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A Study on the Transmission of a Transgene in the Offspring of Transgenic Mice

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형질전환 생쥐의 후손에서 외래 유전자의 유전성에 대한 연구

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요 약

형질전환 동물의 후손에서 transgene은 멘델의 법칙에 따라 유전된다고 일반적으로 인식되어져 왔다. 따라서 본 연구에서는 transgene이 이러한 인식과 일치하는지를 여러 세대를 통하여 확인하고 후손에서 어떻게 유전되는지를 연구하기 위하여 형질전환 생쥐를 생산하여 본 연구의 모델로 삼았다. 수정된 생쥐의 embryo에 DNA를 microinjection하는 방법으로 MMTV-LTR (long terminal repeat), bovine α_{S1}-casein cDNA, 그리고 SV 40 splicing과 polyadenylation site 등의 sequence를 포함한 3.0 kb의 DNA가 주입되었다. 여기에서 태어난 새끼는 dot blot과 Southern blot에 의하여 transgene의 존재여부가 확인되어 founder line이 만들어졌다. 그들의 자손은 PCR에 의해서 transgene이 유전되는지를 확인하였다. F_0의 72마리 새끼중에서 4마리의 Founder가 transgene을 가지고 있었다(5.6%). F_0에서 F_1으로의 유전(transmission)은 각각 33.3, 7.7, 0, 62.5%이었다. Transgene은 F_1에서 F_2로 각각 63.6, 5.9, 68.8% 유전되었고, F_2에서 F_3로 각각 85.7, 0, 88.2% 유전되었다. 따라서 본 연구 모델에 의하면 transgene은 멘델의 법칙을 따르는 경우와 deletion이 되는 경우로 각각 관찰되었다.

I. INTRODUCTION

Over the last two decades, the production of transgenic animals has been a routine procedure in many reseach laboratories all over the world. Methods for transferring foreign genes into embryos by microinjection of the recombinant DNA into the pronucleus of an egg have been rapidly developed for mice, rats, rabbits, swine, sheep, and cattle (for review, see Ebert and Selgrath, 1991). Many lines of transgenic mice were produced for investigations of physiological, genetic, and immunologic research purposes (Palmiter and Brinster, 1986; Westphal, 1989). Nonetheless, the production efficiency remains relatively low for all species (First, 1991). More efficient approaches are being developed and use replication-defective viral vectors (Kim et al., 1993) and embryonic stem cells (Evans et al. 1990; First, 1991).

Regardless of what methods to produce transgenic animals, the aim of gene transfer is germline incorporation of the transgene. Thus,

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gene transfer into the genome of animals may provide an exciting way to make animals with new traits never found in the populations of their species. The power of this technology comes from the rapid propagation of the transgene into the animal population by artificial insemination or embryo transfer (Parrish, 1990). Obviously, a stable incorporation of a transgene into the genome of a transgenic animal will result in transmission of the gene into the offspring. Therefore the transmission pattern of the transgene will be of great interest to those who are trying to apply this technology.

In this report, transgenic mice were produced to study the transmission of a transgene into their offspring. Previously, Yom et al. (1993) reported the expression of the MMTV-α_{S1}-casein cDNA in the transgenic mouse milk. The same recombinant DNA was used in this study. The 3.0 kb linear fragment containing the MMTV LTR, bovine α_{S1}-casein cDNA and SV 40 splicing and polyadenylation site was obtained from the plasmid pSPas1CN. Four founder lines of transgenic mice were produced. The transmission of the transgene into the offspring was studied for 4 generations.

II. MATERIALS AND METHODS

1. Cloning DNA

The cloning strategy was described previously (Yom et al., 1993). Briefly, it utilized the maximum use of sticky end ligations to join the 5′ end of bovine cDNA to the 3′ end of MMTV promoter. Fig. 1 shows the summary of cloning experiment. The pC_{184}, a pBR322 clone with bovine aS1 casein cDNA, was provided by Dr. Mackinlay at University of South Wales (Stewart et al., 1984). The pBluescript with KS polylinker (pBSKS), a derivative of pUC_{19}, was obtained from Stratagene (La Jolla, CA). The pBSas1CN

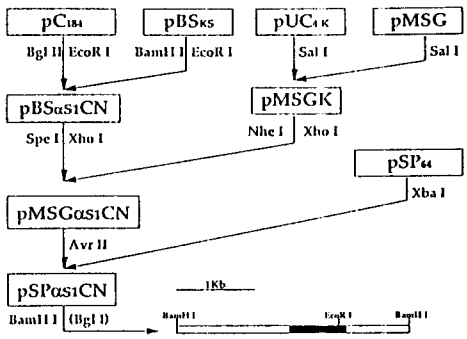

Fig. 1. Summary of cloning strategy for MMTV/aS1 casein. It utilizes the maximum use of sticky ends. All the ligations used here were by compatible ends. 1) Modification of the casein cDNA. 2) Modification of pMSG. 3) Ligation of pBSα_{S1}-CN fragment to pMSGK 4) Subcloning of pMSGα_{S1}CN into pSP_{64}. 5) BamH I digestion, followed by Bgl I, resulted in the 3.0 kb for microinjection. The dark region is the casein cDNA.

was made by ligation of a 2.9 kb fragment from pBSKS and a 0.67 kb fragment from pC_{184}. The pMSG, a mammalian vector with MMTV LTR; and the pUC-4K, a pUC derivative with kanamycin resistance sequence; were purchased from Pharmacia Inc. (Piscataway, NJ). The pSP_{64} was obtained from Promega Co. (Madison, WI). The pSPα_{S1}CN was constructed by ligation of a 3.3 kb fragment from pMSGα_{S1}CN and 3.0 kb fragment from pSP_{64}. Enzymes were from Promega Co. Gene clean kit was obtained from Bio 101 Inc. (La Jolla, CA).

2. Microinjection of embryos

Female mice (C57B6 × B6D2) with 7 to 10 week-old were superovulated with PMSG and hCG as described by Bayna and Rosen (1990) and mated with ICR or B6D2. The plugged females were sacrificed and the embryos were col-

lected from their oviducts. Microinjection was performed by using a Narishige micromanipulator (Narishige Co. Tokyo, Japan) and a Zeiss inverted microscope (Carl Zeiss, Oberkochen, Germany) as shown by Gorden et al. (1980). Microinjected embryos were placed into the oviducts of C57B6 female, which were pseudopregnant by a vasectomized male.

3. Identification of transgene by DNA blots

Dot hybridization was used to screen the transgenes from mice born after microinjection. One half of their tails were removed and digested with pronase K (Sigma Co. MO) overnight. The genomic DNA was purified and subjected to Hybond-N membrane (Amersham Co. IL). A 0.7 Kb fragment, which includes the entire bovine casein cDNA, was isolated from pC_{184}, and nick-translated with P^{32} dCTP (Du pont, DE) for a radioacive probe. The membranes were prehybridized for 4 h and hybridized for 12 h at 65℃ and the final stringency was 0.01X SSC solution. The membrane was exposed to a X-ray film (Kodak) −70℃ for 5 d. As for the positive samples, they were digested with EcoR I overnight and 1.0% agarose gels were run and Southern blots were performed as described above.

4. Polymerase chain reaction (PCR)

Polymerase chain reaction was used to screen the transgene among the offspring of the founders. Mouse tail DNAs were amplified using an automated temperature cycler (Coy Lab. Inc., MI). The primers were two 20 nt long (see *PCR analysis* in Results and Discussion), apart by 584 bp, and within the coding region of the bovine casein cDNA. Amplification was performed for 32 cycles at 92℃ (Denaturation) for 2 min, 47℃ (Annealing) for 1.5 min, and 72℃ (Extension) for 1.5 min. The Taq polymerase and its buffer were from Promega Co. and the dNTPs were from Pharmacia.

5. Breeding

The F_1 animals were produced by mating the transgenic female or male founder to normal ICR partners. As for F_2, transgenic F_1 mice were bred to transgenic parents. F_3 animals were produced by mating between F_2 siblings within the same line. The offspring were screened for the transgene by PCR.

Ⅲ. RESULTS AND DISCUSSION

1. Cloning DNA

The cloning strategy was the maximum use of sticky end ligations to join the 5′ end of bovine cDNA to the 3′ end of MMTV promoter. Fig. 1 shows the summary of cloning. All ligations used here were by compatible ends. Firstly, to maximize the use of sticky end ligations, the Bgl Ⅱ and EcoR I ends of pC_{184} were ligated to the BamH I and EcoR I ends of pBSKS to give pBSas1CN. Secondly, since the Nhe I and Xho I sites at mutiple cloning site of pMSG are only 12 bp apart, it was not possible to cut both sites with the corresponding enzymes. Therefore, the Sal I site of pMSG was spaced by addition of kanamycin resistant sequence with the Sal I site from pUC-4K to give pMSGK. Thirdly, the Spe I and Xho I ends of $pBS\alpha_{S1}CN$ were ligated to the Nhe I and Xho I ends of pMSGK to give $pMSG\alpha_{S1}CN$. Fourthly, since the $pMSG\alpha_{S1}CN$, a mammalian expression vector, gave a low plasmid yield, its Avr Ⅱ ends were ligated to the Xba I of pSP64 to give $pSP\alpha_{S1}CN$. Finally, its BamH I digestion, followed by Bgl I, resulted in the 3.0 kb for microinjection. The open region in Fig. 1 is the promoter region, the dark region the casein cDNA, and the gray region the splicing and poly A tail.

2. Production of transgenic mice

The 3.0 kb fragments were further purified by Gene Clean kit and microinjected into 1 cell embryos. A total of 72 live pups were born and screened by dot blots. Four pups (5.6%), 3 males and 1 female as shown in Table 1, were positive for the transgene and subjected to Southern blots. Fig. 2 shows Southern analysis of one of the transgenic founders. The 7.0 kb band represents the flanking sequence from EcoR I digestion. The 3.0 kb band results from the tandom array of the transgene indicating integration of the multiple copies. An analysis of the intensity of the two bands showed that there were 4 copies of the transgenes in this line. The results of other founders are not shown here. But, the copy numbers of the transgene incorporated in all founders are indicated in Table 1.

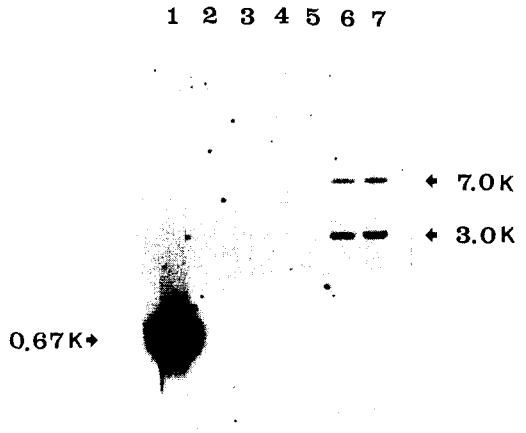

Fig. 2. A Southern analysis of one of the transgenic founders. The 7.0 kb band represents the flanking sequence from EcoR I digestion. The 3.0 kb band results from the tandom array of the transgenes. Lane 1: 0.7 kb casein fragment, 2: blank, 3~5: non-transgenic, 6~7: transgenic from the same line.

3. PCR analysis

PCR was used as a rapid screening method after the founder lines were identified by Southern blots. An example of PCR analysis of mouse genomic DNA is shown in Fig. 3. The two 20 nt primers (159-178, CTATC AAGCA CCAAG GACTC; and 723-742, CCACA GTGGC ATAGT AGTCT) were synthesized within the coding region of the casein. The primers were selected based on GC content (about 50%) and theoretical formation of secondary structures by themselves. The various PCR conditions were optimized for the specific amplification of the 584 bp band, which is shown in lanes 4~17 in Fig. 3.

Table 1. Transmission of the transgene in the offspring of transgenic mice

Founder (F_0)			Transmission (%)		
ID	Sex	Copy no.	F_1	F_2	F_3
1	M	4	33.3 (4/12)[a]	63.6 (7/11)	85.7 (12/14)
2	M	2	7.7 (1/13)	5.9 (1/17)	0 (0/15)
3	M	1	0 (0/15)		
4	F	1	62.5 (5/8)	68.8 (11/16)	88.2 (15/17)

[a] No. of trangenic /Total offspring

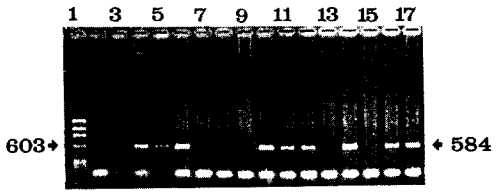

Fig. 3. PCR screening of transgenic (TG) mice. The amplification with 1 U of Taq polymerase was performed for 32 cycles at 92℃ (Denaturation) for 2 min, 47℃ (Annealing) for 1.5 min, and 72℃ (Extension) for 1.5 min. Lane 1: Molecular marker(see 603 bp), 2: No DNA, 3: non-TG, 4: TG, 5~17: Samples. The arrow at 584 bp indicates the specific casein fragment.

4. Transmission analysis

The summary of transmission of the transgene into offspring is shown in Table 1. The rates of transmission from F_0 into F_1 were 33.3, 7.7, 0, and 62.5%. Those from F_1 into F_2 were 63.6, 5.9, and 68.8% and those from F_2 into F_3 were 85.7, and 88.2%. If the transgene follows a Mendelian fashion, the theoretical rates of the transmission for the above breeding will be 50% for F_1, 75% for F_2, and 91.7% for F_3. From the Table 1, one may realize that the animal 1 and 4 follow the theoretical values, where the animal 2 and 3 do not. This means that those animals follow a Medelian principle have a stable incorporation of transgenes into the their germ lines, and that those do not follow it have an unstable incorporation and they are mosaic at their germ lines. Deletion or loss of the transgenes from F_0 in these lines are apparant in the Table 1.

The incorporation of genes into transgenic mice is generally stable and is passed on to next generations in a Mendelian fashion (Palmiter and Brinster, 1986). In this report, the transmission pattern of transgenes in transgenic mice into their successive offspring was demonstrated. It either follows or does not follow a Mendelian fashion and the pattern becomes more apparant to the succeeding generations.

The transgenic animal technology will certainly facilitate the introduction of allelic genes existing in a breed or strain at a low frequency (First, 1991). Many efforts are being made in mapping the genome of animals to identify the DNA sequences or restriction polymorphism linked to production traits (Cowan et al, 1990). Much information may be obtained from the human or animal genomic projects.

Ⅳ. SUMMARY

It is known that the incorporation of genes into transgenic mice is generally stable and is passed on to succeeding generations in a Mendelian fashion. In this report, transgenic mice were set as a model to evaluate whether the transgenes are transmitted in a Mendelian principle in a successive generations and how they are transmitted into their offspring. A 3.0 kb linear DNA fragment, containing the MMTV LTR, bovine aS1 casein cDNA and SV 40 splicing and polyadenylation site; was microinjected into fertilized mouse embryos. The tail DNAs of the resulting pups were subjected to dot and Southern hybridizations to screen transgenic founders. The DNAs of their offspring were anlyzed by PCR to confirm the transmission of the transgene from F_0. Out of 72 live pups four pups (5.6%), 3 males and 1 female, were positive for the transgene. The rates of transmission from F_0 into F_1 were 33.3, 7.7, 0, and 62.5%. Those from F_1 into F_2 were 63.6, 5.9, and 68.8% and those from F_2 into F_3 were 85.7, and 88.2%. In this report, the transmission pattern of transgenes in transgenic mice into their offspring was dem-

onstrated. It either follows or does not follow in a Mendelian fashion. Deletion or loss of the transgenes from F_0 in some lines became apparant to the succeeding generations.

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