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BARD

FINAL REPORT

PROJECT NO. I-797-84

Genetic Improvement of Sheep by Introduction of Foreign Genetic Information into Prolific Breeds

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Genetic improvement of sheep by introduction of foreign genetic information into
prolific breeds.

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Abstract

The mode of expressoion of transgenes in mice, the development of early ovine embryos in vitro and increasing prolificacy in the Awassi and the Assaf dairy breeds were addressed in this work as preparation steps for production of dairy type transgenic sheep. Our studies with the rat skeletal muscle genes demonstrated that one can target foreign gene expression to the correct tissue, provided that sufficient cis acting elements are included in the introduced gene. It appears that appropriate expression in transgenic mice is directly correlated with the presence of sufficient control sequences and less on the chromosomal site of integration. Ovine embryos collected from the oviduct can be cultured succesfully to the blastocyst stage using meduim conditioned by embryonic or oviductal cell. Under those conditions injected embryos can be grown and screened for gene incorporation. Prolificacy in the Awassi and the Assaf breeds can be increased by the inclusion in these breeds, of the F gene from the Booroola Merino or by genes from other prolific breeds. Plasma FSH levels at one month of age are correlated with prolificacy in those breeds and may be used as early physiological marker for high prolificacy.

Objectives:

Following the demonstration that foreign growth hormone gene can dramatically increase growth rate of transgenic mice, it was suggested that introduction of new genes into the germ line of farm animals can be used for genetic improvement of domestic species.

Till now, successful microinjection of DNA into pronuclei has been demonstrated in pigs, sheep and cows. However, in all cases the process was inefficient and the percentage of microinjected eggs that developed into transgenic animals was around 1%. The most frequently investigated structural genes were growth hormone genes and the main promotor used was the metallothionin promotor. Generally, the transgenic animals produced in the different studies were inferior to the control in both production and reproduction traits - pointing out that the key issue for creating successful transgenic livestock is the correct control of the expression of the transgenes. Thus far, most transgenic livestock expressed the foreign gene at variable levels, in an unpredictable manner and throughout development. Such mode of expression can lead to the deleterious effects already observed.

Thus, using transgenic mice carrying rat muscle specific genes as a model system, our first goal in this project was to study the tissue specificity, developmental regulation and chromosomal position of transgens and the possible interrelationship between the transgens and the endogenous genes.

It became evident that production of transgenic livestock is a costly operation since a large number of females are required as embryo donors and recipients, because of the low efficiency of gene incorporation following gene injection. Improving culture conditions in which one cell sheep embryos could develop in vitro up to the blastocyst stage to allow screening for viability and gene incorporation prior to embryo transfer was the second goal of this project.

To achieve maximum economic merit from developing transgenic stock, genes of interest should be initially inserted into animals of breeds which are widely distributed and which are superior in productive traits other than those governed by the transgene. High growth rate, lean carcass and higher milk production were shown to benefit from increasing growth hormone levels. Thus, highly prolific breeds which are widely distributed should be preferred as a recipient of the growth hormone gene. The third goal of this project was to continue to develop highly prolific Awassi and Assaf dairy breed by crossing them with prolific breeds.

Body of the report:

This section is divided into three parts:

1. Studies on transgenic mice
2. Culture of early ovine embryos

Description of cooperation:

The foundation of the joint project were laid in a meeting between the Israeli team and Prof. Foote in Israel, where the main objectives for gene transfer project in sheep were defined. During the course of the project Dr. Gootwine travelled to Utah, U.S.A. to gain experience in tissue culture techniques which were developed by the American collaborators. These techniques were later successfully employed in embryo cultures in Israel. Throughout the project there was continuous and fertile communication and exchange of ideas between the two teams. As it emerged that the transfer of the Booroola Merino F gene into local economically important breeds of sheep is the better way for increasing their prolificacy, Prof. Foote participated in setting up a second BARD project in which the Israeli group and scientists from Illinois University are now collaborating.

Main Achievements:

Our studies with the rat skeletal muscle gene demonstrated that one can target foreign gene expression to the correct tissue, provided that sufficient cis acting elements are included in the introduced gene. In addition, these studies revealed the inhibitory effect of prokaryotic vector DNA sequence on the expression of the transgene. It is now common practice to remove these sequences prior to microinjection. The expression of the rat skeletal muscle actin gene was also developmentally regulated. Thus, this skeletal promoter can serve as a vehicle for foreign gene expression in skeletal muscle of livestock.

Chromosome position effect is considered to be the main cause for the inappropriate expression of foreign genes in transfected somatic cells and in transgenic animals. We have addressed this issue in a more direct way by introducing into the mouse germ line two genes of different tissue specificities, that were cloned on the same DNA fragment and integrated at the same chromosomal site. Each gene included its own set of regulatory elements, that are required for proper expression in transgenic animals.

The results demonstrated that in those transgenic mice that expressed the two genes each retained its tissue specificity despite the close proximity to different control elements. It is also indicated that a single chromosomal site is permissive to the autonomous expression of two genes of different tissue specificity.

It appears therefore, that appropriate expression in transgenic mice is directly correlated with the presence of sufficient control sequences and less on the chromosomal site of integration. Defining these elements in transgenic mice should be a prerequisite for successful production of transgenic livestock. Recently, it was shown that simply by substitution of the metallothionin promoter by a promoter of the gene coding for the tissue specific enzyme phosphoenol pyruvate carboxykinase, the expression of the human growth hormone gene resulted in transgenic pig with no back fat, increased growth rate, increased feed efficiency and no detectable deleterious effect.

An important question that had to be dealt with was the possible interaction between the transgene and the endogenous gene. The purpose of transgenesis is not to interfere too much with the normal physiology of the animal. In many cases this would not be possible if the transgene would affect the mode of expression of the endogenous gene. Our studies have shown that transgens could down regulate the endogenous gene, either as a result of in vivo competition over limited amount of transcriptional factors, or due to negative feedback inhibition as a result of overproduction of the specific products (RNA or protein). Understanding these factors may be important in the future design of the introduced gene.

Embryonic cell lines (EK lines) derived from early embryos can serve as a vehicle for inserting genetic information into embryos. This experimental system may have an advantage over the microinjection system since cells for DNA transfection can be obtained in unlimited number. In order to develop sheep derived EK cell system it was necessary to define appropriate in vitro culture conditions which will support the development of sheep embryos and embryonic cells in culture..

Co-culturing of 2-4 cell ovine embryos with feeder cells obtained from 12-14 day old sheep embryos enable them to successfully develop through the 8-16 cell block stage up to the post hatching stage. Hatched embryos under those culture conditions continued to expand and cellular growth was observed in the region of the trophectoderm adjacent to the inner cell mass. At this stage transformation of normal embryonic cells into EK cells might take place.

High prolificacy should be considered as a preferable trait of genotypes in which transgenic manipulation is performed. In a crossbreeding program aiming at increasing prolificacy of the Awassi breed, early markers for the identification of potentially more prolific ewe lambs are of interest. Our results suggest that higher prolificacy in the Awassi crosses is followed by higher plasma FSH levels at 4-6 weeks of age and by greater ovarian sensitivity to gonadotropins at 5 month of age. Thus, those parameters could be used as physiological markers for high prolificacy.

Inclusion of the F gene into the Awassi and the Assaf breeds was found as a better way for increasing prolificacy in those breeds in comparison with crossbreeding with the Finn and the Romanov breeds.

It is our belief that further progress and success in germ line transformation in farm animals would much depend much on better understanding of the control of gene expression, on the developing of efficient ways to introduce the transgens into embryonic cells and the screening for gene incorporation before embryo transfer, and on the developing of the optimal target genotypes for transgenic manipulations.

List of publications:

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