisms.

(1) Population genetics of malaria parasites

The human malaria parasite *Plasmodium falciparum* infects only humans. Consequently, the evolution of this parasite is presumed to be intimately associated with humans. To examine this association, we are investigating the genetic diversity of *P. falciparum* populations from diverse geographic areas. We have observed a clear negative correlation between the within-population genetic diversity and the geographic distance from East Africa to Asia and Oceania, which mirrors the isolation-by-distance of modern humans. Age estimates support the notion that modern humans carried the parasite along during their colonization of the world.

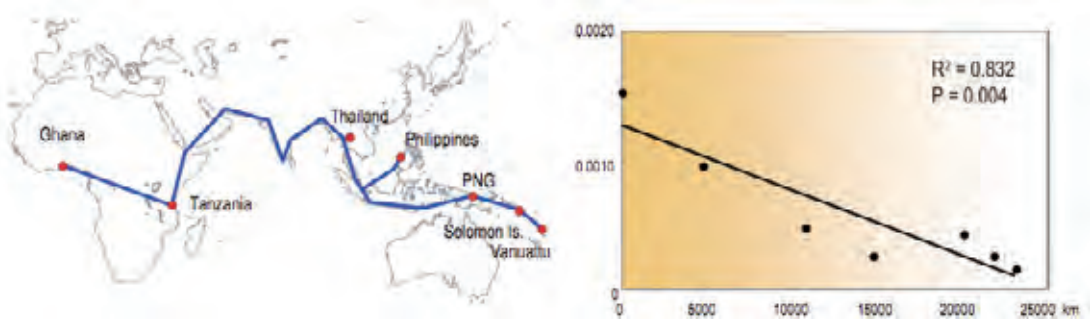



Fig. 1. Negative correlation between the within-population genetic diversity of *P. falciparum* and the geographic distance from East Africa to Asia and Oceania. This suggests that the parasite spread together with modern humans during their out-of-Africa colonization.

(2) The evolution of antigenic polymorphism in malaria parasites

One way malaria parasites evade the host's immune system is through antigenic polymorphism. We are investigating human parasite populations from diverse geographic areas, as well as monkey malaria parasites, to reveal the evolutionary origins and population genetic mechanisms that drive antigenic polymorphism in immune-target genes.

(3) Co-evolution between malaria parasites and their hosts

While malaria parasites infect all classes of terrestrial vertebrates (mammals, birds, and reptiles), each parasite species infects only one specific host. Such wide host range and high host specificity indicate that malaria parasites have a close relationship with their hosts. To examine this parasite-host co-evolution, we compared the evolutionary histories of malaria parasites and their hosts. We found that all of the extant lineages of malaria parasites originated during a period of rapid diversification that involved host switches.

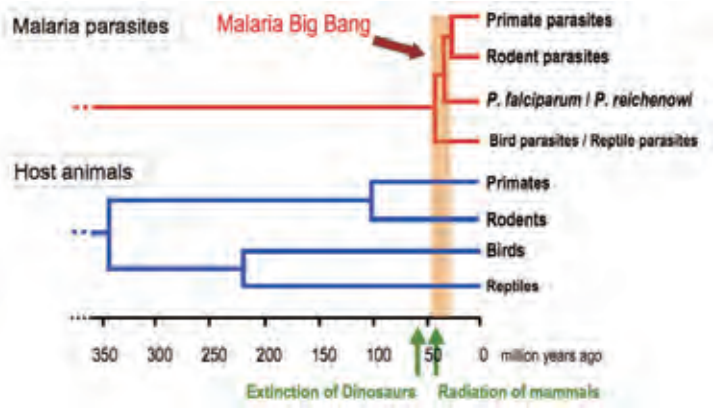



Fig. 2. Malaria big bang in the evolution of extant malaria parasites. The diversification (beige bar) occurred much later than the emergence of host animals, which suggests that host switching led to the diversification of the parasite lineage.

(4) Genome sequencing of a *P. vivax*-related monkey malaria parasite, *P. cynomolgi*

The human malaria parasite *P. vivax* became a parasite of humans by host switching from a monkey. To clarify the genetic changes that are specific to *P. vivax*, we are genome sequencing a *P. vivax*-related monkey malaria parasite, *P. cynomolgi*. The sequencing is currently at the gap closure and annotation step, and a draft genome will be complete soon. Comparative genomics has already revealed gene gains/losses that are specific to *P. vivax*.

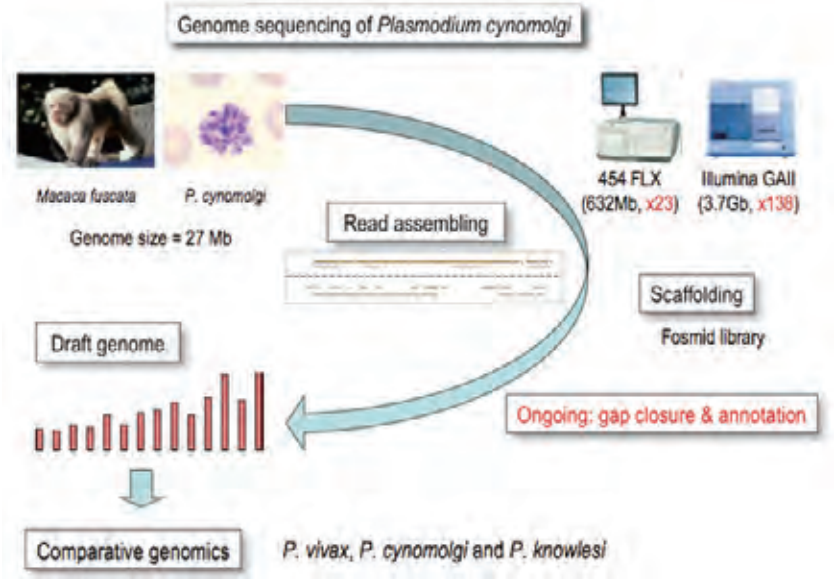



Fig. 3. Genome sequencing of *P. cynomolgi*, a monkey malaria parasite that is closely related to *P. vivax*, by using the new sequencing system (in collaboration with Dr. S. Kawai at Dokkyo Med. Univ.).

## Recent publications


1. Tanabe K, Mita T, Jombart T, Eriksson A, Horibe S, Palacpac N, Randord-Cartwright L, Sawai H, Sakiyama N, Ohmae H, Nakamura M, Ferreira MU, Escalante AA, Prugnolle F, Björkman A, Färnert A, Kaneko A, Horii T, Manica A, Kishino H, Balloux F. *Plasmodium falciparum* accompanied the human expansion out of Africa. Curr. Biol. 2010 (in press).
2. Hikosaka K, Watanabe Y, Tsuji N, Kita K, Kishine H, Arisue N, Palacpac N M Q, Kawazu S, Sawai H, Horii T, Igarashi I, Tanabe K. Divergence of mitochondrial genome structure in the apicomplexan parasites, *Babesia* and *Theileria*. Mol Biol Evol. 2010 May; 27 (5): 1107-16.
3. Sawai H, Otani H, Arisue N, Palacpac N, de Oliveira Martins L, Pathirana S, Handunnetti S, Kawai S, Kishino H, Horii T, Tanabe K. Lineage-specific positive selection at the merozoite surface protein 1 (*msp1*) locus of *Plasmodium vivax* and related simian malaria parasites. BMC Evol Biol. 2010 Feb 19; 10: 52.
4. Culleton R, Ndounga M, Zeyrek F Y, Coban C, Casimiro P N, Takeo S, Tsuboi T, Yadava A, Carter R, Tanabe K. Evidence for the transmission of *Plasmodium vivax* in the Republic of Congo, West Central Africa. J Infect Dis. 2009 Nov 1; 200 (9): 1465-9.
5. Hayakawa T, Culleton R, Otani H, Horii T, Tanabe K. Big bang in the evolution of extant malaria parasites. Mol Biol Evol. 2008 Oct; 25 (10): 2233-9.

# Laboratory of Combined Research on Microbiology and Immunology

Research Group SA Associate Professor Hiroki Nagai, Ph.D.

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 to deliver effector proteins, and this 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 their 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.
There is essentially nothing 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 working towards the structural and functional analysis of the type IV secretion apparatus from Legionella.

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

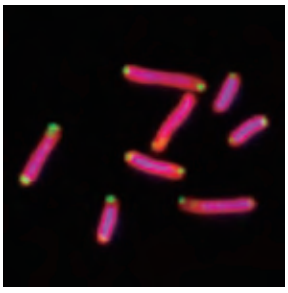


Fig. 1 Type IV apparatus localizes to bacterial poles (Green).

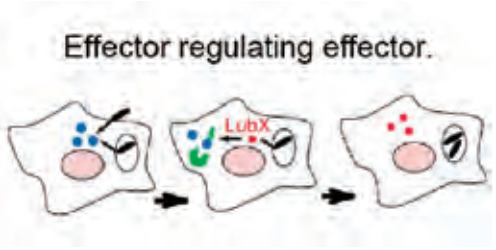



Fig. 2 Discovery of metaeffector.

## Recent publications

1. 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.
2. Nagai, H., Cambronne, E.D., Kagan, J.C., Amor, J.C., Kahn, R.A. and Roy, C.R.. A C-terminal translocation signal required for Dot/Icm-dependent delivery of the Legionella RalF protein to host cells. Proc. Natl. Acad. Sci. USA. 2005;102,826-831.
3. Amor,J.C., Swails, J., Roy, C.R., Nagai, H., Ingmundson, A., Cheng, X., and Kahn, R.A. The structure of RalF, an ARF guanine nucleotide exchange factor from Legionella pneumophila, reveals the presence of a cap over the active site. J. Biol. Chem, 2005;280, 1392-1400.

# Office of Combined Program on Microbiology and Immunology

| | | | |
|---|---|---|---|
| Research Group | 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 in the fields of microbiology and immunology, respectively. These institutes are located next to each other. To take maximum advantage of this situation, our office works to encourage cross-disciplinary microbiology and immunology research as follows:

Research promotion

To directly promote cross-disciplinary microbiology and immunology research, we are implementing the following plans.

1. Organization of the Awaji international forum on infection and immunology, which is held in September annually.
2. Organization of the research progress report, which is produced every two months by our institute.
3. Organization of the symposium and the research presentation, which are held annually.
4. Organization of interactive projects that involve the Institut Pasteur in France, Chonnam University in Korea, and the Research Collaboration Center on Emerging and Re-emerging Infections in Thailand.

These activities aim to facilitate cooperative research on microbiology and immunology by promoting research collaboration, information exchange, personal exchange between laboratories, and preparing the research environment.

Education promotion

To facilitate seamless cross-disciplinary research on microbiology and immunology, we also direct a multidisciplinary graduate program on microbiology and immunology. This task includes designing the curriculum and its contents. We also organize an Open House of the institute and guide new students.

Research Group　Associate Professor　Yoshiko Murakami, MD. Ph.D

I have an additional appointment in the Department of Immunoregulation, where I serve as the leader of the PNH group. This group is performing the following studies (see details on the Department page):

1. Investigation of the pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH), an acquired glycosylphosphatidylinositol (GPI) deficiency.
2. Investigation of the pathogenesis of inherited GPI deficiency.
3. Investigation of the functional significance of GPI-anchored proteins by using the Pgap3 KO mouse in which GPI-anchored proteins fail to localize within the raft due to defective GPI anchor remodeling.

## Recent publications

1. Sena CB, Fukuda T, Miyanagi K, Matsumoto S, Kobayashi K, Murakami Y, Maeda Y, Kinoshita T, Morita YS. Controlled expression of branch-forming mannosyltransferase is critical for mycobacterial lipoarabinomannan biosynthesis. J Biol Chem. 2010 Mar 9. doi: 10.1074/jbc.M109.077297
2. 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.
3. 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).
4. Almeida AM*, Murakami Y*, Layton M, Hillmen P, Sellick G, Maeda Y, Richards S, Patterson S, Kotsianidis I, Mollica L, Crawford D, Baker A, Ferguson M, Roberts I, Houlston R, Kinoshita T, Karadimitris A. Hypomorphic promoter mutation in the mannosyltransferase-encoding PIG-M gene causes inherited glycosylphosphatidylinositol deficiency. Nat. Med., 12:846-851. 2006(* equally contributed).
5. Inoue N, Izui-Sarumaru T, Murakami Y, Endo Y, Nishimura J, Kurokawa K, Kuwayama M, Shime H, Machii T, Kanakura Y, Meyers G, Wittwer C, Chen Z, Babcock W, Frei-Lahr D, Parker C, Kinoshita T. Molecular basis of clonal expansion of hematopoiesis in two patients with paroxysmal nocturnal hemoglobinuria (PNH). Blood 2006 108:4232-4236.

# Fujii Group

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

We are developing novel technologies to address important questions in biology. In addition, we are analyzing mechanisms that regulate the immune system by using state-of-the-art transgenic/knock-out/knock-in technologies.

I. Development of novel technologies to elucidate fundamental principles of the immune system

(1) We developed the inducible translocation trap (ITT) system to identify the signal-induced nuclear translocation of signaling proteins (Fig. 1). ITT is the first non-protein–specific technology that can identify nuclear-translocating proteins; it also enables the analysis of the "translocatome", namely the entire set of proteins that translocate to the nucleus in response to a defined extracellular stimulus. We will use ITT to (i) identify and characterize signal-induced nuclear-translocating proteins, (ii) perform high-throughput screening of small compounds that affect the nuclear translocation of particular signaling proteins, and (iii) screen an RNAi library to identify proteins that regulate the nuclear translocation of signaling proteins.

(2) We are developing the insertional chromatin immunoprecipitation (iChIP) system to isolate specific genomic regions that retain their in vivo conformation. This system will enable us to perform unbiased molecular biological and biochemical analyses of the chromatin structure of specific genomic regions and to identify the molecules (proteins, DNA, RNA, and others) that interact with these regions. iChIP will also help us to elucidate molecular transcriptional regulation, cell differentiation, and lineage commitment mechanisms, especially those involved in lymphocyte development.

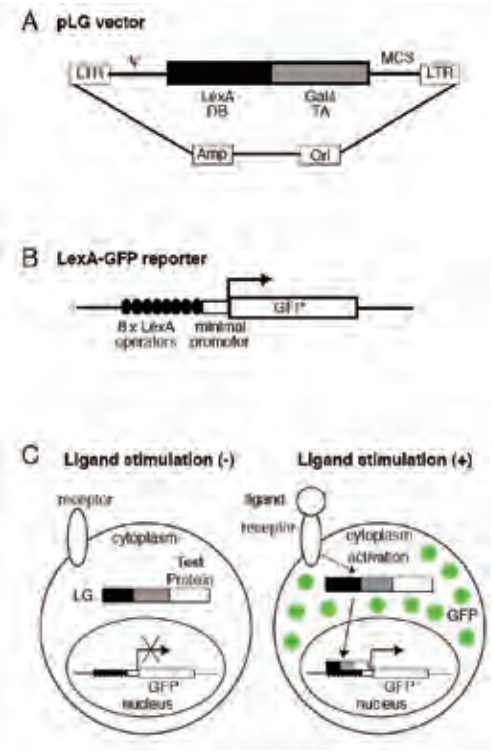



Figure 1. The inducible translocation trap system.

II. Analysis of immune regulation mechanisms and development of therapies for autoimmune diseases

(1) We identified a novel phosphorylated nuclear protein, Cyclon, whose expression is induced in T cells when they are activated. We found that Cyclon regulates the activation-induced cell death of T cells by modulating the expression levels of Fas (Fig. 2). We are currently using transgenic and gene-deficient mice to analyze the in vivo functions of Cyclon and the molecular mechanisms by which it regulates Fas expression. The knowledge generated by these studies will be used to develop effective therapies of autoimmune diseases.

(2) We identified GARP, an activated T-regulatory cell (T-reg)-specific cell surface molecule, and showed that it plays an important role in the immune suppression that is mediated by T-regs. We are currently elucidating the in vivo function of GARP by using transgenic and gene-deficient mice.

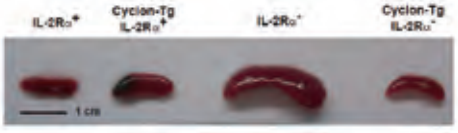



Figure 2. Normalization of the splenomegaly in interleukin-2 receptor $\alpha$-chain–deficient mice by the transgenic expression of Cyclon.

## Recent publications

1. Hoshino A, Fujii H. Insertional chromatin immunoprecipitation: a method for isolating specific genomic regions. J Biosci Bioeng. 2009 Nov;108(5):446-9.
2. 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.
3. Wang R, Kozhaya L, Khaitan A, Fujii H, Unutmaz D. Expression of GARP selectively identifies activated human Foxp3+ regulatory T cells. Proc Natl Acad Sci U S A. 2009 Aug 11;106(32):13439-44.
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 Shigeyuki Hamada, D.D.S., Ph.D.

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


To this end, Osaka University founded the Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI) in the Thai National Institute of Health (NIH), Department of Medical Sciences, Ministry of Public Health of Thailand in 2005.

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

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

P2-level laboratory

P3-level laboratory

# Section of Bacterial Infections

Research Group

| | |
|---|---|
| SA Professor | Shigeyuki Hamada, D.D.S., Ph.D. |
| SA Lecturer | Yumi Kumagai, Ph.D. |
| SA Researcher | Kazuhisa Okada, Ph.D. |
| SA Researcher | Natsuko Kishishita, Ph.D. |
| Research Fellow | Amonrattana Roobthaisong, M.Sc. |
| Research Fellow | Chetsada Boonthimat, M.Sc. |

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

It has been reported that pneumonia, tuberculosis, and acute diarrheal diseases are associated with high morbidity and mortality rates in Thailand. Consequently, in the Program of Promotion of Research Network for Emerging and Reemerging Infectious Diseases during the 2010–2014 fiscal years, we prioritize research on enteric infectious diseases in Thailand that are caused by *Vibirio cholerae* and diarrheagenic *E. coli*.

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

Cholera surveillance in the border of Thailand and Myanmar

## Recent publications


1. 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. May; 82(5):875-8.
2. 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.Apr;138(4):519-24.
3. 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. Feb;66(2):135-9.
4. Maruyama F, Kobata M, Kurokawa K, Nishida K, Sakurai A, Nakano K, Nomura R, Kawabata S, Ooshima T, Nakai K, Hattori M, Hamada S, Nakagawa I. Comparative genomic analyses of Streptococcus mutans provide insights into chromosomal shuffling and species-specific content. BMC Genomics 2009. Aug 5;10:358.
5. Kasai S, Okada K, Hoshino A, Iida T, Honda T. Lateral transfer of the lux gene cluster. J Biochem 2007. Feb;141(2):231-7.

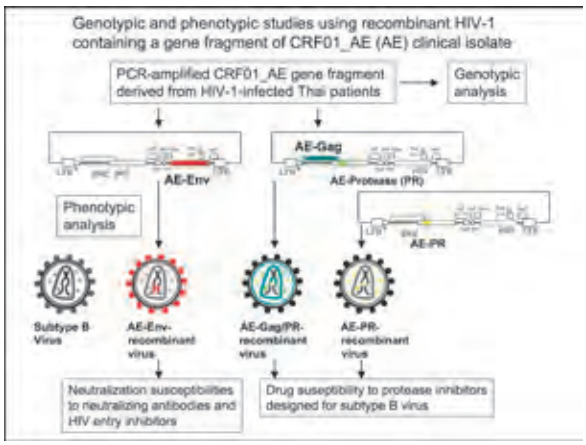
## Section of Viral Infections

| Research Group | | |
|---|---|---|
| | SA Professor | Naokazu Takeda, Ph.D. |
| | SA Professor | Masanori Kameoka, Ph.D. |
| | SA Researcher | Yong-Gang Li, Ph.D. |
| | Postdoctoral Fellow | Uamporn Siripanyaphinyo, Ph.D. |
| | Postdoctoral Fellow | Sompong Sapsutthipas, Ph.D. |
| | Research Fellow | Piraporn Utachee, M.Sc. |
| | Research Fellow | Chris Verathamjamras, M.Sc. |
| | Research Fellow | Samatchaya Boonchawalit, M.Sc. |
| | Research Fellow | Chidchanok Khamlert, M.Sc. |

Intestinal infectious diseases (enteroviral infections): It has recently become difficult to isolate viruses from patients with hand, foot and mouth diseases, and it has been suggested that enteroviruses other than Enterovirus 71 and Coxsackie virus 16 may be involved. To grasp the prevalence of these viruses in Thailand, molecular epideomiological studies are currently underway.

Blood-borne infectious diseases (HIV diseases/AIDS): We are performing basic studies that examine the virological and immunological characteristics of the HIV-1 CRF01_AE strains that are prevalent in Southeast Asia, including Thailand. In addition, the mechanism by which HIV acquires viral drug resistance to anti-retroviral drugs is being studied.

Mosquito-borne infectious diseases (dengue fever): We are constructing infectious molecularly cloned viruses to identify the viral genes that are involved in viral pathogenesis.




### Recent publications


1. Utachee P, Nakamura S, Isarangkura-na-ayuthaya P, Tokunaga K, Sawanpanyalert P, Ikuta K, Auwanit W, Kameoka M. Two N-linked glycosylation sites in 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 CD4 binding domain. J Virol. 2010;84:4311-20.
2. Pongsuwannna Y, Guntapong R, Tacharoenmuang R, Prapanpoj M, Kameoka M, Taniguchi K. A long-term survey on the distribution of the human G type rotavirus in Thailand. J Med Virol. 2010;82:157-63.
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4. Jinnopat P, Isarangkura-na-ayuthaya P, Utachee P, Kitagawa Y, de Silva UC, Siripanyaphinyo U, Kameoka Y, Tokunaga K, Sawanpanyalert P, Ikuta K, Auwanit W, Kameoka M. Impact of Amino Acid Variations in Gag and Protease of Human Immunodeficiency Virus Type 1 CRF01_AE strains on Drug Susceptibility of Virus to Protease Inhibitors. J Acquir Immune Defic Syndr 2009;52:320-8.
5. Auwanit W, Isarangkura-na-ayuthaya P, kasornpikul D, Ikuta K, Sawanpanyalert P, Kameoka M. Detection of drug resistance-associated and background mutations in human immunodeficiency virus type 1 CRF01_AE protease and reverse transcriptase derived from drug treatment-naive patients residing in central Thailand. AIDS Res Hum Retroviruses 2009;25:625-31.

# Laboratory of Molecular Biology (Lifetechnologies Corporation-Endowed Chair)

Research Group

Professor Fumio Imamoto, Ph. D.
Assistant Professor Takefumi Sone, D. Agr. Sc

The cells that form the multi-cellular organism control its responses to various extracellular signals by employing dynamic inter- and intra-cellular networks of biological molecular interactions. The recent development of fluorescence imaging technologies has made it possible to investigate how these molecular network systems function when a signal from the outer world is received. However, in most cases, researchers use cell extracts or membrane-permeable cells to introduce the fluorescence-labeled protein into the intracellular system in question. Moreover, even when the fluorescent proteins are expressed in living cells, this involves the introduction of vectors and the cell is observed under a condition where the protein is over-expressed. In contrast, in living cells, the expression level and cytological localization of each protein is precisely regulated, which facilitates their proper functions. Thus, the observations made by studies such as those described above may not be reflective of the protein in its intrinsic physiological condition. If we wish to analyze the real dynamics of multiple target proteins by introducing their genes (cDNAs) into living cells, we need to integrate the genes, which are regulated by their own promoter, into a definite site on the chromosome, as this is the native state of the intrinsic genes in the genome.

This department has developed further the multisite Gateway (msGW) technology, which was originally created and developed by Invitrogen Co. We are now applying this technology to the construction of multi-gene expression clones that permit the simultaneous introduction of the genes into culture cells. These clones are designed to regulate the expression of the genes at a physiological level. Our research subjects thus include:

1) Construction of msGW vectors for the high-throughput introduction of multiple genes labeled by fluorescence protein tags.
2) Development of proper promoters and IRES signals to ensure expression is at physiological levels, and development and improvement of fluorescence protein tags for bio-imaging.
3) Development of technology to control the expression levels of the transgenes, and development of methods to quantify the fluorescently-tagged products when they are expressed at low levels.
4) Introduction of multiple genes simultaneously and stably into definite sites on the chromosome to obtain superior transformed cell lines.

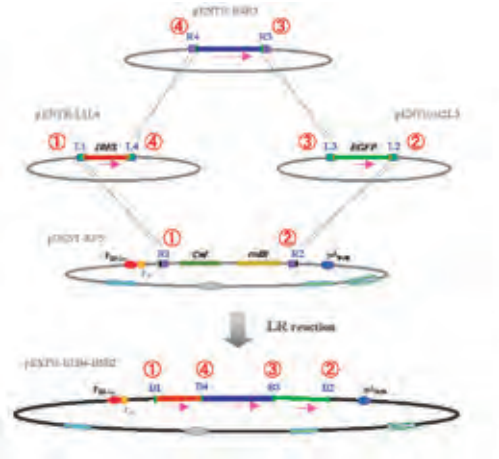



Fig: 1 Multisite Gateway cloning technology.

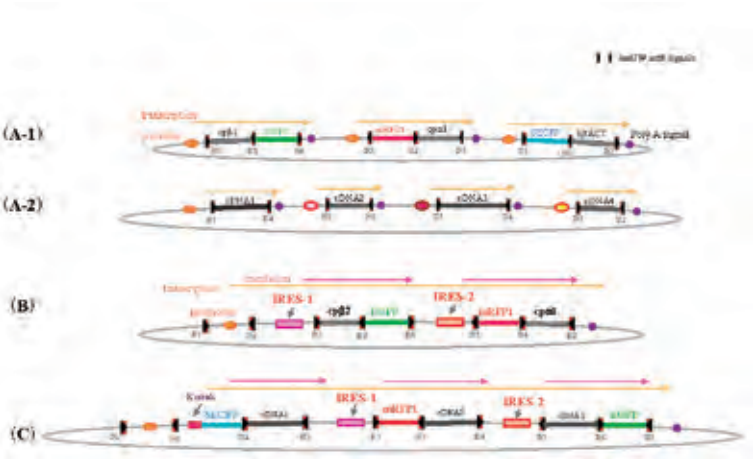



Fig. 2 Generation of msGW clones for multiple purposes.

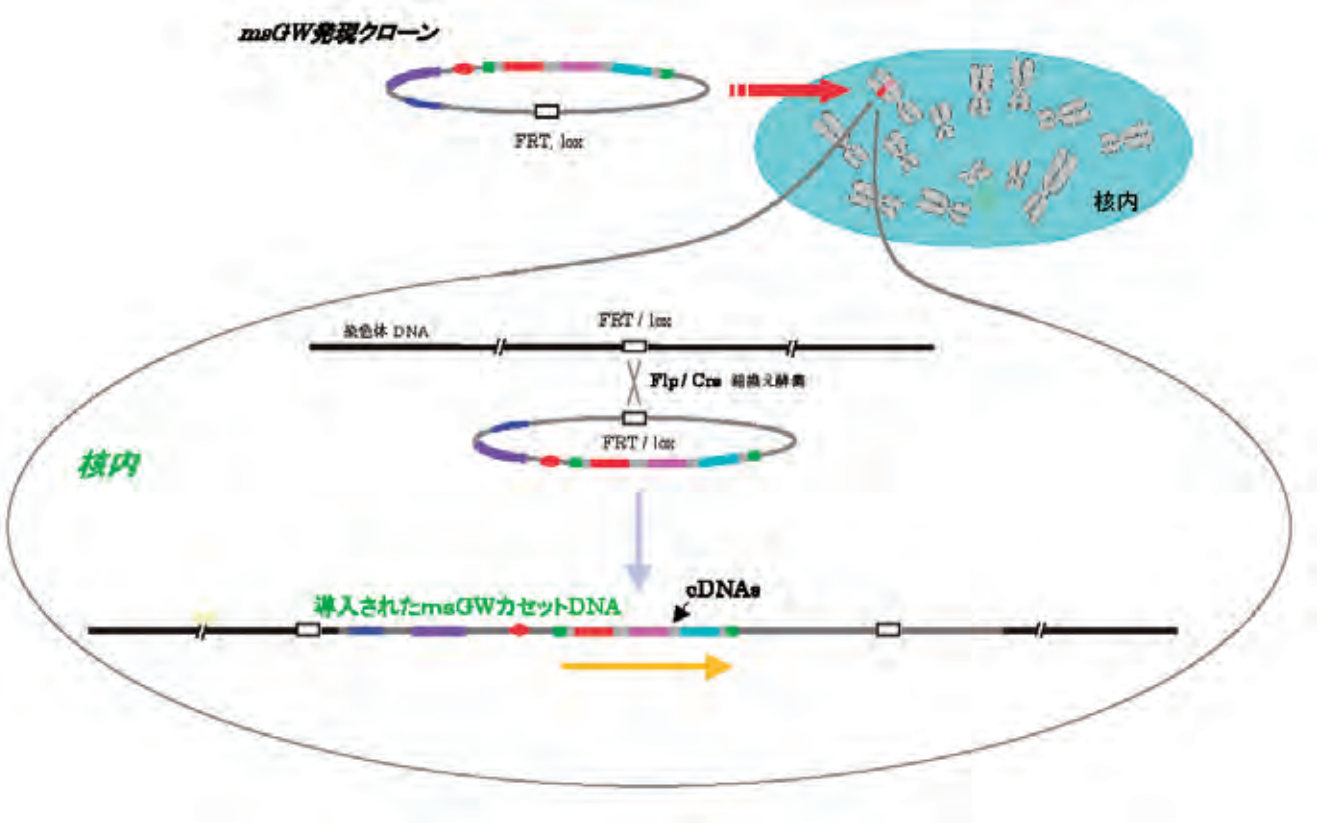



Fig. 3 Introduction of an expression clone into a targeted site of the cellular chromosome.

## Recent publications


1. Inoue K ., Sone T., Oneyama C., Nishiumi F., Kishine H., Sasaki Y., Andoh T, Okada M., Chesnut J.D. and Imamoto F. A versatile nonviral vector system for tetracycline-dependent one-step conditional induction of transgene expression. Gene Therapy 2009 Dec ; 16(12) :1383-1394
2. Nishiumi F., Sone T, Kishine H., Thyagarajan B., Kogure T., Miyawaki A., Chesnut J. D. and Imamoto F. Simultaneous single cell stable expression of 2-4 cDNAs in HeLaS3 using $\phi$C31 integrase system. Cell Structure and Function 2009 Mar 20 ; 34:47-59(online)
3. Sone T., Yahata K., Sasaki Y., Hotta J., Kishine H., Chesnut J.D. and Imamoto F. Multi-gene gateway clone design for expression of multiple heterologous genes in living cells: Modular construction of multiple cDNA expression elements using recombinant cloning. J. Biotechnol.2008 Sep 10 ; 136(3-4):113-121
4. Sasaki Y., Sone T., Yahata K., Kishine H., Hotta J., Chesnut J.D., Honda T. and Imamoto F. (2008) Multi-gene gateway clone design for expression of multiple heterologous genes in living cells: Eukaryotic clones containing two and three ORF multi-gene cassettes expressed from a single promoter. J. Biotechnol. 2008 Sep 10 ; 136(3-4):103-112
5. Tahara K., Takagi M., Ohsugi M., Sone T., Nishiumi F., Maeshima K., Horiuchi Y., Tokai-Nishizumi, Imamoto F., Yamamoto T., Kose S., Imamoto N. Importin-$\beta$ and the small guanosine triphosphatase Ran mediate chromosome loading of the human chromokinesin Kid. J. Cell Biology, 2008 Feb 11 ; 180(3):493-506

# 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.

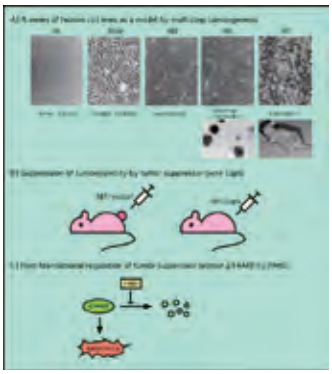
We hope to create an environment where traditional barriers between departments and fields essentially don't exist - where young scholars can quickly and efficiently obtain advice from researchers in different fields.
Major changes like this don't happen by themselves - which is why our faculty will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

Members

| Name<br>Affiliated department, Position title, Specialized field, Academic degree | Division of roles |
|---|---|
| **Yoshihiro Yoneda**<br>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**<br>Graduate School of Medicine (Division of Preventive and Environmental Medicine), Visiting Professor, Mol.Genetics & Neurology, M.D., Ph.D. | Sugar chain modifications and neurodegeneration diseases |
| **Yoshihide Tsujimoto**<br>Graduate School of Medicine (Division of Preventive and Environmental Medicine), Professor, Medical Genetics, Ph.D. | Mechanism of cell death and organelle |
| **Kiyoshi Takeda**<br>Graduate School of Medicine (Division of Preventive and Environmental Medicine), Professor, Immunology, M.D., Ph.D. | Analysis of activity regulation mechanism of the natural immunity system |
| **Kazunori Tomono**<br>Graduate School of Medicine (Division of Preventive and Environmental Medicine), Professor, Clinical Microbiology, M.D., Ph.D. | Analysis of antibacterial activity and development of new antimicrobial agents |
| **Masaya Tohyama**<br>Graduate School of Child Development, Professor, Anatomy & Neurosci, M.D., Ph.D. | Neurological function abnormalities and organella |
| **Toshikazu Nakamura**<br>Center for Advanced Science and Innovation (Joint Research Division for Regeneartive Drug Discovery), Professor, Biochemistry, Molecular Biology, Ph.D. | Treatment strategies against neurodegenerative and renal diseases with HGF |
| **Tetsuo Takehara**<br>Graduate School of Medicine (Division of Internal Medicine), Associate Professor, Gastroenterology & Hepatology, M.D., Ph.D. | Hepatitis onset mechanism and treatment strategies |
| **Eiji Miyoshi**<br>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**<br>Graduate School of Medicine (Division of Internal Medicine), Visiting Professor, Mass Spectrometry, M.D., Ph.D. | Development of sugar chain analysis methods |
| **Yukari Fujimoto**<br>Graduate School of Science (Department of Chemistry), Associate Professor, Organic Chemistry, Ph.D. | Recognition system of bacteria with its glycoconjugate |
| **Naoyuki Taniguchi**<br>The Institute of Scientific and Industrial Research, Endowed Chair Professor, Biochemistry, M.D., Ph.D. | Functional analyses of sugar chains/proteins |
| **Tamotsu Yoshimori**<br>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**<br>Research Institute for Microbial Diseases, Professor, Immunology, M.D., Ph.D. | Study on the dynamics of the acquired immunity |
| **Eisuke Mekada**<br>Research Institute for Microbial Diseases, Professor, Cell Biology, Ph.D. | Analyses of factors related to toxicity manifestation of the diphtheria toxin |
| **Yoshiharu Matsuura**<br>Research Institute for Microbial Diseases, Professor, Virology, Ph.D. | Infection mechanism of Hepatitis C virus and studies on the control methods |
| **Tatsuo Shioda**<br>Research Institute for Microbial Diseases, Professor, Virology, Ph.D. | Studies on host factors related to HIV infection |
| **Yasuhiko Horiguchi**<br>Research Institute for Microbial Diseases, Professor, Bacteriology, Ph.D. | Analyses of functions and structures of bacterial virulence factors |
| **Kazuyoshi Ikuta**<br>Research Institute for Microbial Diseases, Professor, Virology, Ph.D. | Studying emerging viral infections and their pathogenesis |
| **Toshihiro Horii**<br>Research Institute for Microbial Diseases, Professor, Parasitology, Ph.D. | Development of malaria vaccines and analyses of the host-parasite interactions |
| **Shizuo Akira**<br>WPI Immunology Frontier Research Center, Professor, Immunology, M.D., Ph.D. | Studies on innate immunity |
| **Taroh Kinoshita**<br>WPI Immunology Frontier Research Center, Professor, Immunology, Ph.D. | Analysis of significance of GPI anchor in the host-pathogen interactions |
| **Hisashi Arase**<br>WPI Immunology Frontier Research Center, Professor, Immunology, M.D., Ph.D. | Studies on mechanism of immunoregulation by pathogens |
| **Atsushi Kumanogoh**<br>WPI Immunology Frontier Research Center, Professor, Immunology, M.D., Ph.D. | Studies of immunoregulation/regulatory molecules of cell migration |
| **Tadashi Suzuki**<br>RIKEN (Systems Glycobiology Research Group), Team Leader, Biochemistry, D. Sc. | Quality controls of free sugar chains and glycoproteins |
| **Yoshiki Yamaguchi**<br>RIKEN (Syetems Glycobiology Research Group), Team Leader, Structural Biology, D. Pharm. | Structural analyses of glycoconjugates by NMR |

## Cancer Cell Research Group

Research Group
Associate Professor Masuo Yutsudo, Ph.D.
Assistant Professor Shinji Higashiyama, Ph.D.



(1) Analyses of the CapG tumor suppressor gene
We isolated a series of cell lines from a human diploid fibroblast that had been transformed in various ways; these lines included immortalized, anchorage-independent, and tumorigenic cell lines (Figure). Analysis of their gene expression profiles revealed that the tumorigenic cell line had lost CapG protein expression. Analysis of a variety of cancer cell lines revealed that several had also lost CapG expression. When these tumorigenic human cancer cell lines were transfected with CapG cDNA, they all became non-tumorigenic. We also identified a protein that interacts with CapG: this protein is an oncogene product that forms a complex with another tumor suppressor protein. Thus, CapG may suppress tumorigenicity by modulating the activity of a particular oncoprotein/tumor suppressor protein complex.

(2) Cellular dedifferentiation involved in tumorigenesis
It is well known that cancer cells often express genes that are usually only expressed by less differentiated cells. We found that during the malignant progression of our model cell lines, fibroblast-specific gene expression was shut off and the expression of several new genes was switched on. We are currently studying how the alteration of differentiation status relates to tumorigenesis.

(3) Post-translational regulation of tumor suppressor protein p14ARF by PANO
We isolated a novel apoptosis-inducing gene called PANO, which encodes a nucleolar protein. Our studies then revealed that PANO up-regulates the expression of tumor suppressor protein p14ARF by inhibiting its degradation, and that this may induce apoptosis. We are currently investigating whether this gene participates in human tumorigenesis.

### Recent publications

Watari, A., Takaki, K., Higashiyama, S., Li, Y., Satomi, Y., Takao, T., Tanemura, A., Yamaguchi, Y., Katayama, I., Shimakage, M., Miyashiro, I., Takami, K., Kodama, K., and Yutsudo, M. (2006). Suppression of tumorigenicity, but not anchorage-independece, of human cancer cells by new candidate tumor suppressor gene CapG. Oncogene 25, 7373-7380.

## Germ Cell Group

Research Group
Associate Professor Masami Nozaki, Ph. D.

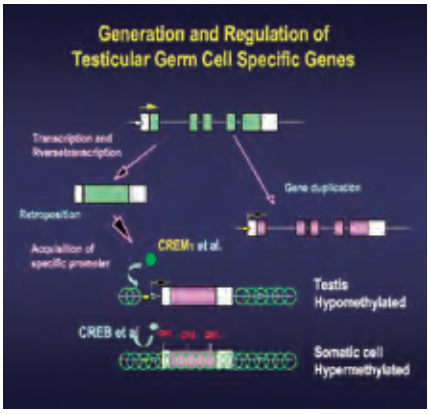



Figure legend. Generation and regulation of testicular germ cell-specific genes. Testicular isoform genes are generated by conventional gene duplication or retroposition. In the case of retroposons, the inserted cDNA, which is reverse-transcribed from mRNA, cannot be expressed in any tissue because the mRNAs lack a promoter in the 5'-flanking sequence of the genomic DNA. This implies that testicular germ cells provide an appropriate environment for retroposon transcription and facilitate gene expression from promoter-like sequences. This in turn suggests that the rules governing gene transcription during the later stages of spermatogenesis differ drastically from those in other cell types.

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

(2) Unique structure of sperm chromatin.
In the mammalian sperm nucleus, the haploid genome is packaged into a highly compact structure that contains protamines and some remaining histones. We are analyzing the physiological importance of the somatic-like, histone-containing regions of sperm chromatin.

(3) Establishment of an in vitro germ cell differentiation system
To examine the genetic requirements that are needed for germ cell formation and epigenetic reprogramming, we are in the process of establishing an in vitro developmental system based on ES cell differentiation.

### Recent publications

Kato Y, Kaneda M, Hata K, Kumaki K, Hisano M, Kohara Y, Okano M, Li E, Nozaki M, Sasaki H. Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse. Hum Mol Genet. 2007 Oct1; 16(19): 2272-80.

# Laboratory of Genome Dynamics

Research Group
Associate Professor Takashi Hishida, Ph.D.
Postdoctoral Fellow Nami Haruta, Ph.D.

DNA damage occurs frequently in all organisms as a consequence of both endogenous metabolic processes and exogenous DNA-damaging agents. Organisms have evolved several repair and tolerance mechanisms that remove and tolerate DNA damage and coordinate cell cycle progression. In the S phase of the cell cycle, replication stress occurs when an active fork encounters DNA lesions or proteins that are tightly bound to the DNA. These obstacles pose a threat to the integrity of the replication fork and are thus a potential source of genome instability that can contribute to tumorigenesis and aging in humans. Confronted with this risk, cells have developed fundamental DNA damage response mechanisms in order to faithfully complete DNA replication. Our group uses Escherichia coli and budding yeast as model systems to examine the cellular responses to DNA damage, with a special emphasis on the mechanisms that maintain genome integrity during DNA replication.

(1) Molecular mechanisms of the post-replication repair (PRR) pathway.
The UV spectrum present in sunlight is a potent and ubiquitous carcinogen that is responsible for most of the skin cancers in humans. In the natural environment, organisms are exposed to chronic low-dose UV light (CLUV), as opposed to the acute high doses that are commonly used in laboratory experiments. Hence, to clarify the biological significance of specific DNA damage response pathways, understanding the cellular response to CLUV exposure is an important approach that complements the more traditional laboratory approaches. An experimental assay that was recently developed to analyze CLUV-induced DNA damage responses was used to show that the PCNA polyubiquitination-dependent error-free PRR pathway plays a critical role in tolerance to CLUV exposure. We are currently analyzing in more detail the role(s) the error-free PRR pathway plays upon CLUV exposure. In addition, we are examining the molecular and structural foundations of PRR functions by combining genetic, biochemical and structural approaches.

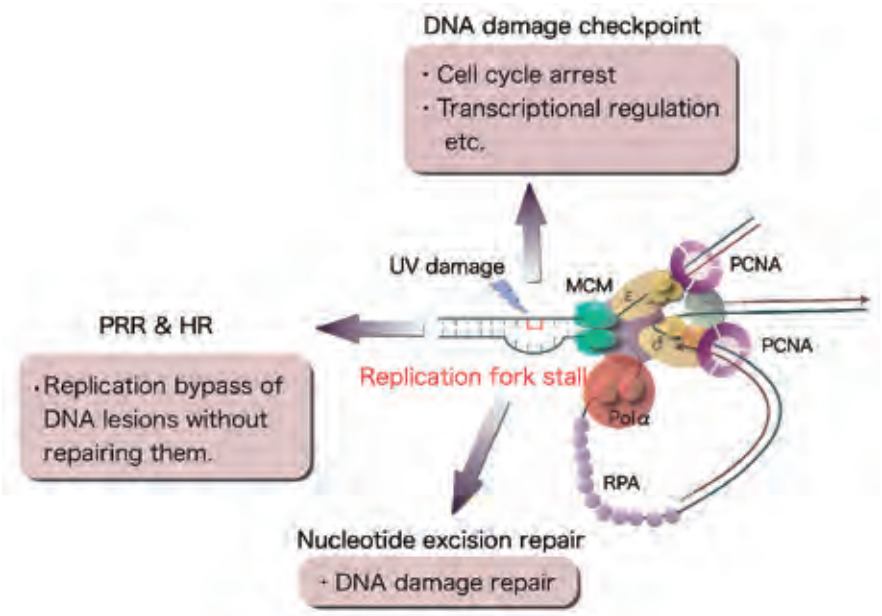



Fig. 1: DNA damage tolerance pathway. The RAD6 pathway consists of at least two different Rad6-Rad18 dependent mechanisms, which include translesion DNA synthesis and Rad5-dependent template switching.

## Recent publications

1. Hishida, T., Kubota, Y., Carr, A. M. and Iwasaki, H. (2009) RAD6-RAD18-RAD5 pathway-dependent tolerance to chronic low-dose UV light. Nature 457, 612-615.
2. Ohya T., Arai, H., Kubota, Y., Shinagawa, H. and Hishida, T. (2008) A SUMO-like domain protein, Esc2, is required for genome integrity and sister chromatid cohesion in Saccharomyces cerevisiae. Genetics 180, 41-50
3. Hishida, T., Ohya, T., Kubota, Y., Kamada, Y. and Shinagawa, H. (2006). Functional and physical interaction of yeast Mgs1 with PCNA: impact on RAD6-dependent DNA damage tolerance. Mol. Cell. Biol. 26, 5509-5517.
4. Hishida, T., Han, Y-W., Fujimoto, S., Iwasaki, H. and Shinagawa, H. (2004). Direct evidence that a conserved arginine in RuvB AAA+ ATPase acts as an allosteric effector for the ATPase activity of the adjacent subunit in a hexamer. Proc Natl Acad Sci USA. 101, 9573-9577.
5. Hishida, T., Han, Y-W., Shibata, T., Kubota, Y., Ishino, Y., Iwasaki , H. and Shinagawa, H. (2004). Role of the Escherichia coli RecQ DNA helicase in SOS signaling and genome stabilization at stalled replication forks. Genes Dev. 18, 1886-1897.

# Animal Resource Center for Infectious Diseases

| Research Group | | |
|---|---|---|
| | Head, Professor (SUP) | 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. |

A key area of microbial disease research is the analysis of the interactions between the host and pathogenic organisms. Such microbiological and immunological research is greatly facilitated by animal models, particularly since molecular biological and biotechnological methods now allow the generation of genetically manipulated mice that can aid our understanding of the mechanisms of infection. These experiments require that experimentally infected animals are managed in a suitable, safe and controlled fashion. 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 three areas: one for animal experimentation with microbial disease models (P2 and P3 level) and an SPF area. The microbial disease model animal experimentation section is completely air-conditioned and maintained at a negative air pressure to minimize the risk of contamination. Each sub-area in the microbial disease model animal experimentation section has an individual pass-through type of autoclave to sterilize all materials before they are removed. Moreover, the exhaust air is filtered to prevent the exterior dissemination of pathogenic microbes. These measures ensure that microbial disease model animals are handled safely without accidental cross-contamination.

Before gaining access to this restricted facility, researchers are required to take an official orientation tour and submit a research plan for committee review. The condition of the animals is inspected regularly.

The facility offers services such as the generation of genetically manipulated animals, in vitro fertilization, and the cryopreservation of mouse strains (Table 1).

Figure 1: Biosafety level 3 room (Building A, 1st floor).
This is the room where disease model animal experimentation occurs under biosafety level 3 conditions. Hemorrhagic fever with renal syndrome-causing virus (HFRSV) was first isolated in this area. In addition, animal experiments for Creutzfeldt-Jakob Disease (CJD), severe acute respiratory syndrome (SARS) and Acquired Immune Deficiency Syndrome (AIDS) can be performed in this facility.

Table 1) No. of mouse lines produced/preserved at the facility

| period | TG mice | KO mice | Cryopreservation |
|---|---|---|---|
| 1995-1997 | 92 | 14 | 83 |
| 1998-2000 | 116 | 23 | 178 |
| 2001-2003 | 101 | 49 | 443 |
| 2004-2006 | 43 | 76 | 331 |
| 2007-2009 | 21 | 69 | 216 |

TG, transgenic; KO, knock-out

# DNA-chip Development Center for Infectious Diseases

Research Group

| | |
|---|---|
| Head, Professor (SUP) | Hiroshi Nojima, Ph. D. |
| Assistant Professor | Daisuke Okuzaki, Ph. D. |
| Assistant 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 requires the 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, namely the Agilent-type and the Affymetrix-type, are available in this center. Our real-time PCR analysis system (ABI, PRISM7900HT-2) is also 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. This system employs a "focused microarray" that examines the expression of ~200 blood cell-specific and disease-related genes.

(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 enables 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 that facilitates the development of novel diagnostic systems for infectious diseases.

Fig. 1 : High density DNA microarray system.

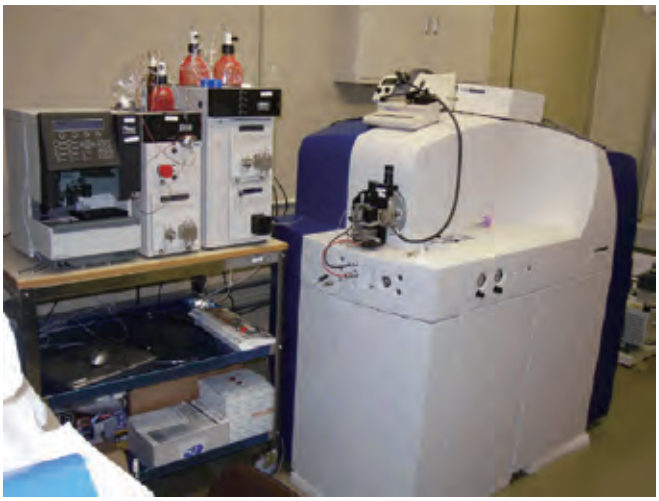


Fig. 2 : MS/MS spectrometer.

## Recent publications

1. 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.
2. 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.
3. 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.
Assistant Professor (SUP) — Hidetoshi Hasuwa, 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.
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) — Atsushi Kumanogoh, M. D., 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 by the functions of proteins produced from many genes. In other words, our health is essentially dependent on the balance of our gene products. Many diseases can therefore be traced to a defect in, or malfunction of, various genes. To find and develop new drugs or new therapies, it is important to identify the function of each gene in vivo. However, at present, the functions of various genes remain poorly understood, which makes it difficult to clarify their relationships with each other, or to analyze in a systematic fashion how they participate in specific diseases.
Gene-disrupted animals can be powerful tools that help us to understand the role of certain genes in vivo. Such animals can be highly useful as models of various human diseases as well as for screening for new drugs. Thus, many countries are producing gene-disrupted mouse lines of entire genes as national research projects in hopes of finding cures for diseases. In this context, the Japanese effort remains small and needs to produce more gene-manipulated animals in order to contribute to the search for cures to diseases. It is very important that Japan makes a reasonable contribution to this area. Given these issues, we created a consortium composed 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). The headquarters are in Kumamoto University. This consortium allows us to share our specialties with each other and aims to produce many gene-manipulated animals for the study of human diseases and their treatment. In our center, we are mainly focusing on genes that participate in reproduction, infection and allergy by taking advantage of an existing disease-screening system in our university; this system includes features such as fluorescent-colored sperm and eggs (Figure 1). We aim to use these gene-manipulated animals to perform the translational research that will eventually lead to new therapeutic systems and the discovery of new drugs.

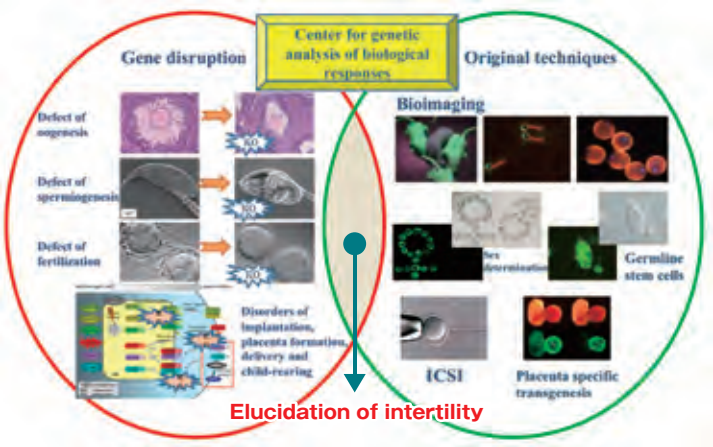



Figure 1. Strategy for elucidating sterility

## Recent publications

1. Inoue N, Kasahara T, Ikawa M, Okabe M. Identification and disruption of sperm-specific angiotensin converting enzyme-3 (ACE3) in mouse. PLoS ONE. 2010. 5, e10301.
2. Fujihara Y, Murakami M, Inoue N, Satouh Y, Kaseda K, Ikawa M, Okabe M. Sperm equatorial segment protein 1, SPESP1, is required for fully fertile sperm in mouse. J Cell Sci. 2010. 123, 1531-1536.
3. Yamaguchi R, Muro Y, Isotani A, Tokuhiro K, Takumi K, Adham I, Ikawa M, Okabe M. Disruption of ADAM3 impairs the migration of sperm into oviduct in mouse. Bio Reprod. 2009. 81, 142-146.
4. Yamaguchi R, Yamagata K, Hasuwa H, Inano E, Ikawa M, Okabe M. Cd52, known as a major maturation-associated sperm membrane antigen secreted from the epididymis, is not required for fertilization in the mouse. Genes Cells. 2008. 13, 851-861.
5. Okada Y, Ueshin Y, Isotani A, Saito-Fujita T, Nakashima H, Kimura K, Mizoguchi A, Oh-hora M, Mori Y, Oagata M, Oshima RG, Okabe M, Ikawa M. Complementation of placental defects and embryonic lethality by trophoblast-specific lentiviral gene transfer. Nat Biotechnol. 2007. 25, 233-237.

# Biken History Museum

Head Professor Hiroshi Nojima, Ph.D.

Biken History Museum, which will open on December 2010, will present historical materials of RIMD since its establishment in February 1934.

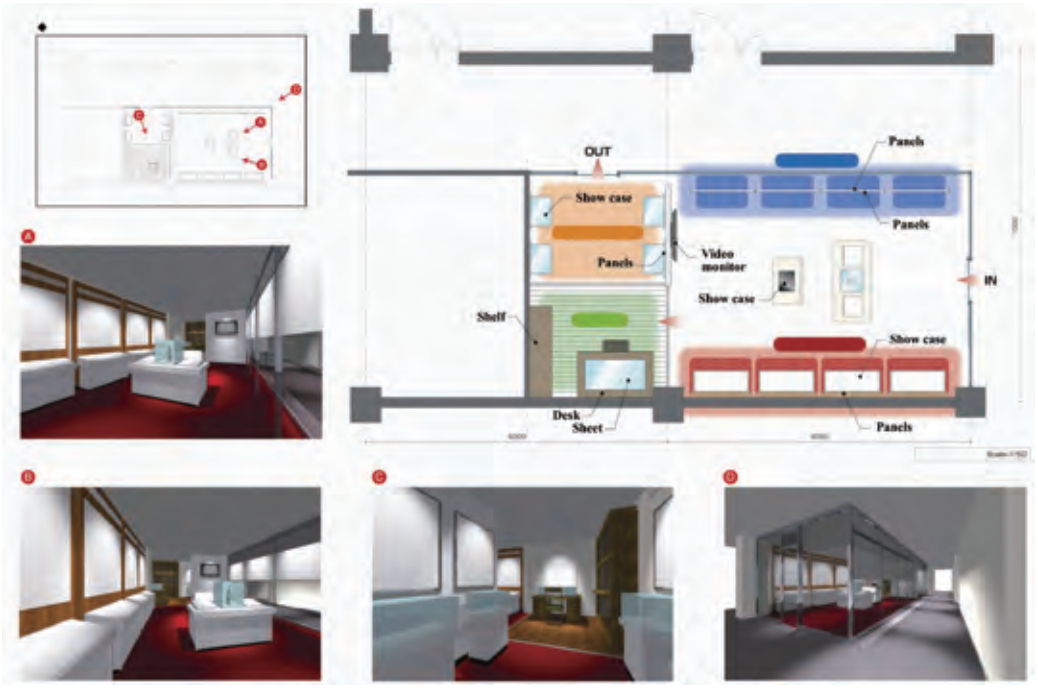



Fig. 1: Perspective Drawing (inside view) of the Biken History Museum.

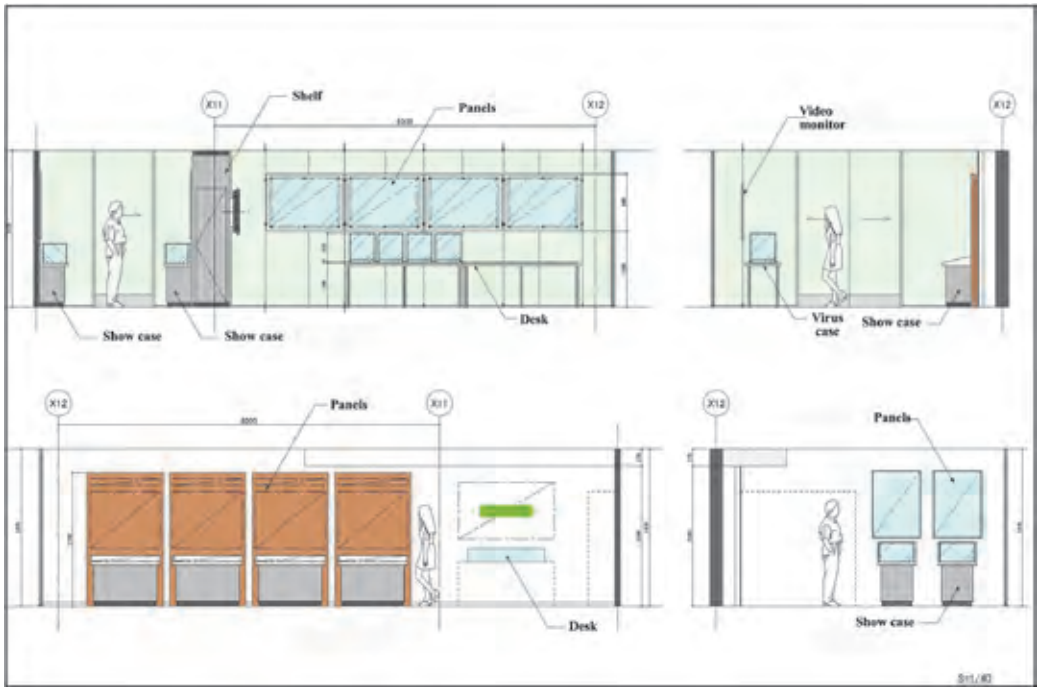



Fig. 2: A stereo view of the blue print.

## Central Instrumentation Laboratory

| | | |
|---|---|---|
| Head | Professor | Masato Okada, Ph. D. |
| | Associate Professor | Masuo Yutsudo, Ph. D. |
| | Assistant Professor | Shinji Higashiyama, Ph. D. |


This laboratory was established around the late 1950's. Since then, it has grown to possess a variety of high-performance instruments, including ultracentrifuges, electron microscopes, a laser microdissection system, cell sorters, automatic plasmid purification systems, DNA sequencers, and a mass spectrum analyzer. This laboratory also provides a room installed with large liquid nitrogen tanks for the preservation of living materials such as cells and viruses, and a room for treating specified injurious chemicals. Several technicians are employed to keep the instruments in proper working condition as well as to provide advice to beginners and ongoing support for researchers. In addition, they execute cell sorting, nucleotide sequencing, observation by electron microscope, and mass spectrometric analyses on samples upon request from Institute researchers. These kinds of services will become more and more important in the future since many instruments are becoming increasingly precise and complicated and require extensive training. Plans to accommodate such changes are currently in progress.

## Radioisotope Laboratory

| | | |
|---|---|---|
| Head | Head, Professor | Masato Okada, Ph.D. |
| | Assistant Professor | Shinji Higashiyama, Ph.D. |

The Radioisotope (RI) Laboratory is located adjacent to the main building of the institute and covers 403 sq.m. It was founded in 1967, and was extended by branch laboratories with a combined space of about 600 sq. m. during the establishment of the North building in 1979 and of the Central Laboratory for Biological Hazardous Microbes in 1983. In 1998, a radiation exposure room was established on the first basement level of the South building. The Genome Information Research Center radioisotope laboratory joined in 2007. At present, the total radiation controlled area is about 1,700 square meters.

The RI Laboratory is designed for biomedical experiments with radioisotopes and plays an important role in the research institute. Its facilities include an RI stockroom, a distribution room, a tissue culture room, and an area for RI- measuring equipment. Safety requirements are met by a stringent security system that involves the use of ID cards and computerized management of radioisotopes. About 200 researchers use the RI 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 the HFRS (hemorrhagic fever with renal syndrome) 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 3-story building that is 550 sq. m. in area. The first floor is reserved for experiments using radioisotopes. The facilities are designed to protect researchers from getting infected with pathogens and to prevent the spread of biohazardous pathogens outside the building.

The supply of fresh air is regulated to keep room interiors at negative pressure. Air is released from the facility through high-quality outlet filters to minimize contamination of the outside environment. Furthermore, each room is equipped with safety cabinets and autoclaves for the sterilization of used materials before their disposal. The entire laboratory has been renovated from 2005 to 2007 to increase numbers of pathogens simultaneously used in this laboratory.

In 2008 and 2009, 64 and 66 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 Hisashi Arase, M.D., 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 June 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 70 and 20 journals published in English and Japanese, respectively. Most of the books are kept on the bookshelves 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. Three librarians handle 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

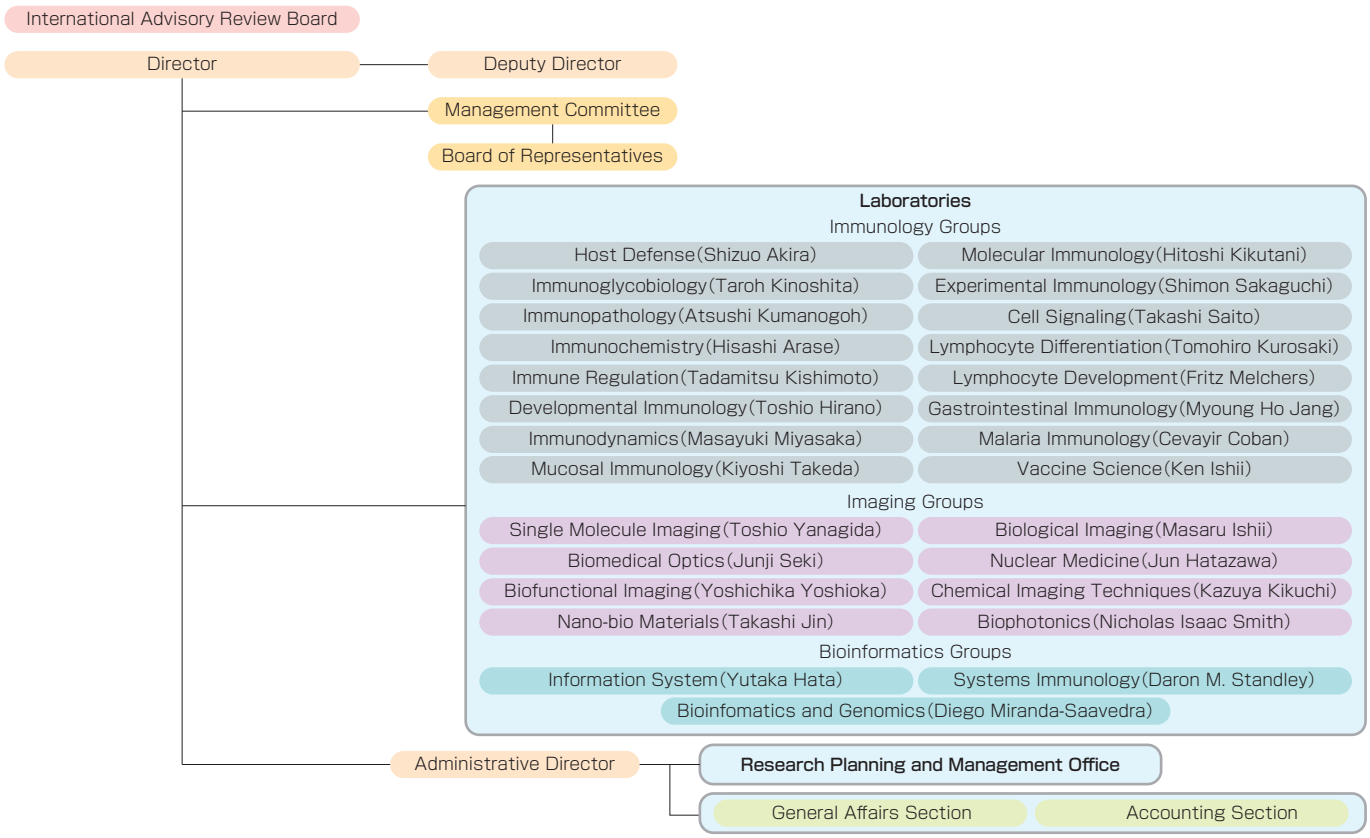
# Immunology Frontier Research Center

## 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 five 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 like AIDS, 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.

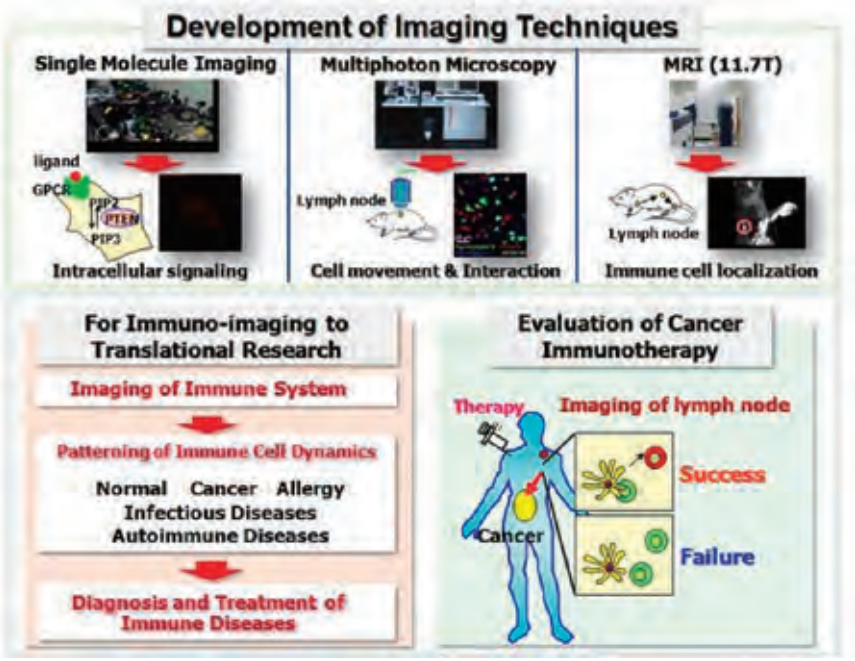


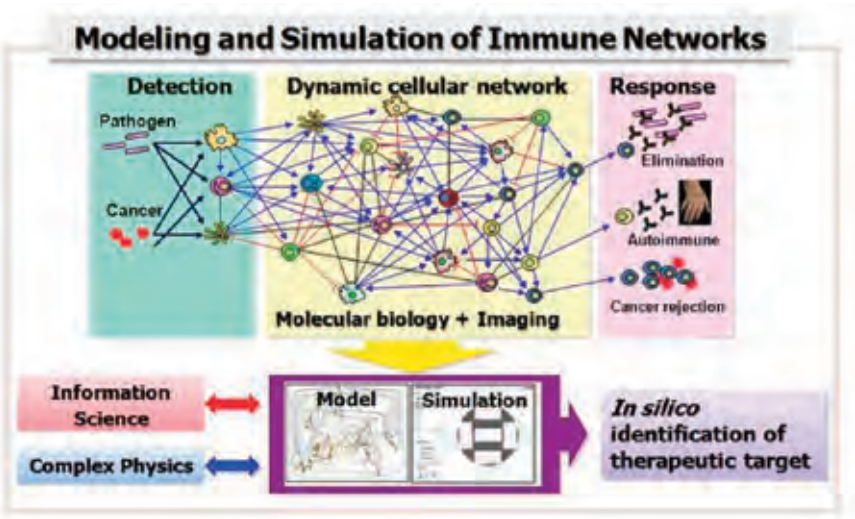
## ●Research fields／Expected Achievements

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 | 2005 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|
| Personnel | 926,795 | 943,574 | 917,415 | 905,437 | 859,673 |
| Non-Personnel | 754,260 | 643,140 | 495,488 | 513,073 | 548,947 |
| Total | 1,681,055 | 1,586,714 | 1,412,903 | 1,418,510 | 1,408,620 |

## Other Grants

(unit : thousand yen)

| Classification | 2005 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|
| Contract Research | 946,305 | 997,753 | 1,175,396 | 1,022,353 | 1,040,180 |
| Donations for Research | 194,528 | 252,863 | 1,215,677 | 187,969 | 343,772 |
| Miscellaneous | 6,408 | 7,499 | 4,591 | 3,406 | 2,090 |
| Total | 1,147,241 | 1,258,115 | 2,395,664 | 1,213,728 | 1,386,042 |

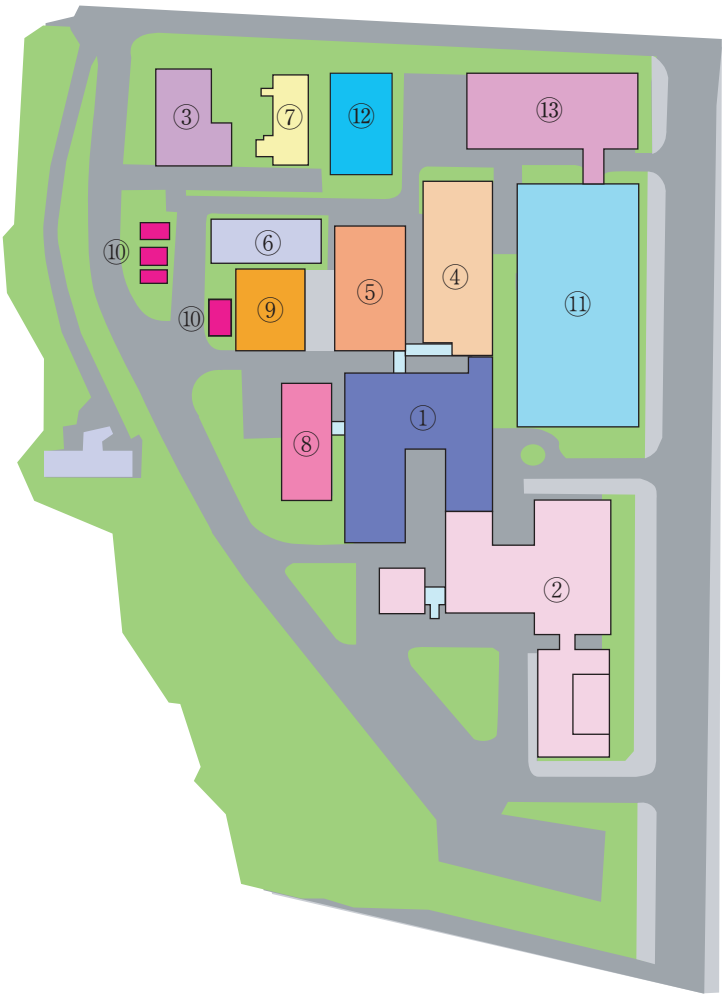
## Grants-in-Aid for Scientific Research

(unit : thousand yen)

| Classification | 2005 | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|---|
| MEXT Research Grants | 604,530 | 578,559 | 613,870 | 863.592 | 688,999 |
| Health and Labor Sciences Research Grants | 136,220 | 156,049 | 237,575 | 163,278 | 118,789 |
| Health and Labor other Research Grants | - | - | - | 18,000 | 13,988 |
| 21st Century COE Program Grants | 174,700 | 192,500 | 196,900 | - | - |
| Global COE Program Grants | - | - | - | 149,599 | 120,037 |
| Total | 915,450 | 927,108 | 1,048,345 | 1,194,469 | 941,813 |

# Building Area

Site Area …… **36,197**㎡ | Building Area …… **7,752**㎡ Gross Floor Area **27,408**㎡



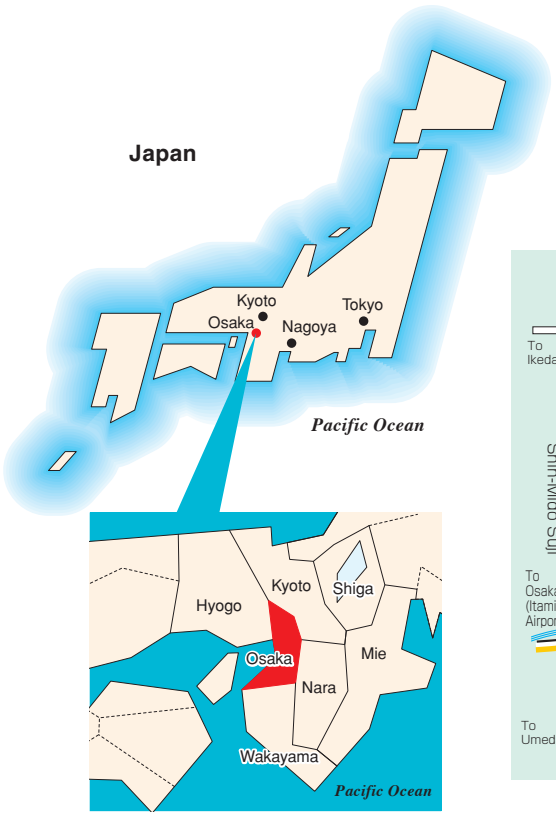

①⑪ Main building(left) and
Integrated Life Science building(right)

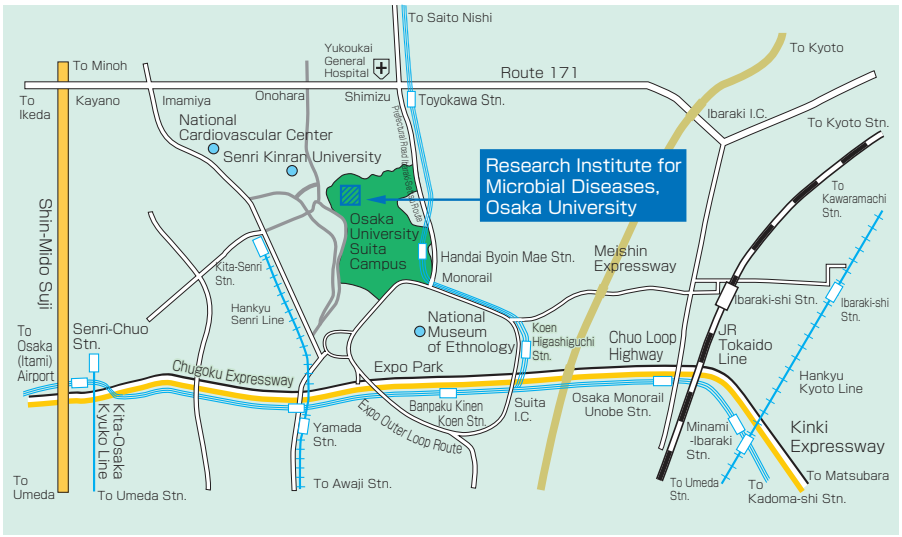
② South building

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

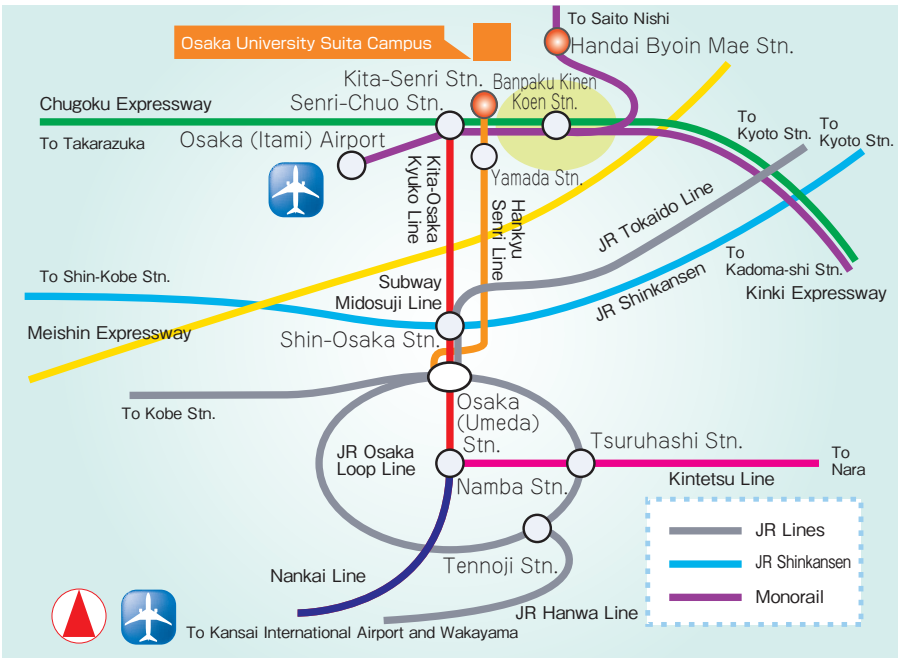
| Building name | Total floor numbers | Building area(㎡) | Total floor area(㎡) |
|---|---|---|---|
| ①Main building | 7 | 1,518 | 6,059 |
| ②South building | 3(1basement) | 1,712 | 4,941 |
| | DNA-chip Developmet Center and Genome Information Research Center are included | | |
| ③North building | 3 | 499 | 1,259 |
| ④Annex | 2 | 771 | 1,548 |
| ⑤Animal Resource Center A | 2 | 640 | 1,293 |
| ⑥Animal Resource Center B | 4 | 354 | 1,430 |
| ⑦Central Laboratory for Biological Hazardous Microbes | 3 | 242 | 550 |
| ⑧Radioisotope Laborator | 1 | 403 | 403 |
| ⑨Central Instrumentation Laboratory | 2 | 378 | 504 |
| ⑩Depository for dangerous chemicals | 1 | 163 | 163 |
| ⑪Integrated Life Science building | 10 | 1,072 | 9,258 |
| ⑫Animal Resource Center C(belonging to IFReC) | 3 ( 1 basement) | 600 | 2,400 |
| ⑬New IFReC building(tentative name) | (Construction to be completed in March, 2011) | | |

## 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 Scienses
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 Scienses
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 Industrual 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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