Affiliated Facilities

Laboratory Animal Research Center
実験動物研究施設

Professor	Chieko Kai D.V.M., Ph.D		教　授	農学博士	甲　斐　知恵子
Associate Professor	Misako Yoneda D.V.M., Ph.D		准教授	農学博士	米　田　美佐子
Assistant Professor	Hiroki Sato Ph.D		助　教	理学博士	佐　藤　宏　樹

Our major research interests are to elucidate molecular mechanisms of pathogenicity and species specificity of minus and single strand RNA viruses (Mononegavirales), and to control viral diseases. For these purposes, we are studying virus replication and identifying viral and host factors important for the expression of pathogenicity using a novel reverse genetics technique. We are also developing new virus vaccines and virus vectors by genetic engineering. In the animal research center, more than 30,000 mice, mainly transgenic or knockout, are kept for research of IMSUT, and the technical staff support their breeding, frozen storage of eggs and microbiological cleaning.

Selective translation of the measles virus nucleocapsid mRNA by La protein.

Inoue Y, Sato H, Fujita K, Tsukiyama-Kohara K, Yoneda M, Kai C.

Measles, caused by measles virus (MV) infection, is the leading cause of death in children because of secondary infections attributable to MV-induced immune suppression. Recently, we have shown that wild-type MVs induce the suppression of protein synthesis in host cells (referred to as "shutoff") and that viral mRNAs are preferentially translated under shutoff conditions in infected cells. To determine the mechanism behind the preferential translation of viral mRNA, we focused on the 5′ untranslated region (UTR) of nucleocapsid (N) mRNA. The La/SSB autoantigen (La) was found to specifically bind to an N-5′UTR probe. Recombinant La enhanced the translation of luciferase mRNA containing the N-5′UTR (N-fLuc), and RNA interference of La suppressed N-fLuc translation. Furthermore, recombinant MV lacking the La-binding motif in the N-5′UTR displayed delayed viral protein synthesis and growth kinetics at an early phase of infection. These results suggest that La induced predominant translation of N mRNA via binding to its 5′UTR under shutoff conditions. This is the first report on a cellular factor that specifically regulates paramyxovirus mRNA translation.

The nucleocapsid protein of measles virus blocks host interferon response.

Takayama I., Sato H., Watanabe A., Omi-Furutani M., Kanki K., Yoneda M. and Kai C.

MV belongs to the genus Morbillivirus of the family Paramyxoviridae. A number of paramyxoviruses inhibit host interferon (IFN) signaling pathways in host immune systems by various mechanisms. Inhibition mechanisms have been described for many paramyxoviruses. Although there are inconsistencies among previous reports concerning MV, it appears that P/V/C proteins interfere with the pathways. In this study, we confirmed the effects of MV P gene products of a wild MV strain on IFN pathways

and examined that of other viral proteins on it. Interestingly, we found that N protein acts as an IFN-α/β and γ-antagonist as strong as P gene products. We further investigated the mechanisms of MV-N inhibition, and revealed that MV-N blocks the nuclear import of activated STAT without preventing STAT and Jak activation or STAT degradation, and that the nuclear translocation of MV-N is important for the inhibition. The inhibitory effect of the N protein was observed as a common feature of other morbilliviruses. The results presented in this report suggest that N protein of MV as well as P/V/C proteins is involved in the inhibition of host IFN signaling pathways.

Evaluation of a recombinant measles virus as the expression vector of hepatitis C virus envelope proteins.

Kasama Y[1], Satoh M[1], Saito M[1], Okada S[2], Kai C, Tsukiyama-Kohara K[1]: [1]Department of Experimental Phylaxiology, Faculty of Life Sciences, Kumamoto University, [2]Division of Hematopoiesis, Center for AIDS Research, Kumamoto University

We constructed the recombinant Edmonston strain of MV (MV-Ed) that expressed hepatitis C virus (HCV) envelope proteins (rMV-E1E2). The rMV-E1E2 successfully expressed HCV E1 and E2 proteins. To evaluate its immunogenicity, NOD/Scid/Jak3null mice that were engrafted with human peripheral blood mononuclear cells (huPBMC-NOJ) were infected with this rMV-E1 E2. Although human lymphocytes could be isolated from the spleens of mock-infected mice during the 2-weeks-long experiment, the proportion of mice that were infected with MV or rMV-E1E2 was decreased in a viral dose-dependent manner. Over 10^3 PFU of virus infection decreased the human PBL to less than 5%. Significant decrease of B cell population in human PBL from rMV-E1E2 infected NOD-SCID mice and decrease of T cell population in those from MV infected mice were observed. Human antibody production in these mice was also examined. Thus, the results in this study may contribute for future improvement of recombinant vaccine using measles virus vector.

Determination of a phosphorylation site in Nipah virus nucleoprotein and its involvement in virus transcription.

Huang M, Sato H, Hagiwara K, Watanabe A, Sugai A, Ikeda F, Kozuka-Hata H, Oyama M, Yoneda M, Kai C.

Many viruses use their host's cellular machinery to regulate the functions of viral proteins. The phosphorylation of viral proteins is known to play a role in genome transcription and replication in paramyxoviruses. The paramyxovirus N protein, the most abundant protein in infected cells, is a component of the N-RNA complex and supports the transcription and replication of virus mRNA and genomic RNA. Recently, we reported that the phosphorylation of MV-N is involved in the regulation of viral RNA synthesis. In this study, we found a rapid turnover of phosphorylation in the Nipah virus N (NiV-N). The phosphorylated NiV-N was hardly detectable in steady-state cells, but was detected after inhibition of cellular protein phosphatases. We identified a phosphorylated serine residue at Ser451 of NiV-N by peptide mass fingerprinting by electrospray ionization-quadrupole time-of-flight mass spectrometry. In the NiV minigenome assay, using luciferase as a reporter gene, the substitution of Ser451 for alanine in NiV-N resulted in a reduction in luciferase activity of approximately 45% compared with the wild-type protein. Furthermore, the substitution of Ser451 for glutamic acid, which mimics a phosphoserine, led to a more significant decrease in luciferase activity-approximately 81%. Northern blot analysis showed that both virus transcription and replication were reduced by these mutations. These results suggest that a rapid turnover of the phosphorylation of NiV-N plays an important role in virus transcription and replication.

Publications

Kodama, A., Yanai, T., Kubo, M., El Habashi, N., Kasem, S., Sakai, H., Masegi, T., Fukushi, H., Kuraishi, T., Yoneda, M., Hattori, S. and Kai, C.: Cynomolgus monkey (*Macaca fascicularis*) may not infected with equine herpesvirus 9. *J. Med. Primatol.* 40(1): 18-20, 2011.

Takano, T., Kohara, M., Kasama, Y., Nishimura, T., Saito, M., Kai, C. and Tsukiyama-Kohara, K. Translocase of outer mitochondrial membrane 70 expression is induced by Hepatitis C virus and is related to the apoptotic response. *J. Med. Virol.*, 83(5): 801-809, 2011.

Satoh, M., Saito, M., Takano, K., Kasama, Y., Nishimura, 1., Nishito, Y., Hirata, Y., Arai, M., Sudoh, M., Kai, C., Kohara, M. and Tsukiyama-Kohara, K. Monoclonal Antibody

2-152a Suppresses Hepatitis C Virus Infection Thorough Betaine/GABA Transporter-1. *J. Infect. Dis.* 204: 1172-1180, 2011.

Huang M., Sato H., Hagiwara K., Watanabe A., Sugai A., Ikeda F., Kozuka-Hata H., Oyama M., Yoneda, M. and Kai, C. Determination of phosphorylation site in Nipah virus nucleoprotein and its involvement in viral transcription. *J. Gen. Virol.*, 92(9); 2133-2141, 2011.

Kasama, Y., Satoh, M., Saito, M., Okada, S., Kai, C. and Tsukiyama-Kohara, K. Evaluation of a recombinant measles virus as the expression vector of hepatitis C virus envelope proteins. *World J. Vaccines*, 1, 98-103, 2011.

Inoue Y, Sato H, Fujita K, Tsukiyama-Kohara K, Yoneda M, Kai C. Selective translation of the measles virus nucleocapsid mRNA by la protein. *Front Microbiol.* 2: 173; 2011.

Sato H, Yoneda M, Honda T, Kai C. Recombinant vaccines against the mononegaviruses-what we have learned from animal disease controls. *Virus Res.* 162(1-2): 63-71. 2011.

Takayama, I., Sato, H., Watanabe, A., Omi-Furutani, M., Kanki, K., Yoneda, M. and Kai, C. The nucleocapsid protein of measles virus blocks host interferon response. *Virology*, 424, 45-55, 2012.

Affiliated Facilities

Amami Laboratory of Injurious Animals
奄美病害動物研究施設

Professor	Chieko Kai, D.V.M., Ph.D.	教　授　農学博士	甲　斐　知恵子
Associate Professor	Shosaku Hattori, D.V.M., Ph.D.	准教授　農学博士	服　部　正　策

The Amami Laboratory of Injurious Animals was established in 1965 at Setouchi-cho in Amami-oshima Island in order to study on endemic diseases involving parasite, arthropods, and venomous snakes in the tropics or subtropics.
The Amami-oshima Island belongs to the Nansei (Southwest) Islands and the fauna is quite different from that in other islands of Japan. Since establishment of the laboratory, trials have been carried out to utilize small mammals found unique in the Amami islands as experimental animals in addition to studies on prevention of Habu bites. As well known, successful eradication of filariasis from this island is one of the monumental works of the laboratory. Our present works are as follows:

1. Research on the Habu control

Shosaku Hattori, Takeshi Kuraishi, Motonori Ohno[1], Naoko Oda-Ueda[2], Takahito Chijiwa[1], Aichi Yoshida[3], Yoshihiro Hayashi[4], Michihisa Toriba[5] and Tomohisa Ogawa[6]: [1]Department of Applied Life Science, Faculty of Bioscience, Sojo University, [2]Department of Biochemistry, Faculty of Pharmaceutical Science, Sojo University, [3]School of Health Science, Faculty of Medicine, Kagoshima University, [4]Department of Veterinary Anatomy, Faculty of Agriculture, University of Tokyo, [5]The Japan Snake Institute, [6]Faculty of Agriculture, Tohoku university

Snake bites by the venomous snake Habu, *Protobothrops flavoviridis*, have been reported annually about 60 cases in the population of 100,000 in the Amami Islands. Moreover, there is no indication that the population of the Habu itself has decreased, despite a campaign for capture of snakes by the Kagoshima Prefectural Government. Rat-baited box traps have been introduced to catch the snakes and found to be quite effective. However, maintenance of live rats requires man power and its cost is expensive. Therefore, our effort has been focused on the development of attractant for Habu. The attractant extracted from rats seems ineffective if compared with use of live rats.

It was known that the Habu survived the injection of the Habu venom since early times, because some proteins in the serum of the Habu blood combine to the elements of the Habu venom. The research of these binding proteins has been initiated with an objective of clinical trials. Phospholipase A_2 and its isozymes isolated from Habu venom have myonecrotic activity and hemorrhagic activity, and metal protease has hemorrhagic activity. The binding proteins isolated from serum of Habu inhibit myonecrotic activity of phospholipase A_2 and its isozymes. We found that protein-HSF and peptide-AHP isolated from the Habu serum effectively control the hemorrhage caused by venom of the Habu, *Ovophis okinavensis, Agkistrodon blomhoffi brevicaudus, Calloselasma rhodostoma, Bitis arietans, Bothrops asper*, and, *Trimeresurus stejnegeri.*

Further, a statistics analysis and the simulation were done with the snakes captured by the Government, and the analysis of population dy-

namics of Habu was attempted. As a result of investigating the individual measurement data of the captured Habu over 9 years, we were able to obtain the generous age composition of the Habu. From analyzing of the age pyramid of the Habu and the result of questionnaire surveys for the inhabitant in the Amami-oshima Island, the total population of the Habu which lives in this island was estimated at about 80,000. By the analysis of the measured data of last nine years, the snake sizes were miniaturized, and the population of young snakes decreased. According to these investigations, the population of the Habu is expected to decrease in the near future.

These studies are supported by grants from the Ministry of Land, Infrastructure and Transport and the Kagoshima Prefectural Government.

2. Measurement of IgE antibodies against venom of Habu (Protobothrops flavoviridis) sephadex fractions and purified venom proteins by means of enzyme-linked immunosorbent assay

Takeshi Kuraishi, Enoki Asanuma[7], Shosaku Hattori: [7]Asanuma clinic, Medical corporation Hekizankai

We have developed an enzyme-linked immunosorbent assay (ELISA) for measurement of IgE antibodies to the Habu (*Protobothrops flavoviridis*) venom. The Habu crude venom was fractionated by a Sephadex G-100 columu and their reactivities with IgE antibodies were investigated with ELISA. High IgE antibody values were observed in fractions with high and middle molecular weight namely, GF1 and GF2. High molecular hemorrhagic protease (HMHP) was purified from GF1 and phospholipase A_2 (PLA_2), Basic Protein I (BPI) and Basic Protein II (BPII) were purified from GF2. IgE-ELISA using these proteins as antigen was performed for 16 sera which obtained from individuals bitten by Habu. In all patient sera, IgE antibody values were higher than that of ordinary person's sera. These purified venom proteins (PLA2, BPI, BPII, HMHP) was suggested to be the Habu venom allergens.

3. Reproduction of squirrel monkeys.

Shosaku Hattori, Takeshi Kuraishi, Kumiko Ikeda, Hazuki Yoshimura and Chieko Kai

The squirrel monkey, *Saimiri sciurea*, is widely distributed in the tropical rainforest in Central and South America between 10 degrees N and 17 degrees S of latitudes. The advantage of using this species for medical researches resides in its small size and gentle behavior. In this laboratory, about 3～7 newborns are given annually by 24 adult females.

The aim is to optimize the use of the non-human primate model in future the Amami Laboratory research activities. The laboratory newly established experimental infection systems which require or can be adapted to the squirrel monkey model, particularly the study of human falciparum malaria. Development of parasites, immune response to malaria parasites and pathological changes were investigated in in-vivo condition, furthermore, in vitro analysis of cell and molecular level was performed. It is also investigating the mechanisms of infection in immunology, vector development, a vaccine production program, and a clinical trials program.

4. Sleep profile in captive owl monkey (Aotus lemurimus).

Sachi Sri Kantha[8], Juri Suzuki[9], Shosaku Hattori, Takeshi Kuraishi and Chieko Kai: [8]Pharmaceutical English Section, Gifu Pharmaceutical University, [9]Center for Human Evolution Modeling Research, Kyoto University Primate Research Institute

In the wild, owl monkeys preferentially sleep in the holes of tree trunks, entanglements of climbers and among dense foliage during the daytime, on average at 10-20 meters above the ground level. This preferential niche has made it difficult to quantitate owl monkey's sleep in the field. Sleep profile of the only nocturnal simian primate, Aotus spp., remains little studied even under captive conditions. Therefore, we are monitoring two sleep parameters namely, total sleep time (TST) and sleep episode length (SEL) of 16 owl monkeys (*Aotus lemurimus*) in breeding colony of Amami laboratory. TST and SEL of these monkeys were quantitated for 14 days (one experiment period) via actigraphy: by tagging an acclerometer-type miniature transmitter (Actiwatch-MINIMITTER) sensitive to omnidirectional movement, to the owl monkey's neck. Furthermore, we will compare TST and SEL data acquired from owl monkeys (Aotus lemurimus) of Amami laboratory with that of owl monkeys (Aotus azarae) of Kyoto University Primate Research Institute.

5. Forest animals (mainly mammal and birds) recorded with camera trap survey at the southern part, a mongoose free area on Amami-oshima Island, southwestern Island of Japan

Takeshi Kuraishi, Ken Ishida[10], Shosaku Hattori: [10]Graduate School of Agricultural and Life Science, University of Tokyo

We examined the ground use of forest animal inhabiting the southern part of Amami-oshima Island (including Mt. Yui and Mt. Kanengo area). We conducted a camera trap survey for 4 years to assess the relative abundance of ground using animals. In this study, we found that the frequency with which Amami Spiny Rat (*Tokudaia osimensis*) was photographed was extremely low at Mt Yui area regardless of a mongoose free zone. Instead, we confirmed that the frequency with which Roof Rat (*Rattus rattus*) was photographed was high at same area. We also confirmed that the relative abundance of Amami Spiny Rat was significantly higher than that of Roof Rat at Mt Kanengo area. Thus, the invasion of the area by the Roof rat may cause a crucial problem for the Amami Spiny Rat community of the forest in Mt. Kanengo area.

Publications

Kodama, A., Yanai, T., Kubo, M., El-Habashi, N., Kasem, S., Sakai, H., Masegi, T., Fukushi, H., Kuraishi, T., Yoneda, M., Hattori, S. and Kai, C. Cynomolgus monkeys (*Macaca fascicularis*) may not become infected with equine herpesvirus 9. J. Med. Primatol. 40: 18-20, 2011.

Yanai, T., Kodama, A., Sakai, H., Fukushi, H., Kuraishi, T., Hattori, S. and Kai, C. Equine Herpesvirus 9 (EHV-9) induced encephalitis in nonhuman primates. In Non-Flavivirus Encephalitis. Edited by Tkachev, S. (InTech). pp 147-156, 2011.

服部正策，倉石武．ハブ毒から精製されたタンパク質SVSP2の生理活性　Flavoxobinとの比較．平成22年度奄美ハブ毒免疫機序研究報告書．（鹿児島県）．pp. 46–48，2011．

服部正策．ハブ毒免疫馬血清の抗BPII活性．平成22年度奄美ハブ毒免疫機序研究報告書．（鹿児島県）．pp. 48–52，2011．

服部正策．合成ペプチドの筋壊死活性．平成22年度奄美ハブ毒免疫機序研究報告書．（鹿児島県）．pp. 52–53，2011．

倉石武，服部正策．ハブ毒免疫ウマ血清中に含まれるBPIIβウイング領域およびC末端領域に対する抗ペプチド抗体検出用ELISAの構築．平成22年度奄美ハブ毒免疫機序研究報告書．（鹿児島県）．pp. 54–59，2011．

倉石武，服部正策，朝沼榎．ハブ粗毒からのゲルろ過溶出画分(高分子量画分/中分子量画分/低分子量画分)と精製ハブ毒タンパク質に対する咬傷者血清中のIgEの間接ELISA法による測定．平成22年度奄美ハブ毒免疫機序研究報告書．（鹿児島県）．pp. 68–71，2011．

服部正策．生態査部会報告．平成22年度ハブとの共存に関わる総合調査事業報告書．（鹿児島県）．pp. 8–45，2011．

森口一，鳥羽道久，服部正策．環境研究部会報告．平成22年度ハブとの共存に関わる総合調査事業報告書．（鹿児島県）．pp. 46–59，2011．

Affiliated Facilities

Laboratory of Molecular Genetics
遺伝子解析施設

Professor	Izumu Saito, M.D., D.M.Sc.	教　授	医学博士	斎　藤　　泉
Assistant Professor	Yumi Kanegae, D.M.Sc.	助　教	医学博士	鐘ヶ江　裕　美
Assistant Professor	Saki Kondo, D.M.Sc.	助　教	医学博士	近　藤　小　貴

This laboratory has two main activities: development and supply of new adenovirus vectors enabling strictly-controlled gene expression useful for studies in various fields including gene therapy, and supporting the researchers by advising on recombinant DNA technology and on biohazards under the safety guidelines.

The purposes of our laboratory are concerned about not only research but also support for all researchers in this institute. Our supporting activity is involved in advising service on gene-manipulation experiments and on biohazards under the safety guidelines and laws. For the research part, we intend to develop novel methods or new experimental systems leading in the field of gene expression and its regulation. We are concentrating mainly on developing efficient adenovirus expression vectors useful for various fields including gene therapy. We are maintaining more than 20 collaborations within and outside of this institute. In these collaborations, we can supply adenovirus vectors (AdVs) enabling strictly regulated gene expression and helper-dependent AdVs (HD-AdVs) of high capacity up to 30 kilobases (kb). Previously we developed a system for construction of E1-deleted AdV using a full-length viral genome with intact viral termini (Fukuda. *et al*., Microbiol. Immunol. 50: 643-654, 2006). This cassette is available from Takara Bio and Nippon Gene. We have also developed a method for ON/OFF switching of gene expression in mammalian cells using a combination of adenovirus vector and Cre/*lox*P system (Kanegae *et al*., Nucleic Acids Res. 23: 3816-3821, 1995; Kanegae *et al*., Gene 181: 207-212, 1996) as well as FLP/frt system (Nakano *et al*., Nucleic Acids Res. 29: e40, 2001; Kondo *et al*., Nucleic Acids Res. 31: e76, 2003; Kondo *et al*., Microbiol. Immunol., 50: 831-843, 2006; Kondo et al., J. Molec. Biol., 2009). These methods continuously promote studies of various fields of molecular biology and medicine. Moreover, we recently identified adenovirus pIX gene as a main cause of inflammation observed in AdV infection (Nakai *et al*., Hum. Gene Ther. 18: 925-936, 2007). The AdV is now called the "low-inflammatory AdV" and will probably become widely used: for example, a widely used Cre-expressing AdV AxCANCre will be replaced by the low-inflammatory Cre-expressing AxEFNCre (Chiro et al., Virus Res. 160: 89-97, 2011). The research activities in 2011 were shown below.

1. Identification of a DNA segment in AdV genome enhancing foreign promoter activity

Aya Maekawa, Shun-ichi Arato, Yohei Ono, Zhng Pei, Saki Kondo, Yumi Kanegae and Izumu Saito

Helper-dependent AdV (HD-AdV) lacks all viral genes and instead contains large foreign DNA of about 30kb. This vector also has another advantage of no immunological responses against the viral gene products expressed after transduction into cells and consequently the expression of an inserted gene continues for a very long period. However, we experienced that the expression level of a gene in HD-AdV may be lower than that in E1-deleted AdV. Meanwhile, Parks *et al*. reported that the expression of a gene in the HD-AdV containing 30% of the AdV genome is higher than that in usual HD-AdV (J. Virol., 73: 8027-8034, 1999). Therefore, we speculated that there may be some DNA sequence within the 30% of the AdV genome that enhances the activity of a foreign promoter. The 30% of the genome consists of two DNA segments of the genome: the left-terminal segment of 5.7 kb and the right segment of 6.2 kb. To examine this possibility, a series of plasmids were constructed containing a unit expressing GFP under the control of various non-viral promoters. Then various DNA fragments derived from the above segments were inserted in these plasmids and the fluorescence of GFP in the transfected cells was measured using Ascent fluorescent meter. We were able to identify DNA regions that not only increase but also, unexpectedly, decrease the GFP expression. We are now trying to narrow down the regions in order to characterize them and to examine the mechanisms.

2. FLPe is more efficient than Cre in recombinase-mediated cassette exchange (RMCE) in vitro and in adenovirus production

Yuki Takata, Saki Kondo, Yumi Kanegae and Izumu Saito

Cre and FLP recombinases mediate not only specific deletions and insertions, but also the recombinase-mediated cassette exchange (RMCE) reaction, which is utilized in cell biotechnology including ES cells and mouse genetics. However, comparison of efficiencies for Cre and FLP in RMCE has not been performed. We here examined the detailed process of RMCE with Cre and FLP *in vitro* using mutant *lox*P 2272 and three mutant FRTs (FRT G, FRT H and FRT F3), and then quantitatively compared the RMCE reactions *in vitro*. Interestingly, in the *in vitro* reactions the RMCE efficiency of Cre reached a plateau level of about 5% and did not proceed further, while that of FLPe reached about 12-13%, showing that FLPe reached a higher level of efficiency than Cre possibly when they were supplied at a very high concentration. Moreover, we quantitatively compared the production efficiency of E1-deleted adenovirus vector using the RMCE method with Cre or FLP. The results showed that FLPe was again found more efficient than Cre in RMCE reaction. Therefore, although Cre is thought more active than, or similar to, FLPe, it may not be necessarily true for RMCE reaction. One possible explanation for the plateau using Cre was that a reverse RMCE reaction occurred with Cre but not with the FLPs in a high concentration. That is, the processed products and intermediates in the Cre reaction may still be able to attach together and function as a reverse reaction, while in the FLP reaction, they may come apart from each other, preventing their involvement in reverse reactions.

3. Conditional gene expression in hepatitis C virus transgenic mice without induction of severe liver injury using non-inflammatory Cre-expression adenovirus vector

Tomoko Chiyo[1], Satoshi Sekiguchi[1], Masahiro Hayashi[1], Yoshimi Tobita[1], Yumi Kanegae, Izumu Saito and Michinori Kohara[1]: [1]Department of Microbiology and Cell Biology, The Metropolitan Institute of Medical Science, Tokyo.

We previously established inducible-hepatitis C virus (HCV) transgenic mice, which exressed the HCV gene (nucleotides 294-3435) encoding the core, E1, E2, and NS2 proteins. The expression of these proteins is regulated by the Cre/*lox*P system and an adenovirus vector (AdV) that expresses Cre DNA recombinase (Cre) controlled by the CAG promoter (AxCANCre). Recent studies have demonstrated that AxCANCre injection alone results in severe liver injury by induction of the adenovirus protein IX (Ad-pIX) gene. As a result, HCV protein expression in transgenic mice livers was only short-term. In contrast, the EF1α promoter-bearing AdV induces slight Ad-pIX gene expression without inducing severe liver injury. Therefore, in the present study, we developed a Cre-expressing AdV that bears the EF1α promoter (AxEFCre) to express HCV protein in the transgenic mouse livers. In the non-transgenic mice injected with AxCANCre, alanine aminotransferase (ALT) levels were elevated and severe liver inflammation occurred; this was not observed in AxEFCre-injected mice. In contrast, AxEFCre-injected HCV transgenic mice showed milder liver inflammatory responses that were clearly due to HCV protein expression. Moreover, the AxEFCre injection enabled the transgenic mice to persistently express HCV protein. These results indicate that use of AxEFCre efficiently promotes Cre-mediated DNA recombination in vivo with-

out a severe hepatitis response to AdV. This inducible-HCV transgenic mouse model using AxEFCre should be useful for research on HCV pathogenesis.

Publications

Kanegae Y, Terashima M, Kondo S, Fukuda H, Maekawa A, Pei Z, and Saito I. High-level expression by tissue/cancer-specific promoter with strict specificity using a single adenoviral vector. Nucleic Acids Res., 39: e7, 2011

Sakamoto S, Yazawa T, Baba Y, Sato H, Kanegae Y, Hirai T, Saito I, Goto T, and Kurahashi K. Keratinocyte growth factor gene transduction ameliorates pulmonary fibrosis induced by bleomycin in mice. Am. J. Respir. Cell Molec. Biol., 45: 489-497, 2011.

Chiyo T, Sekiguchi S, Hayashi M, Tobita Y, Kanegae Y, Saito I and Kohara M. Conditional gene expression in hepatitis C virus transgenic mice without induction of severe liver injury using a non-inflammatory Cre-expressing adenovirus. Virus Res., 160: 89-97, 2011.

Ishijima N, Suzuki M, Acida H, Ichikawa Y, Kanegae Y, Saito I, Boren T, Haas R, Sasakawa C and Mimuro H. BavA-mediated adherence is a potentiator of the Helicobacter pylori type IV secretion system activity. J. Biol. Chem., 286: 25256-25264, 2011.

Takata Y. Kondo S (equal contribution), Goda N, Kanegae Y and Saito I. Comparison of efficiency between FLPe and Cre for recombinase-mediated cassette exchange (RMCE) *in vitro* and in adenovirus vector production. Genes Cells, 16: 765-777, 2011.

Pei Z, Kondo S, Kanegae Y and Saito I. Copy number of adenoviral vector genome transduced into target cells can be measured using quantitative PCR: application to vector titration. Biochem. Biophs. Res. Commun., 417: 945-950, 2012.

Kanegae Y, Ishimura M (equal contribution), Kondo S and Saito I. Influence of *lox*P insertion upstream of the cis-acting packaging domain on adenovirus packaging efficiency. Microb. Immunol., in press, 1012.

Affiliated Facilities

Medical Proteomics Laboratory
疾患プロテオミクスラボラトリー

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Professor	Kouhei Tsumoto, Ph.D.	教　授	工学博士	津　本　浩　平
Associate Professor	Shinobu Imajoh-Ohmi, D. Sc.	准教授	理学博士	大　海　　忍
Associate Professor	Masaaki Oyama, Ph.D.	准教授	医学博士	尾　山　大　明
Assistant Professor	Hiroshi Sagara, Ph.D.	助　教	医学博士	相　良　　洋

The mission of our laboratory is to develop advanced technologies for antibody engineering, mass spectrometry and electron microscopy to perform an integrative protein analysis from a physicochemical, structural and systems biology point of view. Currently, we mainly focus on the researches on functional protein-protein interactions related to a variety of diseases such as cancer and infection.
We are also engaged in collaborative researches regarding mass spectrometry, electron microscopy, peptide synthesis, protein purification and the related functional analyses and have made a substantial contribution to many scientific achievements.

‹Group I›

AIM: Life, as we understand it, requires of a concerted and complex set of interactions between different biological molecules, such as DNA, RNA, proteins, lipids, and carbohydrates. We seek to understand the nature of these interactions at the molecular and energetic level. Our dissecting tools are applied to study a broad range of biological phenomena, and to develop the next generation of therapeutic antibodies in the era of Bio-better and Bio-superior.

1. Nanodiscs as a model system to study membrane proteins

Takeaki Kawai, Jose M.M. Caaveiro, Ryota Abe, Toyomasa Katagiri and Kouhei Tsumoto

ATP-binding cassette (ABC) transporters couple hydrolysis of ATP with vectorial transport across the cell membrane. We have reconstituted ABC transporter MsbA in nanodiscs of various sizes and lipid compositions to test whether ATPase activity is modulated by the properties of the bilayer. ATP hydrolysis rates, Michaelis-Menten parameters, and dissociation constants of substrate analog ATP-γ-S demonstrated that physicochemical properties of the bilayer modulated binding and ATPase activity. This is remarkable when considering that the catalytic unit is located ～50Å from the transmembrane region. Our results validated the use of nanodiscs as an effective tool to reconstitute MsbA in an active catalytic state, and highlighted the close relationship between otherwise distant transmembrane and ATPase modules.

2. Stepwise characterization of the thermodynamics of trichocyte intermediate filament protein supramolecular assembly

Daisuke Ishii, Ryota Abe, Sun-ichi Watanabe, Masaru Tsuchiya, Bernd Nöcker and Kouhei Tsumoto

Trichocyte intermediate filament protein (IFP) is a heterodimeric complex that plays a pivotal role in the hair shaft for its mechanical strength, hair shape, and so on. Trichocyte IFP consists of acidic-type IFP and basic-type IFP, and the well-studied supramolecular assembly process of the complex occurs via the following steps: dimer formation, tetramer formation, formation of the lateral 32mer, and the elongation of the 32mer. Among these interactions, only the dimer formation, owing to coiled-coil interaction, has been described in detail; the nature of other interactions remains unspecified. For each assembly step, we report interaction isotherms obtained by means of isothermal titration calorimetry at various urea and NaCl concentrations. Decreasing the urea concentration generally promotes protein refolding, and we therefore expected to observe endothermic interactions owing to the refolding process. However, exothermic interactions were observed at 4 and 2 M urea, along with various characteristic endothermic interactions at the other urea concentrations as well as NaCl titration. The thermal responses described herein enabled us to analyze the protein supramolecular assembly process in a stepwise manner.

3. Molecular basis of recognition of antibacterial porphyrins by heme-transporters of *Staphylococcus aureus*

Yoshitaka Moriwaki, Jose M.M. Caaveiro, Yoshikazu Tanaka, Hiroshi Tsutsumi, Itaru Hamachi and Kouhei Tsumoto

Antibiotic resistance is increasingly seen as a serious problem that threatens public health and erodes our capacity to effectively combat disease. So-called non-iron metalloporhyrins have shown promising antibacterial properties against a number of pathogenic bacteria including *Staphylococcus aureus*. However, little is known about the molecular mechanism(s) of action of these compounds and in particular how they reach the interior of the bacterial cells. A popular hypothesis indicates that non-iron metalloporphyrins infiltrate into bacterial cells like a "Trojan horse" using heme transport systems. Iron-regulated surface determinant (Isd) is the best characterized heme transport system of S. aureus. Herein we studied the molecular mechanism by which the extracellular heme-receptor IsdH-NEAT3 of Isd recognizes antimicrobial metalloporphyrins. We found that potent antibacterial porphyrins Ga(III)-protoporphyrin IX (PPIX) and Mn(III)-PPIX closely mimicked the properties of the natural ligand heme, namely (i) stable binding to IsdH-NEAT3 with comparable affinities for the receptor, (ii) nearly undistinguishable three-dimensional structure when complexed with IsdH-NEAT3, and (iii) similar transfer properties to a second receptor IsdA. On the contrary, weaker antibacterial porphyrins Mg(II)-PPIX, Zn(II)-PPIX, and Cu(II)-PPIX were not captured effectively by IsdH-NEAT3 under our experimental conditions and displayed lower affinities. Moreover, reduction of Fe(III)-PPIX to Fe(II)-PPIX with dithionite abrogated stable binding to receptor. These data revealed a clear connection between oxidation state of metal and effective attachment to IsdH-NEAT3. Also, the strong correlation between binding affinity and reported antimicrobial potency suggested that the Isd system may be used by these antibacterial compounds to gain access to the interior of the cells. We hope these results will increase our understanding of Isd system of *S. aureus* and highlight its biomedical potential to deliver new and more efficient antibacterial treatments.

4. Refolding single-chain antibody (scFv) using lauroyl-L-glutamate as a solubilization detergent and arginine as a refolding additive

Motonori Kudou, Daisuke Ejima, Haruna Sato, Ryosuke Yumioka, Tsutomu Arakawa and Kouhei Tsumoto

Therapeutic potential of immunoconjugates has opened a new window for antibody-based biopharmaceuticals. Greater tissue penetration and hence enhanced cell toxicity are obtained with a smaller version of antibodies. While the whole antibody can be readily produced via mammalian expression system, antibody fragments often require refolding of insoluble proteins. Here we report a new refolding method for antibody fragments using a novel amino acid-based detergent as a solubilizing agent and arginine-assisted refolding. Inclusion bodies of antibody fragments were solubilized by 2.5% lauroyl-L-Glu (C12-L-Glu) and successfully refolded by multi-step dilution into a buffer solution containing arginine hydrochloride and thiol/disulfide-exchange reagents. Adjustment of temperature was found to be critical for increase in the refolding yield. Although each protein requires appropriate optimization, solubilization by C12-L-Glu and dilution refolding assisted by arginine can generate the native, functional antibody fragments. The procedure should enable us to utilize bacterial expression systems for the large-scale manufacturing.

5. Structural and energetic hot-spots for the interaction between a ladder-like polycyclic ether and the anti-ciguatoxin antibody 10C9Fab

Mihoko Ui, Yoshikazu Tanaka, Takeshi Tsumuraya, Ikuo Fujii, Masayuki Inoue, Masahiro Hirama and Kouhei Tsumoto

The mechanism by which anti-ciguatoxin antibody 10C9Fab recognizes a fragment of ciguatoxin CTX3C (CTX3C-ABCDE) was investigated by mutational analysis based on structural data. 10C9Fab has an extraordinarily large and deep antigen-binding pocket at the center of its variable region. We mutated several residues located at the antigen-binding pocket to Ala, and kinetic analysis of the interactions between the mutant proteins and the antigen fragment was performed. The results indicate that some residues associated with the rigid antigen-binding pocket are structural hot-spots and that L-N94 is an energetic hot-spot for association of the antibody with the antigen fragment CTX3C-ABCDE, suggesting the importance of structural complementarity and energetic hot-spot interactions for specific recognition of polycyclic ethers.

6. Targeted-glycosylation as a means to improve the stability of therapeutic interleukin-11

Saeko Yanaka, Emiko Sano, Norio Naruse, Kin-ichiro Miura, Mutsumi Futatsumori-Sugai, Jose M.M. Caaveiro and Kouhei Tsumoto

Human interleukin-11 (hIL-11) is a pleiotropic cytokine administered to patients with low platelet counts. From a structural point of view hIL-11 belongs to the long-helix cytokine superfamily, which is characterized by a conserved core motif consisting of four α-helices. We have investigated the region of hIL-11 that does not belong to the α-helical bundle motif, and that for the purpose of brevity we have termed "non-core region." The primary sequence of the interleukin was altered at various locations within the non-core region by introducing glycosylation sites. Functional consequences of these modifications were examined in cell-based as well as biophysical assays. Overall, the data indicated that the non-core region modulates the function of hIL-11 in two ways. First, the majority of muteins displayed enhanced cell-stimulatory properties (superagonist behavior) in a glycosylation-dependent manner, suggesting that the non-core region is biologically designed to limit the full potential of hIL-11. Second, specific modification of a predicted mini α-helix led to cytokine inactivation, demonstrating that this putative structural element belongs to site III engaging a second copy of cell-receptor gp130. These findings have unveiled new and unexpected elements modulating the biological activity of hIL-11, which may be exploited to develop more versatile medications based on this important cytokine.

‹Group II›

1. Development of novel antibodies as tools available for in situ analyses of post-translational modification of proteins

After biosynthesis proteins undergo various post-translational modifications, and their functions are modulated. In order to understand such biochemical reactions in a single cell, we have been making modification-specific antibodies as probes for such in situ analyses; cleavage-site-directed antibodies for proteolysis, phosphorylation-site-specfic antibodies, myristoilated peptide-specific antibodies , ubiquitination-specific antibodies, inhibitor-bound enzyme-specific antibodies etc. These antibodies should be useful tools for research in cellular biochemistry.

a. Evaluation of polyclonal cleavage-site directed antibodies and their fractionation into more easy-to use probes

Tsuyoshi Katagiri, Chidzuko Takamura, Nozomi Ichikawa and Shinobu Imajoh-Ohmi

Cleavage-site directed antibodies are convenient tool for in situ analysis of proteolysis, since they do not bind unproteolyzed native proteins that retain the same sequence internally. To obtain such antibodies, peptides corresponding to the terminal regions around the cleavage are chemically synthesized and used for haptens, where molecular design of the peptides is critical for quality of the antibodies. Too short peptide results in generation of useless antibodies recognizing the short peptide but not the terminus of cleaved proteins. On the other hand, when a longer sequence is selected for immunogenic peptide, antibodies raised bind unproteolyzed proteins as well as the cleaved ones. Thus, an evaluation system is necessary for cleavage-site directed antibodies. Phage display libraries were used for evaluation of antigenic specificity of cleavage-site directed antibodies. Randomized sequences of synthetic oligonucleotide were introduced into phage DNA in order that a fusion protein with randomized sequences of amino- or carboxyl-terminal region.

A library was applied to immobilized antibodies, and phages bound were subjected to sequence analysis for terminal regions. When antigenic specificity of a cleavage-site directed antibody was examined by this method, the antibody was found to be a mixture of three or more types of antibodies that bind to terminal and internal regions of the peptide used for immunogen. Quality of the antibody was successfully improved by affinity chromatography immobilized three peptides according to the evaluation method.

b. A novel method for hunting substrates of caspases in apoptotic cells

Maiko Okada, Chidzuko Takamura, Hiroyuki Fukuda, Masahiko Kato and Shinobu Imajoh-Ohmi

Caspases catalyze limited proteolysis of many proteins in apoptotic cells. Hundreds of substrates have been identified as targets of caspases so far. Previously, nonmuscle myosin heavy chain-A and a component of DNA-dependent proein kinase, Ku80, are found to be cleaved during apoptosis in human Jurkat T cells. We used first a cleavage-site derected antibody against the amino-terminal fragment of caspase 3/7-catalyzed calpastatin. Carboxyl-terminal region of caspase-proteolyzed fragments resemble each other, and such antibodies are expected to misrecognize the target molecules. We further investigated the apoptotic Jurkat cells for the anitobody-stained polypeptides. Cells were selectively extracted with salt- and denaturant-containing buffers, and extracts were subjected to two-dimensional gel electrophoresis/immunoblotting. Candidate polypeptides stained with antibodies were digested with trypsin and analyzed by mass spetrometer. Isoforms of ribonucleoprotein were thus identified.

2. Biology of calcium-dependent proteases in *Caenorhabditis elegans*

Caenorhabditis elegans, a free-living nematode in the soil, consists of no more than 1,000 cells, but retains various functions similar to those of higher-order animals. We have been interested in biology of vertebrate calpain, an intracellular proteolytic enzyme activated with calcium ions. Genes related to mammalian calpain are also present in C. elegans, and they are expected to be translated into proteinous forms, but their function have not been characterized biochemically.

a. Identification of cysteine proteases in *Caenorhabditis elegans*

Yohei Kato, Nozomi Ichikawa and Shinobu Imajoh-Ohmi

E64c, [L-3-trans-carbonyloxirane-2-carbonyl]-L-leucine(3-methylbutyl)amide, is a synthetic inhibitor for cysteine proteases such as cathepsins B, H, L and calpain. To inhibit intracellular cysteine proteases E64d, [L-3-trans-ethoxycarbonyloxirane-2-carbonyl]-L-leucine (3-methylbutyl) amide, a membrane-permeable derivative of E64c is used instead of E64c. E64d penetrates into the cell membranes where endogenous esterases convert it to enzyme-inhibitable E64c that covalently binds to the SH group of active center in cysteine proteases. Thus, anti-E64c antibody is a useful probe for *in vivo* analysis of cysteine proteases.

We have succeeded in making an antibody to E64c. First, we tried to establish an antibody against E64c-bound calpain. A peptide corresponding to the active center of calpain was synthesized by means of the multiple-antigen peptide system using Fmoc chemistry. E64c was chemically introduced into the SH group of active center cysteine under reducing conditions. Rabbits were immunized with the E64c-conjugated calpain-derived peptide without further conjugation with a carrier protein. Unexpectedly, an antibody thus prepared reacted not only with E64c-inactivated calpain but also with E64c-bound other cysteine proteases such as papain and cathepsins. Low antigenicity of peptide region in the immunogen may result in such broad specificity of the antibody. Our antibody is expected to be used for identification of E64c-targeted novel proteases. When cells were treated with E64d, cell growth was suppressed and several proteins were labeled by E64c that is visualized with this antibody on immunoblotting. Structural analysis of these proteins may lead identification of novel cysteine proteases.

Homogenates of *C. elegans* were treated with E64c in the presence or absence of calcium ion, and subjected to electrophoresis/immunoblotting using an anti-E64c antibody. A 55-kDa polypeptide (p55) was labelled with E64c in a calcium ion-dependent manner. In *C. elegans* several calpain-related gene products were identified at the mRNA level, but their physiological function remains to be elucidated.

3. Post-translational modification of proteins during apoptotic cell death

Apoptotic cell death involves various biochemical reactions. Among them, post-trans-

lational modification of proteins is intensively investigated in this laboratory. First, intracellular proteolytic enzymes are activated prior to and during apoptosis. Caspases are now established as pivotal apoptosis-executing enzymes that cleave various substrates. Endogenous or viral proteins and synthetic substances inhibitory for caspases suppress the apoptotic cascade and rescue cells from cell death. On the other hand, proteasomes drive the cell cycle by degrading cyclins etc., and also play important parts in apoptosis, since proteasome inhibitors induce apoptotic cell death in growing cells but suppress apoptosis of some cells that is in quiescent state. Furthermore, in some specific cells such as polymorphonuclear leukocytes, other proteases might be involved in cell death.

a. Limited proteolysis of actin in apoptotic neutrophils

Junko Ohmoto and Shinobu Imajoh-Ohmi

Neutrophil actin is proteolyzed to a 40-kDa fragment during preparation/isolation from peripheral blood. The truncated actin lacks amino-terminal region of native protein and presumably cannot copolymerize to F-actin. The 40-kDa actin-derived fragment is apparently related to spontaneous apoptosis of neutrophils. To investigate the role of actin proteolysis, especially cause-and-effect relationship to neutrophil apoptosis, we have made a cleavage-site-directed antibody (#1090pAb) for the 40-kDa form of actin using synthetic peptides as haptens. The antibody reacted with the 40-kDa polypeptide but not with unproteolyzed native actin which remain abundant in the cell. Using this antibody, we have found that (1) the truncated actin is generated during isolation of neutrophils from peripheral blood, (2) neutrophils without the truncated actin can be prepared in the presence of diisopropyl fluorophosphate, and (3) leukocyte elastase is possibly responsible for this limited proteolysis.

Herein we analyzed cellular localization of the truncated actin using #1090pAb. Confocal laser microscopic observation indicated that the plasma membrane of neutrophils were strongly stained with #1090pAb, but that intracellular regions near the membrane were sometimes stained weakly. We examined here whether or not the amino-teriminal region of the 40-kDa actin is on the cell surface of neutrophils using at the same time established antibodies for components of superoxide-generating system composed of transmembranous cytochrome and cytosolic activator proteins. Furthermore, flow-cytometric analysis revealed that #1090pAb stained the cell-surface antigen under the conditions that antibodies for cytosolic proteins did not. Our findings suggest that the truncated actin is, at least in its amino-terminal part, on the surface of neutrophils. However, another antibody against the amino-terminal region of native actin did not stain neutrophils from outside suggesting that the cleavage site is inaccessible to exogenous proteinases.

b. Fas, a death receptor, is polymerized to high-molecular weight forms during Fas-mediated apoptosis in Jurkat T cells

Hidehiko Kikuchi, Fotoshi Kuribayashi and Shinobu Imajoh-Ohmi

An apoptotic receptor Fas mediates death signal from Fas ligand. A cell death-inducing monoclonal antibody CH11 mimics Fas ligand and triggers apoptotic signal mediated by Fas molecule. Plasma transglutaminases are found to involved in down-regulation of apoptosis induced by a cytotoxic anti-Fas monoclonal antibody in Jurkat cells. When cells were treated with the antibody in fetal calf serum-containing media, Fas was polymerized to higher-molecular-weight polypeptides as judged by immunoblotting. Under conditions where the transglutaminase activity was eliminated or supprressed, the polymerization of Fas was not observed, and concurrently cell death was hastened. Furthermore, an antibody against blood coagulation factor XIII strongly accelerated the Fas-mediated apoptosis, indicating that plasma transglutaminases catalyze polymerization of Fas and down-regulate apoptotic cell death.

4. The ATPase activity of nonmusle myosin IIC is regulated by BRCA2 in the midbody ring

Akira Nakanishi[1,2], Yoshio Miki[2,3], and Shinobu Imajho-Ohmi: [1]IMSUT, [2]Depertment of Molecular Genetics, Medical Research Institute, Tokyo Medical and Dental University, [3]Department of Genetic Diagnosis, The Cancer Institute, Japanese Foundation for Cancer Research

The tumor suppressor gene BRCA2 is involved in homologous recombinational DNA repair that contributes to structural chromosomal stability. BRCA2 also participates in the regulation of centrosome and cytokinesis. BRCA2 is localized to the midbody during cytokinesis and interacts with the human nonmuscle myosin heavy chain (NMHC) IIC. The biochemical activity of NMHC IIC originates from its actin-activated MgATPase activity. However, the spe-

cific function of BRCA2 in regulating the biochemical activity of NMHC IIC in the midbody is unclear.

To examine the function of BRCA2 in the regulation of NMHC IIC, we analyzed the effect of BRCA2 on the actin-activated MgATPase activity of NMHC IIC. BRCA2 and NMHC IIC were isolated by immunoprecipitation from BRCA2-FLAG or HA-NMHC IIC-transfected COS7 cells. The actin-activated MgATPase activity of NMHC IIC was analyzed by incubation of the immunoprecipitated HA-NMHC IIC in the presence and absence of BRCA2-FLAG. MgATPase was activated by the addition of BRCA2-FLAG in a does-dependent manner. In contrast, MgATPase was not activated in the absence of BRCA2-FLAG. Based on these results, we suggest that MgATPase activity was likely to be caused by a complex formation between NMHC IIC and BRCA2.

Furthermore, we demonstrated that BRCA2 and NMHC IIC localized to the midbody ring (Flemming body) during cytokinesis. Time-lapse imaging and performed to reveal the ring structure showed that the midbody ring was dynamic and removed from side to side of the midbody. We hypothesized that the midbody ring is composed of NMHC IIC. To test this prediction, we attempted the *in vitro* reconstitution of the midbody ring using recombinant NMHC IIC. We expressed recombinant NMHC IIC-GFP in COS7 cells and placed a few drops of the cell lysates on a cover glass. The lysates were analyzed using high-resolution deconvolution microscopy, which demonstrated that NMHC IIC-GFP was a part of a unique ring-like structure, when both ATP and Mg^{2+} were added. The ring was 1.5-2.0 mm in diameter. However, in the presence of blebbistatin, which is an inhibitor of the myosin II ATPase activity, NMHC II failed to organize into a ring-like structure. The ring-like structure was also not observed in the presence of ATP or Mg^{2+} alone. Next, we coexpressed recombinant NMHC IIC-GFP and BRCA 2-FLAG in COS7 cells and performed immunofluorescence microscopy using anti-GFP and anti-BRCA2 antibodies. The colocalization of the fluorescent signals derived from the anti-GFP and anti-BRCA2 antibodies suggested that the ring-like structure was composed of NMHC IIC-GFP and BRCA2.

5. The high-affinity choline transporter CHT1 is regulated by the ubiquitin ligase Nedd4-2

Haruhiko Yamada[1,4], Shinobu Imajoh-Ohmi and Tatsuya Haga[4]: [4]Department of Life Science, Faculty of Science, Gakushuin University

The high-affinity choline transporter (CHT1), which is specifically expressed in cholinergic neurons, constitutes a rate-limiting step for acetylcholine synthesis. We have found that the exogenous ubiquitin ligase Nedd4-2 interacts with CHT1 expressed in HEK293 cells decreasing the amount of cell surface CHT1 by approximately 40%, and that small interfering RNA for endogenous Nedd4-2 enhances the choline uptake activity by CHT1 in HEK293 cells. These results indicate that Nedd4-2-mediated ubiquitination regulates the cell surface expression of CHT1 in cultured cells and suggest a possibility that treatments or drugs which inhibit the interaction between CHT1 and Nedd4-2 might be useful for diseases involving decrease in acetylcholine level such as Alzheimer's disease.

‹Group III›

1. Global phosphoproteome analysis of glioblastoma stem cells by high-resolution mass spectrometry

Hiroko Kozuka-Hata, Yukiko Nasu-Nishimura[1], Ryo Koyama-Nasu[1], Hiroko Ao-Kondo, Kouhei Tsumoto, Tetsu Akiyama[1] and Masaaki Oyama: [1]Laboratory of Molecular and Genetic Information, Institute of Molecular and Cellular Biosciences, University of Tokyo

Glioblastoma is one of the most aggressive tumors with poor prognosis. In order to systematically elucidate the aberrant signaling machinery activated in this malignant cancer, we investigated phosphoproteome dynamics of glioblastoma stem cells using high-resolution nanoflow LC-MS/MS system in combination with SILAC technology. Through phosphopeptide enrichment by titanium dioxide beads, a total of 3,288 phosphopeptides from 1,568 phosphorylated proteins were identified based on the two peptide fragmentation methodologies of collision induced dissociation and higher-energy C-trap dissociation. The SILAC-based quantification described 283 up-regulated and 141 down-regulated phosphorylated sites upon epidermal growth factor stimulation, including the comprehensive status of the phosphorylated sites on stem cell markers such as nestin. Very intriguingly, our in-depth phosphoproteome analysis led to identification of novel phosphorylated molecules encoded by the undefined sequence regions of the human transcripts, one of which was regulated upon external stimulation in human glioblastoma stem cells. Our results provide a new insight into the protein networks in glioblastoma stem cells and pave the way for exploration of drug targets to develop more ef-

fective treatments for glioblastoma.

2. Integrative analysis of phosphoproteome and transcriptome dynamics defines drug-resistance properties of breast cancer

Masaaki Oyama, Takeshi Nagashima[2], Hiroko Kozuka-Hata, Noriko Yumoto[2], Yuichi Shiraishi[2], Kazuhiro Ikeda[3], Yoko Kuroki[2], Noriko Gotoh[4], Satoshi Inoue[3], Hiroaki Kitano[5] and Mariko Okada-Hatakeyama[2]: [2]RIKEN, [3]Research Center for Genomic Medicine, Saitama Medical University, [4]Division of Systems Biomedical Technology, IMSUT, [5]Sony Computer Science Laboratories, Inc.

Signal transduction system, in orchestration with subsequent transcriptional regulation, widely regulates complex biological events such as cell proliferation and differentiation. Therefore, a comprehensive and fine description of their dynamic behavior provides a fundamental platform for systematically analyzing the regulatory mechanisms that result in each biological effect. Here we developed an integrated framework for time-resolved description of phosphoproteome and transcriptome dynamics based on the SILAC-nanoLC-MS and GeneChip system. In this study, we analyzed cellular information networks mediated by estrogen receptor/ErbB2 pathways, which have long been implicated in drug response of breast cancer. Through shotgun identification and quantification of phosphorylated molecules in breast cancer MCF-7 cells, we obtained a global view of the dynamics regarding breast cancer-related signaling networks upon estrogen (E2) or heregulin (HRG) stimulation. Comparative analysis of wild-type and tamoxifen-resistant MCF-7 cells revealed altered behaviors of signaling hub dynamics, indicating distinct signaling network properties between these two cell types. Pathway and motif activity analyses using the transcriptome data suggested that deregulated activation of GSK3β and MAPK1/3 signaling might be associated with altered activation of CREB and AP-1 transcription factors in tamoxifen-resistant MCF-7 cells. Thus, our integrative analysis of phosphoproteome and transcriptome in human breast cancer cells revealed distinct signal-transcription programs in tamoxifen-sensitive and insensitive tumor cells, which potentially defines drug-resistance properties against tamoxifen.

3. System-level analysis of CagA-dependent signaling network dynamics by Helicobacter pylori infection

Hiroko Kozuka-Hata, Masato Suzuki[6], Kotaro Kiga[6], Shinya Tasaki, Jun-ichiro Inoue, Tadashi Yamamoto[7], Chihiro Sasakawa[6] and Masaaki Oyama: [6]Division of Bacterial Infection, Department of Microbiology and Immunology, IMSUT, [7]Division of Oncology, Department of Cancer Biology, IMSUT

The signal transduction system within a cell regulates complex biological events in response to bacterial infection. The previous analyses of cell signaling in Helicobacter pylori-infected gastric epithelial cells have revealed that CagA, a major virulence factor of Helicobacter pylori, is delivered into cells via the type IV secretion system and perturbs signaling networks through the interaction with the key signaling molecules such as SHP-2, Grb2, Crk/Crk-L, Csk, Met, and ZO-1. Although the biological activity of tyrosine-phosphorylated CagA has intensively been studied, system-wide effects of the virulence factor on cellular signaling have yet to be analyzed. Here we performed time-resolved analyses of phosphoproteome and CagA-interactome in human gastric AGS cells by CagA-positive/negative Helicobacter pylori infection. Our highly sensitive nanoLC-MS/MS analyses in combination with the Stable Isotope Labeling by Amino acids in Cell culture (SILAC) technology defined CagA-dependent perturbation of signaling dynamics along with a subset of CagA-associated possible modulators on a network-wide scale. Our result indicated that the activation level of the phosphotyrosine-related signaling molecules in AGS cells was suppressed overall in the presence of CagA during Helicobacter pylori infection. As Helicobacter pylori infection plays pivotal roles in the progression of gastric diseases including carcinogenesis, a comprehensive and fine description of the signaling dynamics would serve as a fundamental platform to theoretically explore for the potential drug targets through analyzing the regulatory mechanisms at the system-level.

4. Phosphoproteomics-based modeling defines the regulatory mechanism underlying aberrant EGFR signaling

Shinya Tasaki, Masao Nagasaki[8], Hiroko Kozuka-Hata, Kentaro Semba[9], Noriko Gotoh[4], Seisuke Hattori[10], Jun-ichiro Inoue, Tadashi Yamamoto[7], Satoru Miyano[8], Sumio Sugano[11] and Masaaki Oyama: [8]Laboratory of DNA Information Analysis, Human Genome Center, IMSUT, [9]Department of Life Science and Medical Bio-Science, Waseda University, [10]Department of Biochemistry, School of Pharmaceutical Sciences, Kitasato University, [11]Laboratory of Functional Genomics, Department of

Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo

Mutation of the epidermal growth factor receptor (EGFR) results in a discordant cell signaling, leading to the development of various diseases. However, the mechanism underlying the alteration of downstream signaling due to such mutation has not yet been completely understood at the system level. Here, we report a phosphoproteomics-based methodology for characterizing the regulatory mechanism underlying aberrant EGFR signaling using computational network modeling. Our phosphoproteomic analysis of the mutation at tyrosine 992 (Y992), one of the multifunctional docking sites of EGFR, revealed network-wide effects of the mutation on EGF signaling in a time-resolved manner. Computational modeling based on the temporal activation profiles enabled us to not only rediscover already-known protein interactions with Y992 and internalization property of mutated EGFR but also further gain model-driven insights into the effect of cellular content and the regulation of EGFR degradation. Our kinetic model also suggested critical reactions facilitating the reconstruction of the diverse effects of the mutation on phosphoproteome dynamics. This is the first phosphoproteomics-driven mathematical description of the regulatory mechanism of EGFR signaling networks, which could provide a systematic strategy toward controlling disease-related cell signaling.

5. Photo-crosslinking-based proteomics elucidates direct protein-protein interactions involving a defined binding domain

Nobumasa Hino[2], Masaaki Oyama, Aya Sato[2], Takahito Mukai[2], Hiroko Kozuka-Hata, Tadashi Yamamoto[7], Kensaku Sakamoto[2] and Shigeyuki Yokoyama[2]

Signal transduction pathways are essentially organized through the distribution of various binding domains in signaling proteins, with each domain binding to its target molecules. To identify the targets of these domains, we developed a novel proteomic approach, based on photo-cross-linking and mass spectrometry. Through the use of an expanded genetic code, a photoreactive amino acid, ptrifluoromethyldiazirinyl-L-phenylalanine, was site-specifically incorporated into the SH2 domain of the adaptor protein GRB2 in human embryonic kidney cells. By exposing the cells to 365-nm light after an EGF stimulus, the SH2 of GRB2 was cross-linked with the endogenous proteins directly interacting with it. These targets were identified by a comparative mass-spectrometric strategy. Thus, we discovered that GRB2-SH2 directly binds to the GIT1 scaffold protein and the AF6 protein, a putative effector of the RAS protein. Furthermore, heterogeneous nuclear ribonucleoproteins F, H1, and H2 were found to be direct targets of GRB2-SH2.

6. Mass spectrometry-based annotation of the human short ORFeome

Masaaki Oyama, Hiroko Kozuka-Hata, Sumio Sugano[11], Tadashi Yamamoto[7] and Jun-ichiro Inoue

In parallel with the human genome projects, human full-length cDNA data has also been intensively accumulated. Large-scale analysis of their 5′-UTRs revealed that about half of these had a short ORF upstream of the coding region. Experimental verification as to whether such upstream ORFs are translated is essential to reconsider the generality of the classical scanning mechanism for initiation of translation and define the real outline of the human proteome. Our previous proteomics analysis of small proteins expressed in human K562 cells provided the first direct evidence of translation of upstream ORFs in human full-length cDNAs (Oyama et al., Genome Res, 14: 2048-2052, 2004). In order to grasp an expanded landscape of the human short ORFeome, we have performed an in-depth proteomics analysis of human K562 and HEK293 cells using a two-dimensional nanoLC-MS/MS system. The results led to the identification of eight protein-coding regions besides 197 small proteins with a theoretical mass less than 20 kDa that were already annotated coding sequences in the curated mRNA database. In addition to the upstream ORFs in the presumed 5′-untranslated regions of mRNAs, bioinformatics analysis based on accumulated 5′-end cDNA sequence data provided evidence of novel short coding regions that were likely to be translated from the upstream non-AUG start site or from the new short transcript variants generated by utilization of downstream alternative promoters. Protein expression analysis of the *GRINL1A* gene revealed that translation from the most upstream start site occurred on the minor alternative splicing transcript, whereas this initiation site was not utilized on the major mRNA, resulting in translation of the downstream ORF from the second initiation codon. These findings reveal a novel post-transcriptional system that can augment the human proteome via the alternative use of diverse translation start sites coupled with transcriptional regulation through alternative promoters

or splicing, leading to increased complexity of short protein-coding regions defined by the human transcriptome (Oyama et al., Mol Cell Proteomics, 6: 1000-1006, 2007).

‹Group IV›

1. Analysis of neural circuits in the retina using electron microscopic tomography and x-ray microscopy

Hiroshi Sagara and Haruo Mizutani[1]: [1]Sience Integration Program, Organization for Interdisciplinary Research Projects, University of Tokyo

Neural circuits in the central nervous system are the basis of various high-order brain functions. It is also true in case of retin. In the retina, six main classes of cells connect each other systematically to make up complex neural circuits. Characteristics of the retinal functions have been examined precisely by the electrophysiological methods and models of cell connectivity have been proposed. But morphological studies of the actual neural connection, which constitute the physiological properties of higher order neurons, still not enough. In this study, we are trying to reveal the actual neural circuit morphologically by using electron microscopic computed tomography (CT) and X-ray microscopy. This year, we improved the specimen preparation procedure for electron microscopic tomography and X-ray microscopy by modifying that of the conventional electron microscopy to gain higher membrane contrast and more stability. By using improved procedure we could identify synapse structures in the 500nm thick sections of neural tissue. The improved methods will help collecting the information to decipher the wiring diagram of the retina.

2. Collaborative and supportive researches as electron microscope core-laboratory

This group is also engaged in collaborative researches using electron microscope. We offer supports for the research projects those need electron microscopic analysis. The services available in this group are the conventional thin section transmission electron microscopy, immuno-electron microscopy, negative staining techniques and scanning electron microscopy. By using these individual technique or combination of some of these, we can offer direct visual evidence that cannot be acquired by other methods. This year, 24 projects in 17 laboratories were performed as core-laboratory works.

a. Thin section electron microscopy

Thin section electron microscopy is the most widely used technique to observe the inner structure of cells and tissues. In this method, samples are fixed and embedded in epoxy resin, thin sections with about 70nm thickness are cut and observed in the electron microscope. In case of immuno-electron microscopy, thin sections are obtained by similar procedure, and the antigen epitopes exposed on the surface of the sections are marked by sequentially reacted with appropriate primary antibodies and colloidal gold labeled secondary antibodies. This year, thin section electron microscopy combined with immuno-electron microscopy were used in many collaborative works.

a-1. Ultrastructural analysis of entry and assembly of Herpes Simplex Virus.

We have been performing several studies with research groups in Dr. Kawaguchi[2]'s laboratory: [2]Department of Infectious Disease Control, International Research Center for Infectious Diseases, regarding the infection/replication processes of herpes simplex virus (HSV). This year, thin section electron microscopy was used to analyze the trans-nuclear membrane processes of the newly formed viruses. By analyzing the virus forming processes in some mutant host cells, we could find candidate host molecules those may be involved in the trans-nuclear process of the HSV.

a-2. Morphological and immuno-electron microscopic analysis of the mucosal M-cells.

We have been performing several studies also with research groups in Dr. Kiyono[3]'s laboratory: [3]Division of Mucosal Immunology, Department of Microbiology and Immunology. In these studies, we analyzed the ultrastructure of the M-cells in the airway and intestinal epithelium by thin section transmission electron microscopy and scanning electron microscopy. Molecular characteristics of the M-cells were also analyzed using Immuno-electron microscopy (ref. Kim *et al*). In another study, several species of proteins were induced to express in rice and examined the localization within the cell with immuno-electron microscopy. We revealed that the expressed epitopes were accumulated in the different compartments depending on the kind of the expressed protein.

Some other collaborative research works using thin section electron microscopy and/or immuno-electron microscopy were performed

with Dr. Noda[4] *et al* in [4]Division of Virology, Department of Microbiology, regarding the structure of the influenza viruses and ebola virus, Dr. Sanada's group[5], in [5]Department of Gerontological Nursing/Wound Care Management, Graduate School of Medicine, The University of Tokyo (ref. Ibuki *et al*, Nakagami *et al*), Dr. Hoshina[6] in [6]Division of Oncology, regarding the structure of the synapses, Dr. Kunieda[7] in [7]Laboratory of Physiological Chemistry, Department of Biological Sciences, Graduate school of Science, regarding the morphology of the Tardigrades, and so on.

b. Negative staining techniques

Negative staining techniques are simple and quick method to observe the morphology of the macro molecules. In the collaborative work with Dr. Noda[4] *et al*., this technique was used to analyze the morphology of the influenza virus with or without ultracentrifugation (ref. Sugita *et al*). The negative staining techniques were also used in some works to analyze the structure of the purified proteins and the proteins integrated in the plasma membrane.

c. Scanning electron microscopy

Scanning electron microscopy is a technique used to examine the surface structure of the cells, tissues or other non-biological materials. The collaborative works using scanning electron microscopy were done with Dr. Nishizumi-Tokai[6] *et al*, regarding the surface structure of the egg cells. Other works are in progress with Dr. Sanada[5] *et al*, to analyze the effects of diabetes or bacterial infection during wound repair. Scanning electron microscopy was also used to analyze the non-biological materials as a collaborative work with Dr. Cheng[8] in [8]Olympus Co.

Publications

‹Group I›

Nishina T., Komazawa-Sakon S., Yanaka S., Zheng D., Piao X., Piao J., Yamashina S., Sano E., Doi T., Ueno T., Ezaki J., Ushio H., Ernst M., Tsumoto K., Okumura K., Nakano H. Interleukin 11 links oxidative stress and compensatory proliferation. Sci. Signal, 5, ra 5 (2012).

Kawai T, Caaveiro JMM, Abe R, Katagiri T, and Tsumoto, K. Catalytic activity of MsbA Reconstituted in Nanodisc Particles is Modulated by Remote Interactions with the Bilayer. FEBS Lett, 585: 3533-3537, 2011.

Ishii D, Abe R, Watanabe S, Tsuchiya M, Nöcker B, and Tsumoto K. Stepwise characterization of the thermodynamics of trichocyte intermediate filament protein supramolecular assembly. J Mol Biol, 408: 832-838, 2011.

Moriwaki Y, Caaveiro JMM, Tanaka Y, Tsutsumi H, Hamachi I, and Tsumoto K. Molecular Basis of Recognition of Antibacterial Porphyrins by Heme-Transporter IsdH-NEAT3 of *Staphylococcus aureus*. Biochemistry 50: 7311-7320, 2011.

Kudou M, Yumioka R, Ejima D, Arakawa T, and Tsumoto K. A novel protein refolding system using lauroyl-l-glutamate as a solubilizing detergent and arginine as a folding assisting agent. Protein Expr Purif, 75: 46-54, 2011.

Ui M, Tanaka Y, Tsumuraya T, Fujii I, Inoue M, Hirama M, and Tsumoto K. Structural and energetic hot-spots for the interaction between a ladder-like polycyclic ether and the anti-ciguatoxin antibody 10C9Fab. Mol Biosyst, 7: 793-798, 2011.

Yanaka S, Sano E, Naruse N, Miura K-I, Futatsumori-Sugai M, Caaveiro JMM, and Tsumoto K. Non-core Region Modulates Interleukin-11 Signaling Activity: Generation of Agonist and Antagonist Variants. J Biol Chem, 286: 8085-8093, 2011.

Rajan R.S., Tsumoto K., Tokunaga M., Tokunaga H., Kita Y., and Arakawa T. Chemical and Pharmacological Chaperones: Application for Recombinant Protein Production and Protein Folding Diseases. Curr Med Chem, 18: 1-15, 2011.

Nagatoishi S., Isono N., Tsumoto K., and Sugimoto N. Contribution of loops in DNA G-quadruplex on the binding of protein. Biochimie, 93: 1231-1238, 2011.

Yoshino A., Tashiro S., Ogino A., Yachi K., Ohta T., Fukushima T., Watanabe T., Katayama Y., Okamoto Y., Sano E. and Tsumoto K. Gene expression profiles predicting the response to IFN-β and a combination of temozolomide and IFN-β in malignant gliomas. Int J Oncol, 39: 529-542, 2011.

Suzuki T., Kim M., Kozuka-Hata H., Watanabe M., Oyama M., Tsumoto K. and Yamamoto T. Monoubiquitination of Tob/BTG family proteins competes with degradation-targeting polyubiquitination. Biochem Biophys Res Commun, 409: 70-74, 2011.

Nagatoishi S., Isono N., Tsumoto K., and Sugimoto N. Hydration is Required in DNA G-Quadruplex-Protein Binding. ChemBioChem,

12 (2011), 1822-1826.
Ohtake S., Tsumoto K., Tokunaga M., Kita Y., and Arakawa T. The mechanism of elution by $MgCl_2$, ethylene glycol and arginine in affinity chromatography. Global J Biochem. 2: 1-27, 2011.
Ohtake S., Kita Y., Tsumoto K., and Arakawa T. Amino Acids, Peptides and Proteins in Organic Chemistry Chapter, Solvent Interactions with Proteins and Other Macromolecules,Volume 5 - Analysis and Function of Amino Acids and Peptides, Wiley (2011), 273-356.
中木戸誠，津本浩平「生化学事典」(担当部分)「クロマトグラフィー」「界面活性剤」朝倉書店，印刷中.
津本浩平「薬系免疫学」(担当部分)「抗体が抗原を見分けるしくみ」「抗体医薬品」南光堂，印刷中.
津本浩平「新機能抗体開発ハンドブック」(担当部分)「抗体の構造」「アフィニティの測定」「SPR−ビアコア」「カロリメトリー」NTS，印刷中.
津本浩平「ドラッグデリバリーシステムの新展開」(担当部分)「次世代抗体創製のための分子認識機構の解明」シーエムシー出版，印刷中.
津本浩平「抗体の安定性向上技術」BIOINDUSTRY，28(7)(2011)，28−34.
津本浩平「タンパク質相互作用解析：等温滴定型熱量測定と表面プラズモン共鳴」生物工学，89：391−394.
津本浩平「タンパク質化学を基にした創薬開発」実験医学，(2011)，印刷中.
津本浩平「表面プラズモン共鳴」日本DDS学会誌，印刷中.

‹Group II›

Kikuchi H, Kuribayashi F, Takami Y, Imajoh-Ohmi S, and Nakayama T. GCN5 regulates the activation of PI3K/Akt survival pathway in B cells exposed to oxidative stress via controlling gene expressions of Syk and Btk. Biochem Biophys Res Commun, 405: 657-661, 2011.
Mori E, Fukuda H, Imajoh-Ohmi S, Mori T, and Takasaki S. Purification of N-acetyllactosamine-binding Activity from the Porcine Sperm Membrane: Possible Involvement of an ADAM Complex in the Carbohydrate-binding Activity of Sperm. J Reprod Dev, 58: 117-125, 2012.
Yamada H, Imajoh-Ohmi S, and Haga T. The high-affinity choline transporter CHT1 is regulated by the ubiquitin ligase Nedd4-2. Biomed Res, 33: 1-8, 2012.
Hayashi H, Naoi S, Nakagawa T, Nishikawa T, Fukuda H, Imajoh-Ohmi S, Kondo A, Kubo K, Yabuki T, Hattori A, Hirouchi M, and Sugiyama Y. Sorting nexin 27 interacts with multidrug resistance-associated protein 4 (MRP4) and mediates the internalization of MRP4. J Biol Chem, in press.

‹Group III›

Oyama M, Nagashima T, Suzuki T, Kozuka-Hata H, Yumoto N, Shiraishi Y, Ikeda K, Kuroki Y, Gotoh N, Ishida T, Inoue S, Kitano H, Okada-Hatakeyama M. Integrated quantitative analysis of the phosphoproteome and transcriptome in tamoxifen-resistant breast cancer. J Biol Chem, 286: 818-829, 2011.
Hino N, Oyama M, Sato A, Mukai T, Iraha F, Hayashi A, Kozuka-Hata H, Yamamoto T, Yokoyama S, Sakamoto K. Genetic incorporation of a photo-crosslinkable amino acid reveals novel protein complexes with GRB2 in mammalian cells. J Mol Biol, 406: 343-353, 2011.
Abe S, Nagasaka K, Hirayama Y, Kozuka-Hata H, Oyama M, Aoyagi Y, Obuse C, and Hirota T. The initial phase of chromosome condensation requires Cdk1-mediated phosphorylation of the CAP-D3 subunit of condensin II. Genes Dev, 25: 863-874, 2011.
Suzuki T, Kim M, Kozuka-Hata H, Watanabe M, Oyama M, Tsumoto K, and Yamamoto T. Monoubiquitination of Tob/BTG family proteins competes with degradation-targeting polyubiquitination. Biochem Biophys Res Commun, 409: 70-74, 2011.
Katsumata Y, Kawaguchi Y, Baba S, Hattori S, Tahara K, Ito K, Iwasaki T, Yamaguchi N, Oyama M, Kozuka-Hata H, Hattori H, Nagata K, Yamanaka H, and Hara M. Identification of three new autoantibodies associated with systemic lupus erythematosus using two proteomic approaches. Mol Cell Proteomics, 10: M110.005330, 2011.
Suzuki M, Kiga K, Kersulyte D, Cok J, Hooper CC, Mimuro H, Sanada T, Suzuki S, Oyama M, Kozuka-Hata H, Kamiya S, Zou QM, Gilman RH, Berg DE, and Sasakawa C. Attenuated CagA oncoprotein in Helicobacter pylori from Amerindians in Peruvian Amazon. J Biol Chem, 286: 29964-29972, 2011.
Huang M, Sato H, Hagiwara K, Watanabe A, Sugai A, Ikeda F, Kozuka-Hata H, Oyama M, Yoneda M, and Kai C. Determination of a phosphorylation site in Nipah virus nucleoprotein and its involvement in virus transcription. J Gen Virol, 92: 2133-2141, 2011.
Iwasa A, Halfmann P, Noda T, Oyama M, Kozuka-Hata H, Watanabe S, Shimojima M, Watanabe T, and Kawaoka Y. Contribution of Sec61α to the life cycle of Ebola virus. J Infect Dis, 204: S919-S926, 2011.
Kozuka-Hata H, Tasaki S, and Oyama M. Phosphoproteomics-based systems analysis of signal transduction networks. Front Physiol, 2: 113, 2011.
Gorai T, Goto H, Noda T, Watanabe T, Kozuka-Hata H, Oyama M, Takano R, Neumann G,

Watanabe S, and Kawaoka Y. F1Fo-ATPase, F-type proton-translocating ATPase, at the plasma membrane is critical for efficient influenza virus budding. Proc Natl Acad Sci USA, in press.

Ao-Kondo H, Kozuka-Hata H, and Oyama M. Emergence of the Diversified Short ORFeome by Mass Spectrometry-Based Proteomics In *Computational Biology and Applied Bioinformatics* (ed. Heitor Silverio Lopes and Leonardo Magalhães Cruz), InTech, 2011.

Yamamoto T, Saito M, Kumazawa K, Doi A, Matsui A, Takebe S, Amari T, Oyama M, and Semba K. ErbB2/HER2: Its Contribution to Basic Cancer Biology and the Development of Molecular Targeted Therapy In *Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways* (ed. Mehmet Gunduz and Esra Gunduz), InTech, 2011.

尾山大明「Phos-tagを使った定量プロテオミクスによるリン酸化シグナルネットワークの統合システム解析」生物物理化学，印刷中

尾山大明，秦裕子「高精度定量プロテオミクスによる翻訳後修飾シグナルの包括的動態解析」実験医学，印刷中

‹Group IV›

Ibuki A, Akase T, Nagase T, Minematsu T, Nakagami G, Horii M, Sagara H, Komeda T, Kobayashi M, Shimada T, Aburada M, Yoshimura K, Sugama J, and Sanada H. Skin fragility in obese diabetic mice: possible involvement of elevated oxidative stress and upregulation of matrix metalloproteinases. Exp Dermatol, Epub Early View Nov 4. 2011.

Sugita Y, Noda T, Sagara H, and Kawaoka Y. Ultracentrifugation deforms unfixed influenza A virions. J Gen Virol, 92: 2485-2493, 2011.

Chen X, Yoshida T, Sagara H, Mikami Y, and Mishina M. Protein tyrosine phosphatase σ regulates the synapse number of zebrafish olfactory sensory neurons. J Neurochem, 119: 532-543, 2011.

Kim DY, Sato A, Fukuyama S, Sagara H, Nagatake T, Kong IG, Goda K, Nochi T, Kunisawa J, Sato S, Yokota Y, Lee CH, and Kiyono H. The airway antigen sampling system: respiratory M cells as an alternative gateway for inhaled antigens. J Immunol, 186: 4253-4262, 2011.

Nakagami G, Morohoshi T, Ikeda T, Ohta Y, Sagara H, Huang L, Nagase T, Sugama J, and Sanada H. Contribution of quorum sensing to the virulence of Pseudomonas aeruginosa in pressure ulcer infection in rats. Wound Repair Regen, 19: 214-222, 2011.

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Publications

K. Arima, A. Kinoshita, H. Mishima, N. Kanazawa, T. Kaneko, T. Mizushima, K. Ichinose, H. Nakamura, A. Tsujino, A. Kawakami, M. Matsunaka, S. Kasagi, S. Kawano, S. Kumagai, K. Ohmura, T. Mimori, M. Hirano, S. Ueno, K. Tanaka, M. Tanaka, I. Toyoshima, H. Sugino, A. Yamakawa, K. Tanaka, N. Niikawa, F. Furukawa, S. Murata, K. Eguchi, H. Ida, and K. Yoshiura Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo-Nishimura syndrome. Proc. Natl. Acad. Sci USA 108: 14914-14919, 2011

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