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 and species-specificities have been 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 virus yield 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. The effect of Rinderpest virus nucleocapsid protein and phosphoprotein on the species specific pathogenicity

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We have previously demonstrated that Rinderpest virus (RPV) haemagglutinin (H) protein plays an important role in determination of host range but other viral proteins are clearly required for full RPV pathogenicity to be manifest in different species from its natural host. To examine the effects of the RPV nucleocapsid (N) protein and phosphoprotein (P protein) on its cross species pathogenicity, we have newly constructed two recombinant viruses. They were rescued from cDNAs of the RPV-RBOK strain that is avirulent in rabbits, in which the H and P or H, N and P genes were replaced with those from the RPV-L strain that is highly pathogenic in rabbits, designated as the rRPV-lapHP or the rRPV-lapNHP, respectively. Rabbits inoculated with the RPV-L became feverish, showed a decrease in body weight gain and leucopenia. Clinical signs of infection were never observed in rabbits inoculated with either the RPV-RBOK or with the rRPV-lapH. However, Rabbits inoculated with either the rRPV-lapHP or with the rRPV-lapNHP became pyrexic and showed leucopenia. Further, high virus titer and histopathological lesions in lymphoid tissues were clearly observed in those of infected with rRPV-lapHP and rRPV-NHP, although those were not observed in rabbits infected with rRPV-lapH. Therefore, RPV P protein is considered to have a key role in the replication of the virus in the cross species infection.

3. Mutations in the N, P, L genes of Rinderpest virus have altered pathogenicities in vivo.

Misako Yoneda, Takahiro Seki, Fusako Ikeda, Reiko Sato, Kyoko Tsukiyama-Kohora, Chieko Kai

We previously established highly virulent cloned virus of the rinderpest virus (RPV-Lv strain) for rabbits by plaque purification of the RPV-L strain and established a new reverse genetics system based on the RPV-Lv strain. By the virus cloning, we have also obtained an attenuated clone virus (RPV-La strain) in the rabbit model. Comparing to the gene sequence of the Lv strain, the La strain possessed only six nucleotide mutations in all genes: one in the N gene, one in the P and three substitutions in the L gene. To examine which substitutions are implicated in the reduced pathogenicity of the RPV -La strain, we recovered seven infectious RPVs from recombinant cDNAs of RPV-Lv with exchanged N, P and/or L gene from the RPV-La strain by using the reverse genetics system of the RPV-Lv strain. The recombinant viruses in which P and/or L protein caused severe clinical signs in inoculated rabbits, however the all of four recombinant viruses possessing N protein of the La strain showed lower pathogenicity in rabbits compared to those caused by the RPV-Lv strain. These findings suggest that the substitution in N protein of RPV-L strain affect virus pathogenecity in vivo.

4. The effect of recombinant swine interleukin -4 on swine immune cells and on pro-inflammatory cytokine productions in pigs

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The *in vitro* effect of recombinant swine IL-4 (rSwIL-4) was characterized in various swine cells and the *in vivo* influence of rSwIL-4 pretreatment in nursery pigs on LPS-induced end toxic shock and pro-inflammatory cytokine productions. *In vitro* experiment, the rSwIL-4 induced a proliferation of CD4 positive T cells in mitogen-prestimulated PBMC. In addition, the rSwIL-4 produced from insect cells in combination with granulocyte macrophage-colony stimulating factor (GM-CSF) promoted the differentiation of monocytes into immature dendritic cells. Furthermore, the rSwIL-4 successfully suppressed the LPS-induced secretion of TNF-α, IL-1β, IL-6, IL-8, and IL-18 from swine alveolar macrophages when rSwIL-4 was treated at the same time with LPS. *In vivo* experiment, s.c. pretreatment of rSwIL-4 produced from baculovirus expression system in nursery pigs enhanced the severity of respiratory failure system with endotoxic shock and increased the production of TNF-α and IL-18 in response to inoculation with LPS. These results indicated that the rSwIL-4 is biologically active both *in vitro* and *in vivo*. The regulation of pro-inflammatory cytokine productions by IL-4 can be either inhibitory or stimulatory depending on the administration time.

5. Phylogenetic analysis of the hemagglutinin (H) proteins of canine distemper viruses from wild masked palm civets (*Paguma larvata*) in Japan.

Kyoko Hirama, Masashi Uema, Ryuichi Miura, and Chieko Kai

Hemagglutinin (H) gene of two CDV strains (the Haku93 and Haku00 strains) isolated from masked palm civets was genetically characterized. Both H genes of two CDVs had one open reading frame (ORF) of 1821 nucleotides encording 607 amino acids. Nucleotide sequences of H gene ORF of the Haku93 and Haku00 strains revealed more than 98% identity to those of the Yanaka and Tanu96 strains. On the other hand, homology of nucleotide sequences between the isolates from masked palm civets and the Onderstepoort vaccine strain was approximately 91%. Predicted amino acid sequences of the H protein of the Haku93 and Haku00 strains showed over 97% homology to those of the recent field isolates in Japan, and 89.7% identity to the vaccine strain. Twelve cysteine residues and one major hydrophobic region were conserved in these strains like other CDV strains. Moreover, nine potential asparagines (N)-linked glycosylation sites were found at the same positions on the recent Japanese isolates. By phylogenetic analysis, the CDV strains derived from masked palm civets were classified into the group of recent Japanese isolates from dogs. These results suggested that wild masked palm civets were exposed to CDV prevailing in domestic dogs and other wild animals in Japan.

6. Establishment of recombinant measles virus expressing hepatitis C virus envelope glycoproteins.

Takahiro Seki, Kyoko Tsukiyama-Kohara, Kohsuke Izumi, Fusako Ikeda, Michinori Kohara[1] and Chieko Kai: [1]The Tokyo Metropolitan Institute of Medical Science.

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.

For the prevention of hepatitis C, we performed to establish of recombinant measles viruses encoding envelope protein of HCV; rMV-E 1, 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. We previously established a rescue system of measles virus (MV) using a field-isolated strain. Using this system, we rescued three replication-competent recombinant MVs expressing either or both of HCV-E1 and E 2 (rMV-E1, rMV-E2 and rMV-E12) in order to obtain a basic knowledge for the development of divalent vaccines against HCV and MV. The replication of each of the rMV-Es in B95a cells was slightly slower than that of the parental rMV and reached its maximum virus titer at 4 d.p.i., while the parental rMV did so at 3 d.p.i. All three rMV-Es properly expressed the respective HCV protein(s) as indicated by western blotting, immunofluorescent assay (IFA), immunoprecipitation and endoglycosidase treatment. Therefore, HCV envelope glycoproteins E 1 and E2 in rMV-Es were processed correctly by

posttranslational glycosylation and formed the noncovalent heterodimer complexes. Moreover, double IF staining revealed that the expressed HCV glycoproteins were properly co-localized with protein disulfide isomerase, which resides in the endoplasmic reticulum. These results suggest that the rMV could be useful expression vector for HCV envelope glycoproteins.

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. They also possess the possibility to apply for the measles virus vaccination in the HCV infection highly prevalent area, such as China and Mongolia.

7. Identification of cell surface antigens expressing in full-length Hepatitis C virus gene persistently expressing cells.

Kyoko Tsukiyama-Kohara, Kohsuke Izumi, Takashi Takano, Kentaro Saito, Ying Huang, Michinori Kohara[1] and Chieko Kai: [1]The Tokyo Metropolitan Institute of Medical Science.

Hepatitis C virus (HCV) frequently causes chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Since the effective infection system of HCV has not been established *in vitro* and *in vivo*, we previously established the conditional full-length HCV gene expressing HepG2 cells. The HCV expressed and passaged cells increased their tumorigenicity after 48 days passage. In order to clarify the existence of cell surface antigens whose expression level has been changed according to the tumorigenicity, we produced monoclonal antibodies (mAbs) by immunizing the passaged cells. Over one thousand monoclonal antibodies were established, and their reactivities to HCV gene expressing cell lines were characterized. Several mAb clones with significantly different reactivities to HCV gene expressed and passaged cells were obtained. Among them, the mAbs 2-152, 243, and 433 recognized 55, 70 and 30 killodalton (kDa.) molecules, respectively. All these molecules were expressed on the surface of hepatoma cell line higher than that of normal hepatic cell line. Expression levels of these molecules were also examined in cancerous and non-cancerous region of tissues from hepatocellular carcinoma in patients' liver with or without HCV and Hepatitis B virus (HBV) infection. Upregulation of the p55 molecule expression in cancerous regions in liver of all HCV infected patients was observed. Thus, p55 molecule might be implicated in occurrence of HCC in HCV positive patients.

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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 the Habu control

Shosaku Hattori, Yuko Katakai, Hiroshi KIhara[1], Motonori Ohno, Naoko Ueda[2], Shigenari Terada[3], Hiro Yonezawa[4], Yoshihiro Hayashi[5], Michihisa Toriba[6], Hiroyuki Takahashi[7], and Tomohisa Ogawa[8],: [1]Bioscience research Institute, Takara Shuzo Co., Ltd., [2]Departmen of Applied Microbiology, Kumamoto Institute of Technology, [3]Department of Biochemistry, Faculty of Science, Fukuoka University, [4]Department of biochemistry, Faculty of Science, Kagoshima University, [5]Department of Veterinary Anatomy, Faculty of Agriculture, University of Tokyo, [6]The Japan Snake Institute, [7]Department of Genetics, [8]Faculty of Agriculture, Tohoku university

Snake bites by the venomous snake Habu, *Trimeresurus flavoviridis*, have been reported annually about 80 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 A2 and its isozymes isolated from Habu venom have myonecrotic activity and hemorrhagic activity, and metal 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 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 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 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. Reproduction of squirrel monkeys.

Shosaku Hattori, Takesi Kuraishi, Yuko Katakai[9], Mamoru Ito[10], and Chieko Kai[11]: [9]Research Institute, International Medical Center of Japan, [10]Laboratory of Immunology, Central Institute of Experimental Animals, [11]Laboratory Animal Research center

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 25 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 adenoviruses 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 adenovirus vector and Cre/*loxP* 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, e 40, 2001). 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 2003 were shown below.

1. Development of an efficient method for constructing first-generation adenovirus vectors using double-reciprocal recombination mediated by Cre recombinase.

Masakazu Nakano, Yukari Nishiwaki[1], Yuzuka Takahashi, Yumi Kanegae and Izumu Saito: [1] School of Pharmaceutical Sciences, Kitasato University

We have developed a new method for constructing first-generation adenovirus vectors (E1-substituted type) using double-reciprocal recombination mediated by Cre/*loxP* system. We have reported a mutant *loxP* V, which contains two certain transversion mutations in its 34-nucleotide sequences. The mutant *loxP* V recombines efficiently with the identical mutant *loxP* V but not with wild-type *loxP* (Lee and Saito, Gene, 216:55-65, 1998). A pair of DNA sequences flanked with wild-type *loxP* and mutant *loxP* V can efficiently be exchanged through

double-reciprocal recombination mediated by Cre recombinase. We constructed a parent virus, called a "recipient virus", which contains an inserted gene at the E1 region flanked with wild-type *loxP* and mutant *loxP* V. In addition, the packaging signal of the virus is flanked with a pair of wild-type *loxP*. We also constructed a "donor plasmid" containing viral packaging signal and a gene to be transferred onto the adenovirus genome, both of which were flanked as a unit with wild-type *loxP* and mutant *loxP* V. A 293 cell line constitutively expressing Cre recombinase was infected with the recipient virus and then transfected with a donor plasmid containing a marker gene. Some of the recipient viruses lacking their packaging signal by Cre-mediated excision restored the signal together with the marker gene from the donor plasmid through the double-reciprocal recombination. The resulting marker gene-containing recombinant adenovirus became >97% of the virus stock after three cycles of serial passages through the Cre-expressing 293 cell line. Therefore, this method works for efficient construction of the first-generation adenovirus vector. Advantages of this method are: (i) this method uses a small donor plasmid rather than a large cosmid, and (ii) the method requires simple infection and transfection followed by serial passages. Especially, the method can be applied for construction of a large number of recombinant adenoviruses using multiple plates: a 96-well plate can generate 96-different recombinant adenoviruses by transfecting 96-different plasmids followed by serial "replica passage". Moreover, the method of double-reciprocal recombination using the mutant *loxP* V may be useful for construction of viral vectors of larger size, such as herpesvirus vectors.

2. Application of double-reciprocal recombination mediated by Cre recombinase for constructing helper-dependent adenovirus vectors.

Masakazu Nakano, Mika Wakisawa[1], Michiko Koshikawa, Saki Kondo, Yumi Kanegae and Izumu Saito:

Although the first-generation adenovirus vector has been extensively used in various fields of basic research and for gene therapy, the next-generation adenovirus vector has also been reported. The new adenovirus vector, called "gutted vector", is devoid of all the viral genes but retains only about 0.5-kb of the left terminal and 0.2-kb of the right terminal of the viral genome. These terminal regions of the virus genome contain viral replication origins and the signal for packaging into the viral capsid. The gutted vector does not express any viral gene products and is consequently expected to cause only minimum immune reaction against its host and to achieve prolonged gene expression. However, because the gutted vector requires helper virus, which supplies all the viral gene products *in trans*, the method for construction and production of gutted vector is still difficult to handle and the production yield of the gutted vector is sometimes too low to obtain gutted virus sufficient for animal experiments.

We have developed a new method for constructing first-generation adenovirus vector using double-reciprocal recombination mediated by Cre/*loxP* system described above. We are trying to construct gutted vector using this strategy. We constructed a new recipient virus containing both a pair of wild-type *loxP*s near the left end of the genome and a mutant *loxP* V at 0.2 kb downstream from the right end of the genome. We also constructed a donor cosmid where 28-kb DNA containing expression units is flanked with wild-type *loxP* and mutant *loxP* V. Transfection of the new recipient virus and the donor cosmid into Cre-expressing 293 cells and subsequent serial passages with addition of the recipient virus successfully generated desired gutted virus, though the titer obtained was rather low. Further investigation is under way.

3. Generation efficiency of adenovirus vector tagged with various lengths of oligonucleotides at the termini of the virus genome.

Hiromitsu Fukuda, Hirohito Umemoto[2], Yumi Kanegae and Izumu Saito: [2]School of Science, Kitasato University

Among the method of constructing recombinant adenoviruses, the COS-TPC method developed by us and the method involving transfection of full-length viral genome with intact termini (transfection method) are well known. Although the COS-TPC method is most efficient, this method requires an expensive material of adenovirus DNA-terminal protein complex. Although the transfection method is less efficient than the COS-TPC method, the former is simpler and more convenient. In this method, an intact vector genome must be excised prior to transfection at the both termini of viral genome from a cosmid cassette pAxcwit with the restriction enzyme that does not cleave the vector genome at all. We previously developed the dual cosmid cassette that can be used both for the COS-TPC method and for the transfection method. But the transfection method cannot be

applied in case that an inserted gene is by chance cleaved by the restriction enzyme for excising virus genome from the cassette. Therefore, if two or more restriction enzymes can be used for excising the virus genome, application of the transfection method will extend further.

Here we constructed a cosmid cassette in which the viral genome can be excised by four different restriction enzymes. The average number of oligonucleotides attached at the both termini of viral genome varied from 3 to 24. Our results showed that the generation efficiency of the adenovirus vector depended on the number attached on the both termini. Generation efficiency of the genome attached with even 24 nucleotides at both termini gave no less than a half or one third of that with 3 nucleotides. Moreover, the 24 nucleotides attached at the both termini of excised viral genome were completely removed in the recovered viral genome. Our result showed that a cassette can be developed in which two or more restriction enzymes can be chosen for excising the adenovirus vector genome containing a desired foreign gene.

Publications

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