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Analysis of foreign DNA transferred by direct injection into mouse testis.

Katsuhiko Hayashi [1)], Msaki Maehara[1,2)], Norihiro Tada[3)], Shyoso Ogawa[2)], Daisuke Kitamura[1)]

1) Division of Molecular Biology, Reseach Institute for Biological Science, Sience University of Tokyo. Chiba 278-0022 Japan.
2) Laboratory for Reproduction Engineering, Department of Animal Production, Meiji University, Kanagawa 214-0033 Japan.
3) Medicinal Research Laboratorys, Molecular Biolgy laboratory, Taisho Pharm. Co. Ltd. Saitama 330-8530 Japan.

Abstract

Liposome-encapsulated plasmid pCAG-Cre was repeatedly injected by means of a microsyringe with a needle into mature mouse testes. Male mice injected with the plasmid were mated to normal females at 4, 7, 14, 17 and 30 days after the first injection, respectively, and then newborns were generated. On PCR analysis of genomic DNA from a total 89 newborn mice, pCAG-Cre sequence was detected in 40-80% of newborns generated by mating at 4, 7, 14 and 17 days after injection. The integration of the injected DNA into genome was confirmed by Southern blot analysis in 7 of 19 newborns derived from the mating at 17 days after injection. These findings indicated that foreign DNA directly injected into mature testes was efficiently introduced into the genome of offspring and demonstrated a feasibility of this simple method to generate transgenic animals.

Keywords: Testis-madiated gene transfer, DNA injection into testis, Liposome, Transgenic mice.

Introduction

An alternative method for highly efficient production of transgenic animals, instead of microinjection into egg, has long been aspired (1,2,3). Recently we demonstrated that foreign DNA directly injected into mature testes [testis mediated gene transfer (TMGT) method] was efficiently introduced into blastocysts(-80%) (4) fetuses(30-70%)(5)and postpartum progeny(40-55%) (5).However the foreign DNA injected into testes was detectable by PCR analysis but not by Southern blot analysis. The foreign DNA injected into testis may be mosaically present in somatic tissue of newborn. We report here that PCR and Southern blot analysis revealed that a high copy number of the foregin DNA could be introduced into genome of the postpartum progeny by TMGT method.

Materials and Method

Preparation of liposome/DNA complex

The plasmid pCAG-Cre contains cytomegalovirus enhancer/chicken B-actin promoter (CAG) and a cording sequence for Cre recombinase which catalyses si specific recombination (6,7,8). The plasmid pCAG-Cre purified with Gene Clea Kit(BIO101, CA, USA)was diluted with phosphate-buffered saline to make a fin DNA concentration of 0.5ug/ug. The DNA solution was added to an equal volun of transfection reagent (LIPOFectin;GIBCO BRL,MD, USA) to form liposome/DNA complex. The complex was kept at room temperature for 1 hour prior to injection.

Injection of DNA into testes and matingprotocol

BDF1(C57BL/6NXDBA/2N; SLC Japan, Inc.) male mice at 10 weeks of age we used for this experiment. Injection of liposome/DNA complex into testis was performed as previously described (4). Briefly, the complex was directly injected with a 1/6 needle 3-4mm in depth through the scrotum under anesthesia for three times with 3 days intervals. The injected mice were mated with ICR(CLEA Japa Inc.) females at 4, 7, 14, 17 and 30 days, respectively, after the first injection.

Isolation of the genomic DNA from newborns, polymerase chain reaction(PCR) and Southern blot analysis

The genomic DNAs from the tail of newborn were isolated, according to the method described by Blim and Stafford(9). For PCR analysis, genomic DNAs(0. 0.3ug) were subjected to 32 cycles of amplification (each cycle consisting of 0.5min at 95 C, 1min at 62 C and 1min at 72 C) on thermal cycler. The PCR primers(Cre5:5-GGACATGTT CAGGGATCGCCAGGCG-3/Cre3 5-GCATAACCAGTGAAACAGCATTGCTG-3) were designed to amplify a 270b fragments from Cre coding sequence(8). The products were analyzed on 2% agarose gels. Southern blot analysis was carried out according to the standard protocols(10). Briefly, approximately 25ug of the genomic DNAs were digested with *Eco*RI, electrophoresed on 0.8% agarose gel and then, transferred to BioDy nylon membranes(PALL, NY, USA). The membrane were probed with 32P-labeled Cre DNA fragment.

Result and Discussion

We examined whether newborn mice generated by mating with males which had undergone injection of DNA possessed the introduced DNA. On PCR of genomic DNA isolated from a total of 89 newborns, pCAG-Cre sequence was detected in 40-80% of newborns derived from mating at 4, 7, 14 and 17 days after injection, but not at 30 days(table 1. figure 1).

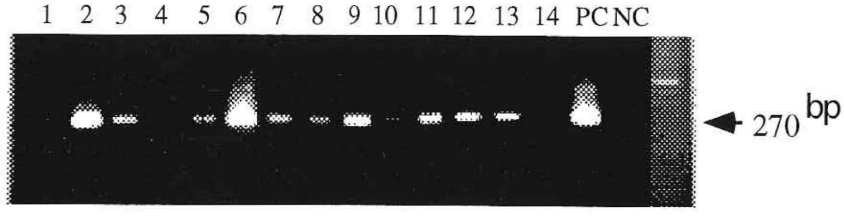

figure 1. PCR analysis of genomic DNAs isolated from tails of newborns produced by mating at 17 days after injection. Note the presence of amplified products of 270 bp derived from pCAG-Cre.

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Southern blot analysis revealed that the integration of the injected DNA into genome was observed in 7 of 19 newborns derived from the mating at 17 days after injection. (figure 2). These findings suggested that the injected DNA was continuously ejaculated for 17 days and a high copy number of the injected DNA was introduced into newborn at 17 days after injection. Although the plasmid pCAG-Cre was not detected in newborns generated by mating at 30 days after injection, it was detected by PCR analysis in spermatozoa in caudal epididymis of males at 30 days after injection of DNA(data not shown). Considering the process of spermatogenesis, it can be hypothesized that most of the injected DNA, which was not integrated in chromosome of spermatozoa but associated with the head of spermatozoa, was ejaculated until 17 days after injection and the injected DNA was slightly remained in caudal epididymis at 30 days after injection.

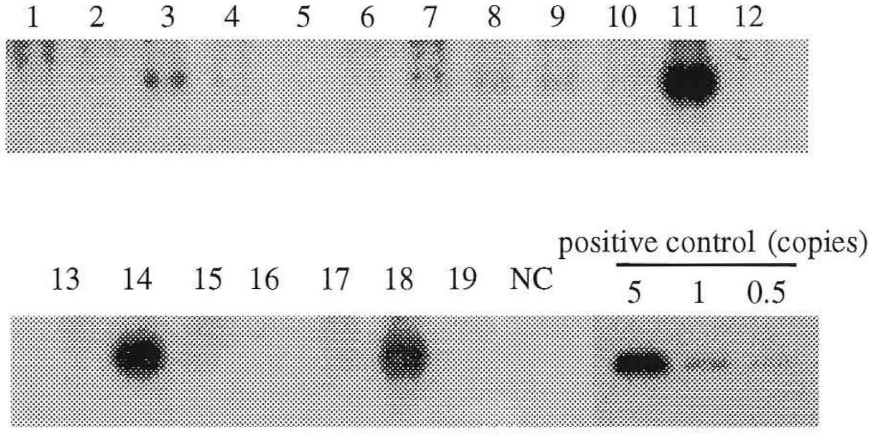

Figure 1. Southern blot analysis of genomic DNA isolated from tails of newborns produced by mating at 17 days after DNA injection. Each genomic DNA(25ug) was digested with *Eco*RI. Southern blot analysis revealed that more than 5 copies of pCAG-Cre sequence was transmitted to 3 newborns(11,14,18) and low copy number of the sequence was transmitted to 4 newborns(3,7,8,9)

Perhaps, the foreign DNA which remained in caudal epididymis at 30 days after injection was introduced to maturing spermatozoa (i.e.elongated spermatid) in testis. Baciller et al. demonstrated that liposome-encapsulated DNA can be incorporated into mouse sperm head after incubation with isolated epididymal spermatozoa, but the sperm which had incorporated DNA failed to generate transgenic mice(11). Sato et al. demonstrated direct injection of Ca-phosphate-precipitated DNA into mouse testis, however, it was impossible to produce transgenic mice by this method as well(12). We show here that the liposome-encapsulated DNA was incorporated into spermatozoa *in vivo*, and introduced to newborn. It is necessary to study the mechanism of introduction of the liposome-encapsulated DNA into spermatogenetic cells. In this study, the injected DNA was introduced to newborn with high efficiency(40-80%) by TMGT method. Then, Southern blot analysis revealed that more than 5 copies of pCAG-Cre was integrated in the genome of three newborns. These findings indicated that TMGT method can be useful tool for the production of transgenic mice. In order to make this technique practically useful, some questions must be answered, including the following: Dose expression of foreign DNA occur in the tissues of adult stages? Dose foreign DNA transmit to next generation? and is this technique applicable to production of transgenic animals other than mice?

Table.1 Efficiency of detection of the foregin DNA in newborns

Date of mating (days after injection)	No of newborns	No. of newborns detected the foregin DNA	
		PCR(%)	Southern(%)
4	12	5/12(41.6)	0/12(0)
7	11	8/11(72.6)	0/11(0)
14	24	13/24(54.1)	0/24(0)
17	19	15/19(78.9)	8/19(42.1)
30	23	0/23(0)	0/23(0)

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