International Research Center for Infectious Diseases
感染症国際研究センター

Professor Yoshihiro Kawaoka, D.V.M., Ph.D. | センター長　獣医学博士　河　岡　義　裕

The number of problematic infectious diseases was once thought to be declining. However, due to the appearance of antibiotic-resistant bacteria and newly emerging viruses, humans are again facing an onslaught of disease threats caused by microorganisms. The International Centers for Infectious Diseases has as it primary objective understanding the pathogenesis of microbial diseases. By studying pathogens and their interactions with hosts at the molecular level and by developing control measures for infectious diseases, we hope to provide information necessary to counteract threats posed by microorganisms and to improve the welfare of society.

International Research Center for Infectious Diseases

Department of Infectious Disease Control
感染制御部門

Professor	Tetsuro Matano, M.D., Ph.D.	教　授	医学博士	俣　野　哲　朗
Professor	Aikichi Iwamoto, M.D., D.M.Sc	教　授(兼)	医学博士	岩　本　愛　吉
Professor	Chihiro Sasakawa, D.M.Sc	教　授(兼)	医学博士	笹　川　千　尋
Associate Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	助教授	獣医学博士	川　口　　寧
Associate Professor	Kazumiti Nakagawa, D.D.S., Ph.D.	助教授	歯学博士	中　川　一　路

To date, approximately 130 herpesviruses have been identified, affecting most animal species. These viruses are associated with a variety of diseases such as encephalitis, malignancy and mucocutaneous diseases in human and animals. The objective of our research is to understand the mechanisms by which herpesviruses replicate in cells, survive and manifest diseases in their hosts. Our goal is to apply our fundamental findings for control of herpesvirus infections and development of viral vectors and manipulated viruses in human therapy.

1. Identification of Proteins Phosphorylated Directly by the Us3 Protein Kinase Encoded by Herpes Simplex Virus 1.

Akihisa Kato, Mayuko Yamamoto,[1] Takashi Ohno, Hiroshi Kodaira,[2] Yukihiro Nishiyama,[1] and Yasushi Kawaguchi: [1]Department of Virology, Nagoya University Graduate School of Medicine, [2]Pharmaceutical Department, Yakult Honsha Co., LTD

We have developed a system to analyze the specific protein kinase activity of herpes simplex virus 1 Us3 *in* vitro, and showed that Us3 directly phosphorylates viral proteins UL34, ICP22 and Us9 and the cellular protein Bad, previously reported to be putative substrates. Using this system, we determined the phosphorylation sites of UL34 and identified UL31 as a previously unreported, novel substrate of Us3. This system will be useful for further identification of Us3 substrates and their phosphorylation sites, clarification of the role of Us3 in viral replication and identification of additional Us3 function(s).

2. The Role of Protein Kinase Activity Expressed by the UL13 Gene of Herpes Simplex Virus 1: The Activity Is Not Essential for Optimal Expression of UL41 and ICP0.

Michiko Tanaka,[3] Yukihiro Nishiyama,[1] Tetsutaro Sata,[3] Yasushi Kawaguchi: [3]Department of Pathology, National Institute of Infectious Disease

Herpes simplex virus 1 (HSV-1) UL13 is a viral protein kinase that is packaged into virions and regulates optimal expression of ICP0 and a subset of late (γ) proteins, including UL41 in infected cells. In the present study, we investigated the role(s) of the protein kinase activity of UL13 in viral replication using a recombinant virus expressing enzymatically inactive UL13 after an amino acid substitution in the invariant lysine of UL13. The recombinant virus carrying this mutation formed smaller plaques, yielded 10-fold less progeny than wild-type virus but could not be differentiated from wild-type virus with respect to accumulation of UL41 and ICP0 in infected cells. These results indicate that the

protein kinase activity of UL13 plays a role in viral replication in cell culture, but the activity is not essential for the optimal expression of UL41 and ICP0.

3. Herpes Simplex Virus Type 1 UL51 Protein Is Involved in Maturation and Egress of Virus Particles.

Naoki Nozawa,[1] Yasushi Kawaguchi, Michiko Tanaka,[3] Akihisa Kato, Ai Kato,[1] Hiroshi Kimura,[1] Yukihiro Nishiyama.[1]

The *UL51* gene of herpes simplex virus type 1 (HSV-1) encodes a phosphoprotein whose homologs are conserved throughout the herpes virus family. Recently, we reported that UL51 protein colocalizes with Golgi marker proteins in transfected cells and that targeting of UL51 protein to the Golgi apparatus depends on palmitoylation of its N-terminal cysteine at position 9. However, its role in the HSV replication cycle was unknown. Here, we generated UL51-null mutants (FDL51) in HSV-1 to uncover the function of UL51 protein. We show that the mutant plaques were much smaller in size and that maximal titers were reduced nearly 100-fold compared to wild-type virus. Electron microscopy indicated that the formation of nucleocapsids was not affected by the deletion of UL51 but that viral egress from the perinuclear space was severely compromised. In FDL51-infected cells, a large number of enveloped nucleocapsids were observed in the perinuclear space, but enveloped mature virions in the cytoplasm, as well as extracellular mature virions, were rarely detected. These defects were fully rescued by reinsertion of the *UL51* gene. These results indicate that UL51 protein is involved in the maturation and egress of HSV-1 virus particles downstream of the initial envelopment step.

Publications

Kato A, Yamamoto M, Ohno T, Kodaira H, Nishiyama Y, Kawaguchi Y. Identification of Proteins Phosphorylated Directly by the Us3 Protein Kinase Encoded by Herpes Simplex Virus 1. J. Virol. 79: 9325-9331, 2005.

Nozawa N, Kawaguchi Y, Tanaka M, Kato A, Kato A, Kimura H, Nishiyama Y. Herpes Simplex Virus Type 1 UL51 Protein Is Involved in Maturation and Egress of Virus Particles. J. Virol. 79: 6947-6956, 2005.

Kato A, Yamamoto M, Ohno T, Tanaka M, Sata T, Nishiyama Y, Kawaguchi Y. Herpes Simplex Virus 1-Encoded Protein Kinase UL13 Phosphorylates the Viral Us3 Protein Kinase and Regulates uclear Localization of Viral Envelopment Factors UL34 and UL31. J. Virol. (in press)

Tanaka M, Nishiyama Y, Sata T, Kawaguchi Y. The role of protein kinase activity expressed by the UL13 gene of herpes simplex virus 1: The activity is not essential for optimal expression of ICP0 and UL41. Virology 341: 301-312, 2005.

Koshizuka T, Kawaguchi Y, Nishiyama Y. The HSV-2 membrane protein UL56 associates with the kinesin motor protein KIF1A. J. Gen. Virol. 86: 527-533, 2005.

Matsuzaki A, Yamauchi Y, Kato A, Goshima F, Kawaguchi Y, Yoshikawa T, and Nishiyama Y. The Us3 protein kinase of herpes simplex virus type 2 is required for the stability of the UL46-encoded tegument protein and its association with virus particles. J. Gen. Virol. 86: 1979-1985, 2005.

Shaku F, Matsuda G, Furuya R, Kamagata C, Igarashi M, Tanaka M, Kanamori M, Nishiyama Y, Yamamoto N, Kawaguchi Y. Development of a Monoclonal Antibody against Epstein-Barr Virus Nuclear Antigen Leader Protein (EBNA-LP) that Can Detect EBNA-LP Expressed in P3HR1 Cells. Microbiol. Immunol. 49: 477-483, 2005.

Arii J, Hushur O, Kato K, Kawaguchi Y, Tohya Y, Akashi H. Construction of an infectious clone of canine herpesvirus genome as a bacterial artificial chromosome. Microbes and Infection (in press)

International Research Center for Infectious Diseases

International Research Center for Infectious Diseases Pathogenic Microbes Repository Unit
病原微生物資源室

Professor	Chihiro Sasakawa, D.M.Sc.		教　授	医学博士	笹　川　千　尋
Research Associate	Sei Yoshida, D.M.Sc.		特任助手	医学博士	吉　田　　整

This laboratory was established as the Laboratory of Culture Collection in 1972 to collect, conserve and supply various standardized pathogenic bacteria. Since its establishment, we have distributed pathogenic bacteria to universities, public research institutes, hygienic laboratories of local governments, medical correlation technologist training schools, hospital laboratories, food laboratories, and research laboratories of companies of all over Japan. Our laboratory was recently renamed the International Research Center for Infectious Diseases, Pathogenic Microbes Repository Unit in April 2005 with a cooperation system with the Research Institute for Microbial Diseases, Osaka University.

Our society is always threatened by emerging and reemerging infectious diseases with various kinds of altitude pathogenic microbes owing to increased foreign tourism, import increase including food, food poisoning such as the O-157 epidemic, and bioterrorism. In addition, by advanced medical developments, the aging society, and increased HIV infection, the quick identification of and therapy for opportunistic infection causative agents and multiple drug resistance bacteria have become important in the medical field.

The need for researchers and clinical practitioners specialized in bacteriology and infectious diseases has risen remarkably, and the substantial study and education required is an emergent problem. For thorough study and education, knowledge of bacteriology, a system of collecting pathogenic microorganism strains of reliable origin, to maintain and save them appropriately, and to provide them to cutting-edge researchers or educational establishments is indispensable. However, in Japan, research into pathogenic microorganisms and infectious diseases is performed mainly in universities, where there is no system for conservation and supply. Therefore, valuable bacterial strains have faced disappearance. Furthermore, under the CARTAGENA PROTOCOL ON BIOSAFETY for conventions of biological diversity, the provision and purchase of pathogenic microorganisms from foreign countries has become difficult.

In such circumstances, we are collecting, saving, and analyzing the pathogenicity of microorganisms and distributing pathogenic bacteria to 1) offer type cultures as a positive control in research, education and examinations, 2) prepare pathogenic bacterial strains that have socially high importance, and 3) offer microbes to universities or public research organizations for training or research. We possess about 1,500 strains that almost cover the main pathogenic microbes, including strains valuable internationally such as pathogenic *E. coli* of Orskov's collection, which is stored only in our laboratory in Japan. Furthermore, it is important to secure

their utility as type cultures by preparing genomic and genetic information about the pathogenicity of our bacterial collection based on the researches of the Division of Bacterial Infection. Thus, our laboratory is expected to contribute to countermeasures against infectious disease, and to the education and research of medical microbiology in our country.

Collection, preservation and data management of bacterial strains

It is necessary for us to collect representative type strains and the derivatives of pathogenic microbes corresponding to the following six items.

a) Comprehensive collection of genome sequencing strains.
b) The causative agents of hospital-acquired (nosocomial) infection, such as opportunistic infectious bacteria and antibiotic-resistant bacteria.
c) Pathogenic *Escherichia coli* associated with the intestinal and urinary tract or meningeal infections, including *Shigella*, EPEC and EHEC O-157.
d) Intracellular bacterial pathogens such as *Mycobacterium avium* and obligate intracellular bacteria.
e) Zoonotic agents causing brucellosis (*Brucella*), leptospirosis (*Leptospira*), and so on.
f) Pathogens causing newly emerging infections and outbreaks, such as *Helicobacter pylori, Salmonella* spp. and *Clostridium perfringens.*

We dissect the biochemical properties of bacterial strains collected by deposition, and maintain them appropriately. We are also opening the database of our collection to the public.

Distribution of bacterial strains

We are distributing bacterial strains to research organizations, hospital laboratories, and medical educational institutions throughout the country. In addition, under cooperation with the Japanese Society for Bacteriology, we are distributing authorized bacterial strains.

Value-added creation of a bacterial strain collection by pathogenic analysis

We are analyzing the pathogenicity of pathogenic microorganisms, especially pathogenic *E. coli*, the pathogenicity of new bacterial infection causative agents in cooperation with the Division of Bacterial Infection. Our collection has original added value by offering this information to users.

Participation with The National BioResource Project (NBRP) "Pathogenic Microorganisms (Bacteria, Fungi and Protozoa)"

The National BioResource Project (NBRP) aims to enable Japan to structurally provide the systematic accumulation, storage and provision of nationally recognized bioresources, which are used widely in life science researches as materials. This project started in July 2002 as part of the "Research Revolution 2002 (RR2002)" project by the Ministry of Education, Culture, Sports, Science and Technology. In addition to our institute, pathogenic bacteria are collected, stored and provided by the Research Institute for Microbial Diseases of Osaka University and the Graduate School of Medicine of Gifu University, pathogenic fungi and actinomycetes by Chiba University Research Center for Pathogenic Fungi and Microbial Toxicoses, and protozoan parasites by the Institute of Tropical Medicine of Nagasaki University. The border between pathogenic and non-pathogenic microbes is vague, such that taxonomic studies can be conducted using both types of microorganisms. Therefore, the project is performed in cooperation with the Japan Collection of Microorganisms of the Riken Bioresource Center. The National Institute of Genetics (NIG) has built a database for microbial strains and bacterial toxins.