GENE TRANSFER IN RABBITS AND PIGS

G. BREM, B. BRENIG, H.M. GOODMAN, R.C. SELDEN, F. GRAF, B. KRUFF, K. SPRINGMANN, J. HONDELE, J. MEYER, E.-L. WINNACKER and H. KRÄUßLICH, WEST GERMANY

Institut für Tierzucht und Tierhygiene
Ludwig-Maximilians-Universität München
Veterinärstr. 13
D-8000 München 22

SUMMARY

One-cell ova were recovered from superovulated rabbit and pig donors. For microinjection a DNA fragment consisting of the structural gene of the human growth hormone and the promotor region of the mouse methallothionein gene was used.

After transfer of injected eggs 37 (9.6 %) offspring were born in rabbits and 15 (3.4 %) in pigs. 5 rabbits and 1 piglet were found to be transgenic by dot hybridisation and Southern blotting.

INTRODUCTION

Gene transfer in farm animals will have a considerable impact upon the future of livestock production. Efficient production of transgenes and the development of systems for an appropriate regulation of transgene expression in transgenic animals are the most important presuppositions for the realization of these projects.

Potential applications will involve traits of economic importance, like growth, disease resistance and efficiency of reproduction or feed utilization. Production of transgenic rabbits and pigs by microinjection into pronuclei has been reported for the first time last year (Hammer et al. 1985, Brem et al. 1985).

GENE CONSTRUCT

For microinjection a 4.0kb linear Eco RI/Eco RI DNA fragment was used consisting of the structural gene of the human growth hormone (hGH) and the promotor or regulatory region of the mouse methallothionein gene I (MT) fused in a Bgl II site. This fragment has been inserted into a 4.31kb hybrid vector containing a 2.35kb Eco RI/Bam HI fragment of BPV and a 2.96kb fragment of pBR327 to give the 9.31kb plasmid pXGH-1 (Fig. 1). This plasmid was used to transform the E. coli strain HB 101.

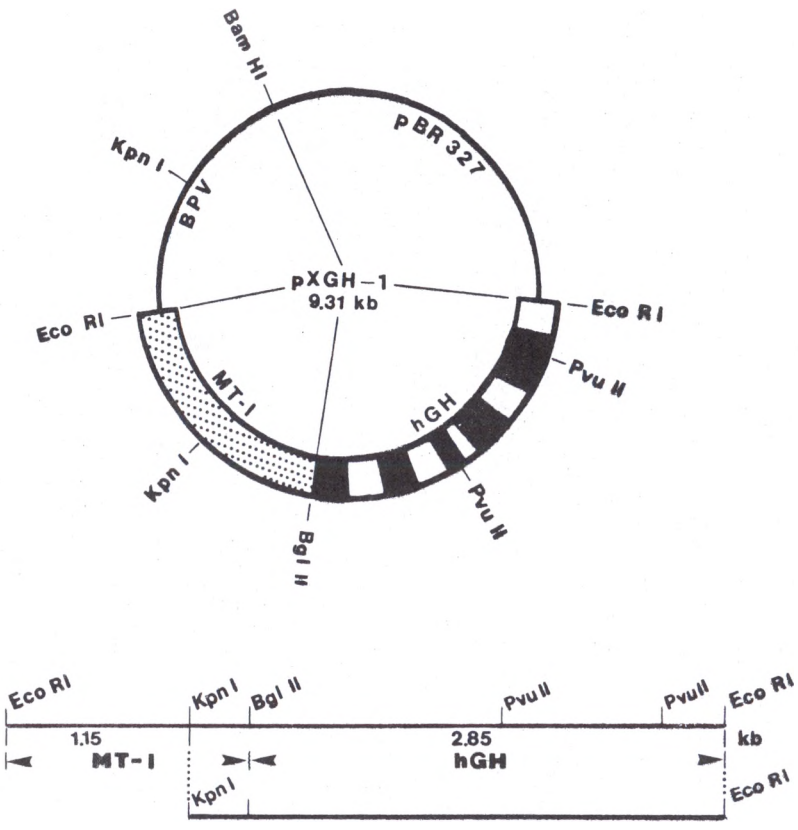

Figure 1: Gene construct used for microinjection in rabbit and pig zygotes

MICROINJECTION

Rabbits were superovulated with 150 I.U. PMSG and embryos were flushed from the oviducts 20 h after mating. Superovulated pigs (1250 I.U. PMSG, 600 I.U. HCG) were flushed 60 h after HCG-injection. In both species only one cell ova (zygotes) were used for the microinjection procedure. Pig zygotes were centrifuged at 15000 g for 3 min to visualize the pronuclei (Wall et al. 1985).

Microinjection was carried out under X 630 magnification using a ZEISS Inverted Microscope ICM 405 and Nomarski interference contrast optics. Zygotes were placed on a depression slide in a drop of flushing medium. Zygotes were attached to a holding pipette by suction, and the injection pipette (diameter 1-2 μm) was inserted into one of the pronuclei. Injection was accomplished by using compressed air. The expansion of the pronuclei demonstrated the successful injection of several pikoliters of DNA solution (4 μg DNA/ml).

After injection the zygotes were cultured for 1 to 4 h and porcine embryos were transported to the location of transfer. Injected zygotes were transferred to the oviducts of synchronized pseudopregnant foster mothers.

RESULTS

Isolation of total nucleic acid was done by powdering small samples of fetal liver or tail in liquid nitrogen. Powdered tissues were homogenized and incubated in a 0.5 M EDTA, 0.5% Sarkosyl, 0.01% proteinase K buffer, pH 8.0, for 2-3 hours (50°C, 230 U/min.) in a water bath shaker. After incubation the homogenates were extracted twice with 10 mM Tris-HCl, 1 mM EDTA, pH 8, saturated phenol, twice with chloroform/phenol and once with chloroform/isoamyl alcohol 24:1. All samples were then dialyzed against 10 mM Tris-HCl, 1 mM EDTA, pH 7.8 over a period of 15-24 hours at 4°C changing the buffer several times. This procedure resulted in 1-1.8 ml of high molecular DNA solution with a concentration of 90-300 μg/ml.

Another method of isolating DNA from blood samples, especially in pigs, yielded very good results. 5-10 ml freshly taken blood was centrifuged through a Ficoll gradient to seperate erythrocytes from white blood cells. The latter were collected and treated as described above.

Animals carrying MT-hGH sequences were first detected by dot blotting. 10 μg of DNA were heated to 100°C for 2 min. in a NaOH-NaCl buffer, chilled on ice, neutralized and then spotted directly onto nitrocellulose filters, which have been soaked in 10xSSC and dried at room temperature prior to use.

Positive animals were then analysed by the Southern technique. Therefore 20 µg DNA were digested with Eco RI (40-50 U, 3-5 h) and simultaneously treated with RNase A. After digestion samples were electrophoresed on a 1% agarose gel and transferred to nitrocellulose as described elsewhere (Southern, 1975).

Filters were hybridized with 1 µg of pcGH-1, nicktranslated with 32-P labeled a-dCTP and autoradiographed after stringent washing for 15-30 hours.

- Rabbit

An average of 23 ova was recovered per donor doe. 82 % of the ova showed both pronuclei and were used for microinjection. About 90 % of all zygotes survived microinjection and were transferred into the oviducts of pseudopregnant recipients. An average of 20 embryos was transferred per recipient, 10 into each oviduct. The pregnancy rate was 26 % and out of 6 litters 37 offspring were born. The survival rate (offspring/transferred embryos) being 9.6 % including to all recipients In pregnant foster mothers 30 % of the zygotes survived to term.

Up to now 27 rabbits, born after transfer of microinjected zygotes, were analysed and 5 transgenes could be detected. The frequency of mMT-hGH integration was 18 %.

- Pig

After centrifugation 74 % of pig ova showed one or two pronuclei. 9 control tranfers were made with centrifuged eggs, the pregnancy rate being 78 % (20 zygotes/recipient) and survival rate of zygotes 20 % respectively.

To investigate the effects of centrifugation, transport and microinjection a 4 day in-vivo culture in pig oviducts was carried out. Approximately 54 % of centrifuged and transported porcine zygotes developed to morulae and blastocyst stages. Out of 251 injected and transferred eggs 162 (65 %) could be recovered. 31 % had cleaved at least twice and 18 % developed to morulae and blastocyst stages. After transfer of the cultured embryos 3 out of 4 recipients became pregnant.

Up to now 25 transfers of injected porcine zygotes without in-vivo culture led to 6 pregnant recipients (Table 1 and 2) and to 3 litters with a total of 15 piglets born; the survival rate in pregnant foster mothers being 20 %. 1 of these 15 piglets was shown to be transgenic by dot hybridisation and Southern blotting. This piglet died after several weeks.

Table 1: Pregnancy of pigs with regard to the number of corpora lutea per recipient

Number of corpora lutea per recipient	Number of transfers abs.	Zygotes transferred	Recipients pregnant	%
≤ 10	10	352	1	10 %
> 10	15	511	5	33 %
Total	25	863	6	24 %

Table 2: Pregnancy rate of pigs after transfer of different numbers of zygotes per recipient

Zygotes per recipient	Number of recipients	Zygotes transferred	Recipients pregnant abs.	%
< 30	12	295	4	33
30 - 39	4	136	1	25
40 - 49	6	250	1	17
> 50	3	182	-	-
	25	863	6	24

CONCLUSIONS

Direct microinjection can be used to produce transgenic rabbits and pigs. Survival rates of eggs after injection procedure are higher in these species than in mice but integration rate of the new DNA seems to be lower. In our laboratory up to now the expression of the new gene was only shown in transgenic mice. Analysing serum samples for hGH by RIA, 2 transgenic mice had hGH serum levels of more than 20 ng/ml. Transgenic mice also grew faster and transgenic offspring were between 1.5 and 2.4 times heavier than normal litter mates, whereas transgenic rabbits and pigs, at least so far, show no higher live weight than controls.

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