Laboratory Animal Research Center
実験動物研究施設

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Morbilliviruses in the family Paramyxoviridae including canine distemper virus, rinderpest virus and measles virus are highly infectious among their natural hosts. We have succeeded in establishing a system of reverse genetics for these three morbilliviruses, using originally isolated strains. The studies on the functions of viral proteins in replication, pathogenicity, and species-specificities have performed. We have also performed the basic research for prevention of Hepatitis C virus infection and hepatocellular carcinoma. In addition, more than 30,000 mice, mainly transgenic and gene-targeted ones, are always kept for researches of IMSUT and the technical staffs contribute to their maintenance and breeding.

1. Development of reverse genetics of morbilliviruses and application to pathological analysis

Misako Yoneda, Motohiro Shiotani, Ryuichi Miura, Fusako Ikeda, Hiroki Sato, Kentaro Fujita, Masahi Uema, Takahiro Seki, Tomoe Katsuo, Reiko Satoh, Akiko Takenaka, Naoki Kohriyama, Kyoko Tsukiyama-Kohara, and Chieko Kai.

The genus morbillivirus, in the *Paramyxoviridae* family, includes 7 viruses; measles virus (MV), rinderpest virus (RPV), canine distemper virus (CDV), peste des petits ruminants virus, and three aquatic mammalian viruses. Morbilliviruses are highly contagious and are considered one of the most important pathogens in each host animal. For a decade from the late 1980s, serious epidemics with mass mortality occurred among both seal and lion populations. These outbreaks were attributed to infection with CDV, although big felids had not previously been considered susceptible to CDV. The mechanisms of pathogenicity and cross-species infection for morbilliviruses are still major problems to be solved. Development of rescue systems of nonsegmented single and negative-strand viruses (*mononegavirales*) since 1994 have opened vast new fields of analysis for a wide range of previously inaccessible areas of these viruses.

We established an excellent animal model for RPV infections using rabbits, which exhibit natural symptoms to experimental peripheral infection. To investigate the pathogenicity of RPV, we isolated ten virus clones from homogenates of infected rabbit lymph nodes by plaque cloning in B95a cells. Among the 10 clones, 2 clones were highly virulent, and one clone was avirulent, defined by histopathology and virus growth in lymphoid tissues. In B95a cells, 8 clones showed almost identical growth curves, while the growth rate of one virulent clone was high and of the avirulent clone was markedly low. Clinical features, virus growth and histopathological changes of rabbits after infection of each virus clones were correlated with the virus growth in B95a cells.

Using the full genome cDNA of the most virulent clone of the RPV-L, we succeeded to develop a reverse genetics system (rRPV-Lv). We first applied a system for the analysis of the role of the V protein produced from the P gene by RNA editing. The V

deficient Sendai virus was shown to be avirulent in infected mice and the V deficient Measles virus was shown to induce significantly fewer and milder clinical symptoms with a lower mortality rate than the parental virus in newborn mice. The V deficient rRPV-Lv replicated to the same extent as the parental virus in B95a cells *in vitro*. The rabbits infected with the V deficient rRPV-Lv showed clinical symptoms including fever or immunosuppression and reduced body weight, similar to those infected with the parental strain. In this study, the V protein was shown to exert little effect on virulence in RPV, in contrast with the protein's role in Sendai and Measles virus.

In addition, we previously established a monkey model for MV. This was the first model to demonstrate measles rash with other natural symptoms such as immunosuppression following experimental infection with MV isolated from affected humans. A reverse genetics system has been successfully developed using a field isolate, the HL strain. This system with the animal model could offer another powerful tool for the investigation of the mechanisms of immunosuppression and for the development of polyvalent vaccines for significant human diseases, after the reduction of virulence by genetic engineering.

2. Identification of neutralizing epitopes on the hemagglutinin protein of canine distemper virus

Ryuichi MIURA, Fusako Ikeda, and Chieko KAI

The hemagglutinin (H) viral glycoprotein on envelop of CDV is involved in cell attachment to specific cellular receptors. The H protein is the main target of neutralizing antibodies and plays a central role in induction of protective immunity. Previously we reported that the prevalence in Japan mainly caused new types of CDV which were distinguished from vaccines and old types of CDV by serological and genetic analyses. Furthermore, one of three neutralizing monoclonal antibodies (MAb) failed to recognize the vaccine strain, suggesting the change of at least one of neutralizing epitopes. To analyze the localization of neutralizing MAb epitopes and functional domains, we constructed the recombinant CDVs expressing 8 kinds of chimeric H protein and selected CDV mutants escaping from the neutralizing MAbs. The chimerical H genes were constructed with that of the CDV Ondersteproot strain which MAb JD-5 does not neutralize and that of the CDV Yanaka strain which is a recent field isolate in Japan and the JD-5 completely neutralizes. All of the recombinant CDVs were rescued, but showed the distinct viral growth and size of CPE. The JD-5 failed to neutralize the recombinant CDVs containing the residue of the Ondersteproot H protein coding 440-606 amino acid. Additionally the H gene of JD-5 resistant-CDV mutant was also changed in the sequence encoding the 440-606 amino acid. The results suggest that the epitope in the region on recent field isolates may be immunodominant.

3. Cytotoxic T-Lymphocyte Activity Specific for Hemagglutinin (H) Protein of Canine Distemper Virus in Dogs

Kyoko Hirama, Ken-ichi Togashi, Chiaki Wakasa, Misako Yoneda, Toshiya Nishi, Yasuyuki Endo, Ryuichi Miura, Kyoko Tsukiyama-Kohara, and Chieko Kai

Cytotoxic T lymphocytes (CTL) play an important role in the immune system for protection against morbilliviruses. CTL epitopes and responses to measles virus (MV) infection have been well studied in a mouse model. The major target antigen for CTL in mice was identified as the nucleocapsid (N) protein by several groups. H protein of MV and CDV also suggested to possess the CTL epitopes in mice. However, Jaye et al. reported that the fusion (F) and hemagglutinin (H) proteins were important targets for measles CTL responses in humans, a natural host of MV, who receiving measles polypetides. On the other hand, CTL responses in infection with another morbillivirus, RPV, were also analyzed in mice and cattles. A CTL response against RPV N protein was detected and a CTL epitope within the N protein was identified in the mouse model. However, the role of N protein in protection and induction of CTL responses against RPV infection in cattle was limited. The H protein in cattle is more effective and its effect lasts for at least two years. These discrepant results suggested that the CTL response in the mouse model does not always reflect that in the natural host, and analyses concerning the development of CTL activity using the virus and the natural host are required.

Restriction of the major histocompatibility complex (MHC) in CTL assay makes it difficult to develop an assay system in outbred animals. Recently, an easy method to produce recombinant adenoviruses based on a replication-deficient adenovirus vector was established, and this method has been shown to be useful for delivery of vectors for gene therapy and protein expression. The adenovirus vector possesses useful characteristics such as high level of protein expression, broad host cell range, applicability to non-replicating cells including neural cells, easy preparation of virus in high yield, insertion of large DNA fragments (approximately ~30 kb), and most importantly the viral vector is noncytopathic because of its proliferation deficiency. Therefore, it can be used as a powerful expression vector to prepare target cells for CTL assay in outbred animals. The function of CTL against CDV infection has not yet been studied and thus the major target protein in dogs, the natural host animal, has

not been determined. We focused on the H protein for analysis of its inducibility of specific CTL responses to CDV in dogs using a recombinant adenovirus protein expression system.

Skin fibroblasts were isolated from two dogs and were infected with recombinant adenovirus bearing the CDV-H gene (Ade-CDVH). CTL assay was performed using fibroblasts expressing CDV-H protein as target cells and peripheral blood lymphocytes (PBL) collected from the same dogs one week after immunization of CDV as effector cells. Specific cytotoxic activity was observed against autologous but not heterologous fibroblasts expressing CDV-H protein. These results indicate that the CTL epitope(s) were localized in the H protein.

4. Up-regulation of acyl-coenzyme A:cholesterol acyltransferase (ACAT) mRNA expression and accumulation of cholesterol esters induced by canine distemper virus infection

Hoshi Miho, Tomoe Katsuo, Misako Yoneda, Kenichi Togashi, Ryuichi Miura, Kyoko Tsukiyama-Kohara and Chieko Kai

MV and CDV is neurovirulent and induces encephalitis with demyelination in their natural hosts. Recently small animal models for MV and CDV were developed and the mechanism of encephalitis induced by virus infection was studied, but pathological mechanism of demyelination remains unknown. It was reported that one of the human genomic diseases, adrenoleukodystrophy (ALD), is a result of progressive demyelination of central and peripheral nervous systems. The brain from the ALD patients contained high level of cholesterol ester. Interestingly, the accumulation of cholesterol esters was observed in the brain of MV infected rat. Furthermore, based on the component of fatty acids, those cholesterol esters were not derived from serum. Based on these results, we were interested in the intracellular cholesterol metabolism and analyzed the implication of CDV infection in the induction of synthesis of cholesterol esters in brain. Cholesterol esters are derived from cholesterol by catalysis with an intracellular enzyme ACAT. ACAT activities are observed in various tissues, such as liver, intestines, adrenal glands and aorta. We focused on ACAT to analyze the mechanism of increased cholesterol esters after CDV infection using mouse model with two kinds of CDV strains, which cause neurovirulence to mice. One is the CDV-KG1 strain which was adapted to human oligodendroglioma (KG-1) cells and the other is the CDV-Bjal which was adapted to mouse brain. Both viruses are highly neurovirulent and induce encephalitis in mouse. After CDV-KG1 inoculation to mice, clinical signs such as body weight loss and neurological disorder, and encephalitis were observed. In cerebrum of CDV inoculated mice, cholesterol esters increased. Interestingly, its fatty acids composition indicated that they were not derived from serum but synthesized intracellularly. The significant increase of mRNA expression of ACAT in the cerebrum of CDV-KG1 inoculated mice was observed using real-time quantitative polymerase chain reaction (PCR) analysis. We evaluated these phenomena more in detail using CDV-Bjal inoculated mice. The mice showed clinical signs and encephalitis like CDV-KG1 inoculated mice, but much more severely. Compared to CDV-KG1 inoculated mice, both of the cholesterol esters and the expression level of ACAT mRNA also increased significantly after CDV-Bjal infection.

We analyzed the implication of virus growth for the ACAT mRNA expression level using human glioma cells (MGC) cells after CDV infection. The expression of ACAT mRNA increased following the virus growth. Therefore, increase of ACAT mRNA in mouse brain might be caused by the viral growth in neuronal cells.

5. Expression of swine IL-4 and IL-6 and establishment of ELISA system for these cytokines

Athipoo Nuntaplasert, Yasuyuki Mori, Kentaro Fujita, Misako Yoneda, Ryuichi Miura, Kyoko Tsukiyama-Kohara and Chieko Kai

Cytokines are natural host proteins that control the type of immune response following infection or vaccination. Therefore, use of cytokines should be a crucial key as the alternative method to control swine bacterial infectious disease instead of using antibiotics. The information from a variety of species indicates that interleukins including interleukin-4 (IL-4) and interleukin-6 (IL-6) are key regulators in the inflammation and immune response during bacterial infections or injury process.

We performed the expression of recombinant swine IL-4 (rSwIL-4) and recombinant swine IL-6 (rSwIL-6) in three kinds of recombinant expression systems; *E. coli*, baculovirus, and eukaryotic cells. The one-step purifications of the bioactive rSwIL-4 and rSwIL-6 from *E. coli* for use in the production of specific antibodies and from baculovirus system were established for basic characterization and clinical applications of these cytokines. The biological activity of the recombinant proteins on human TF-1 cells *in vitro* was also confirmed. We established a sandwich enzyme-linked immunosorbent assay (ELISA) for measuring concentration of SwIL-4 and SwIL-6 in samples and their detection limits were 78 and 49 pg/ml, respectively. We also established an enzyme-linked immunospot (ELISPOT) assay for detecting IL-4-secreting cells using a mAb and a polyclonal IgG from goat. These systems are expected to be powerful tools for clinical use.

6. Prevalence of canine distemper virus, feline immunodeficiency virus and feline leukemia virus in captive African lions (*Panthera leo*) in Japan

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Recently, many cases of interspecies transmission of viral diseases between human to animals and animals to animals have been reported and wildlife was not an exception. The incident that threatened the lives of African lions happened at Serengeti national park of Tanzania in 1994 and it is estimated that approximately three thousand lions were lost. Roelke-Parker *et al*. and others clarified that CDV, which is originally a causative agent for neuronal and pulmonary diseases in dogs, infected to the lions breaking through the species barrier and induced the death of infected lions in this area. Furthermore, the source of the virus was identified as hyenas or domestic dogs harboring in or around the park.

Most of large felids were housed in zoos in Japan. It is important for them to understand the status of virus infections in the points of epidemiological, virological and economical views. Sero-prevalence of CDV, Feline immunodeficiency virus (FIV) and Feline leukemia virus (FeLV) were evaluated in 20 captive lions in two Japanese zoos. All lions were healthy and showed no clinical signs, however, anti-CDV antibody was detected in 12 of 20 lions and these sero-positive lions had also possessed CDV specific neutralization antibody. We could pursue antibody responses against CDV in three lions back to 1996. In 1996, sera from three lions were negative for anti-CDV antibody, however, all of them showed sero-conversion in 2000. This result suggested that the epidemic of CDV infection in this zoo might have happened between 1996 and 2000. The lions were also examined for FIV and FeLV infections. We had no evidence for FeLV infection but eight lions were sero-positive for anti-FIV antibody. Although both of the detection of provirus and isolation of virus were unsuccessful, it was strongly suggested that the lions were exposed previously to FIV or related lentiviruses. Our findings clarified existence of the major canine and feline viral infections even in the zoo animals, thus further continuous and careful observations will be required to avoid epidemics and the appearance of virulent mutants.

7. Basic research for the prevention of Hepatitis C

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Hepatitis C virus (HCV) is one of the major etiological agents of chronic hepatitis, which is frequently resulting in liver cirrhosis and hepatocellular carcinoma. About 200 million people around the world, and more than 2 million people in Japan have been already infected with HCV. Currently efficient therapy is alpha interferon (IFN-α) in conjunction with ribavirin, but these are expensive, effective in less than 50% of patients, and carry the potential for significant side effects. Therefore, prevention of hepatitis C is in a high priority.

We approached for the prevention of hepatitis C by mainly two directions. One is the establishment of recombinant measles virus encoding envelope protein of HCV; rMV-E1, rMV-E2, and rMV-E1E2. HCV-E1 and 2 proteins were estimated to be receptor binding proteins and important for protection of virus infection. The genes encoding the HCV E1 and E2 proteins were amplified by PCR and cloned into between N and P gene of the MV-based vector. rMV-Es were rescued from their respective full-length cDNA in 293 cells. The *in vitro* growth characteristics of rMV-Es were compared with those of parental virus. The parental rMV reached its maximum titer at 48 h.p.i. while rMV-Es grew slowly and reached its maximum titer at 72 h.p.i. All three constructs properly expressed the respective protein(s) which were located in the endoplasmic reticulum, as indicated by western blotting and indirect immunofluorescence staining. We are currently examining the ability of these viruses to induce immune responses to HCV envelope proteins, *in vivo*. These recombinant MV vectors might be useful for the characterization of biological effects of HCV-E1, E2 proteins in cells and also give us the basic information to vaccine development of HCV. They also possess the possibility to apply for the measles virus vaccination in the HCV infection highly prevalent area, such as China and Mongolia.

The another approach for Hepatitis C is the characterization of hepatocellular carcinoma (HCC) antigen. HCV causes persistent infection in hepatocytes, and this infection in turn is strongly associated with the development of hepatocellular carcinoma. Therefore, identification of HCC antigen is significant to understand the basic mechanism of HCC formation and also important for the clinical application, such as diagnosis and therapy of HCC. We previously established the conditional expression system of human liver cells and found that passage of hepatocytes expressing replicable full-length HCV

RNA caused upregulation of tumorigenicity. We are now characterizing surface antigens of these cells by establishing specific monoclonal antibodies

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

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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 Habu control

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Snake bites by the venomous snake Habu, *Trimeresurus flavoviridis*, have been reported annually about 100 cases in the population of 80, 000 in the Amami Islands. Moreover, there is no indication that the population of 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 Habu survived the injection of Habu venom since early times. Because, some proteins in the serum of Habu blood combine to the elements of Habu venom. The research of these binding proteins has been initiated with an objective of clinical trials. Phospholipase A2 and its isozymes isolated from Habu venom have myonecrotic activity and hemorrhagic activity, and T2 protease has hemorahagic activity. The binding proteins isolated from serum of Habu inhibit myonecrotic activity of phospholipase A2 and its isozymes. We found that protein-HSF and peptide-AHP isolated from the Habu serum effectively control the hemorrhage caused by Habu, *Ovophis okinavensis*, *Agkistrodon blomhoffi brevicaudus*, *Calloselasma rhodostoma*, *Bitis arietans*, *Bothrops asper*, and, *Trimeresurus stejnegeri* venom. Further, a statistics analysis and the simulation were done with the snakes captured by

the Government, and the analysis of population dynamics 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 Habu and the result of the 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 100, 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 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. Reproduction of squirrel monkeys

Shosaku Hattori, Takesi Kuraishi, Yuko Katakai, Yoshitugu Matsumoto[9], Mamoru Ito[10], and Takahisa, Furuta[11]: [9]Department of Molecular Immunology, Faculty of Agriculture, University of Tokyo, [10]Laboratory of Immunology, Central Institute of Experimental Animals, [11]Department of Parasitology

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 5 newborns are given annually by 30 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, further more, 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.

3. Research of wild mammals.

Shosaku Hattori, Yoshihisa Noboru, Hideki Endo[12], Kimiyuki Tsuchiya[13], Nobuo Ishii[14] and Fumio Yamada[15]: [12]Department of Zoology, National Science Museum, [13]Experimental Animal Center, Miyazaki Medical College, [14]Japan Wildlife Research Center, [15]Wildlife Ecology Laboratory, Forestry & Forest Products Research Institute

Amami-oshima Island is a habitat of animals and plants indigenous to the Nansei Islands. These animals occur originally in the Oriental region of Asia and include the Amami rabbit, *Pentalagus furnessi*, the Ryukyu spiny rat, *Tokudaia osimensis*, the Okinawa long-haired rat, *Diplothrix legata*, the Watase's shrew, *Crocidura watasei*, and the Musk shrew, *Suncus murinus*. These mammals are used for researches on comparative anatomy, taxonomy, and development of experimental animals. Besides, these mammals are valuable species biologically as survivors from the Miocene about 10, 000, 000 years ago. We have initiated the investigation for these species to protect from extinction. We have documented the feasibility of recovering large numbers of oocytes from the Watase's shrew, and some of oocytes can be induced to mature in vitro. Recently, the Java mongoose, *Herpetologica javanicus* grew in the wild as invasive carnivore in the Amami-oshima Island. The population of the mongoose increases every year and the habitat range is extending to south area in the Island. It is necessary to remove the invader to defend nature. Then we are investigating the influence which the mongoose gives to wildlife in the Island. Since hairs such as Amami rabbit, Ryukyu spiny rat, Akahige were confirmed from the excrement of the mongoose, the necessity of the urgent ridding countermeasure of the mongoose was indicated. From 2000, the capture project of the mongoose was started by Ministry of Environment in order to protect Amami-oshima's endemic species.

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Laboratory of Molecular Genetics
遺伝子解析施設

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This laboratory has two main activities, developing efficient expression vectors for gene therapy and supporting the researchers by advising on recombinant DNA technology under the safety guideline.
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 under the safety guideline. 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 aiming gene therapy. We are maintaining more than 20 collaborations within and outside of this institute. In these collaborations, we offer and supply our efficient method (COS-TPC method, Miyake et al., Proc. Natl. Acad. Sci. USA, 93, 1320-1324, 1996) to construct recombinant adenoviruses expressing various genes efficiently. Ten years ago, we constructed 44 recombinant adenovirus for 14 months using this method; this number was more than double constructed in the world per year at that time. More recently we have developed a method for ON/OFF switching of gene expression in mammalian cells using a combination of Cre/loxP system and adenovirus vector (Kanegae et al. Nucleic Acids Res. 23, 3816-3821, 1995; Kanegae et al. Gene 181, 207-212, 1996). The method will promote many fields of molecular biology and medicine and may open a new field of "intracellular gene manipulation". The research activities in 2002 were shown below.

1. Multiple gene regulation on cell chromosome mediated by two independent site-specific recombinases

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Although the site-specific recombinase Cre is powerful tool for regulation of gene expression, simultaneous or sequential gene regulation can not be achieved when using Cre/loxP system alone. Last year, we reported efficient simultaneous gene regulation system introduced in the chromosome of cultured mammalian cells using Cre/loxP together with the mutant loxP V that does not recombine with the wild-type loxP. But even if this system can control multiple gene expression, it is not useful for sequential gene regulation. We showed two strategies of the sequential gene regulation on mammalian cell chromosomes by using Cre/loxP and another site-specific recombinase FLP/FRT. The combined target unit is designed to regulate a given gene as

OFF- ON- OFF. We isolated HeLa cell lines containing a combined target unit for single-gene regulation, where an entire FLP target unit bearing a pair of FRTs was flanked by a pair of loxPs. We also isolated CV-1 cell lines containing two target units for independent regulation of multiple genes, "FLP target unit" bearing the GFP gene flanked by a pair of FRTs and "Cre target unit" bearing stuffer genes flanked by a pair of loxPs. After infection of two kinds of these cell lines firstly with FLP-producing adenovirus at MOI 30-50, and secondly Cre-producing adenovirus at MOI 3, we observed expected ON/OFF regulation of gene expression from each of the expression units. Southern blot analysis showed that each recombination mediated by two-independent recombinase occurred in almost all targets located on cell chromosomes. Therefore, we concluded that the sequential gene regulation system mediated by two independent site-specific recombinase Cre and FLP was highly efficient and reliable in mammalian cells.

2. Generation of adenovirus vector based on different construction methods using a single cassette

Miho Terashima, Saki Kondo, Yumi Kanegae and Izumu Saito

Among the method of constructing recombinant adenoviruses, the COS-TPC method is the most efficient because it utilizes adenovirus DNA-terminal protein complex (Ad DNA-TPC) in stead of cloned adenovirus genome. Recently, the method of transduction of cloned intact viral genome became available as commercial kits. Although such method is less efficient than that of COS-TPC, it is more convenient and simple. Here we developed a cosmid cassette, pAxcwit, which can dually be used both for the COS-TPC method and for transduction of intact genome. The cassette is derived from the COS-TPC cassette pAxcw and has intact terminal sequences. Recombinant adenoviruses can be generated using this pAxcwit cassette as a high efficiency of 90 to 340 clones per 10 μg of an insert-bearing cassette DNA. And the cassette pAxcwit was proven to be used for constructing recombinant adenoviruses as efficient as the original cassette pAxcw. Therefore, the pAxcwit cassette was found useful as a "dual cassette", served as a convenient intact-genome cassette and also as an efficient COS-TPC cassette. In case that recombinant adenoviruses are difficult to obtain, the high-efficient COS-TPC method can immediately be applied. Moreover, we isolated some insert-deleted recombinant adenoviruses when used the pAxcwit cassette for the method of transduction of intact genome. The result showed that isolation of viral single clone is still necessary even when employing the method of intact-genome transduction. The pAxcwit cassette will be obtained from Riken Gene Bank.

3. Gene regulation using Cre-expressing adenovirus under the control of various promoters

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On/off regulation of gene expression mediated by Cre/loxP system has already been used in the various fields of basic science and study of gene therapy because of its excellent efficiency and fidelity. A Cre-expressing recombinant adenovirus under the control of CAG promoter, AxCANCre, is often used in experiments *in vitro* and *in vivo*. However, it was experienced that some of virus stock of this virus showed very low titer and at higher MOI cell toxicity is suspected from morphological changes of infected cells. To elucidate the effective and toxic doses, we constructed new recombinant adenoviruses expressing Cre under the control of SRα promoter and SV40 early promoter (SVE). The expression level of SVE-Cre virus is very low and the optimal range of infection dose is around MOI 2, 500, only slightly below the toxic dose by viral infection. The CAG-Cre virus express Cre enzyme very efficiently and optimum dose is found at MOI 0. 5 to 10. Above MOI 30, toxicity to cells is evident; this toxicity is due to overexpression of Cre because the toxic range is far below the viral toxic dose. The SRα-Cre virus, AxSRNCre, gave an optimal range of MOI 50 to 2, 500 and did not cause Cre-mediated toxicity with doses of usual viral infection. Therefore, both AxCANCre and AxSRCre are useful but when using AxCANCre overdose should carefully be avoided. AxSRCre can be used at moderate range of MOIs without Cre-mediated toxicity in cultured mammalian cells.

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