Proceedings of the New Zealand Grassland Association 49: 63-65 (1988)

PROSPECTS FOR BIOTECHNOLOGY IN ANIMAL PRODUCTION

J. W. Forrest and T. E. Broad
Biotechnology Division, DSIR, Palmerston North

Abstract

Through recent advances in molecular and reproductive biology selected genes from one animal or species can be inserted into another animal's genome to produce a 'transgenic' animal. Striking increases have been achieved in the rate of growth of mice containing additional copies of the rat growth hormone gene. Such results raised the possibility of using similar techniques to modify the expression of existing genes and introduce novel gene(s) into domestic livestock for Improved productivity.

The introduction of multiple copies of the growth hormone genes into pig embryos has resulted in live births of pigs that grew 10-20% larger and reached market weight three weeks earlier than litter-mates. The introduced genes were passed from generation to generation. In sheep cysteine supplementation into the abomasum produced at 10.20% increase in wool growth rate. Genes responsible for cysteine biosynthesis have been isolated from yeast, and a modified gene suitable for expression in sheep produced. By engineering the cysteine biosynthetic pathway into the sheep genome there is scope to considerably increase wool production.

Keywords: gene transfer, transgenesis, recombinant DNA, embryo manipulation.

INTRODUCTION

The term biotechnology can be defined as "the application of science to biological organisms to give new and improved agricultural and industrial production". The application of biotechnology to farm animals is exciting; offering a mechanism whereby genetic material isolated from any animal, plant or bacterial source can be transferred to a recipient animal resulting in the altered activity of an existing gene within the animal or the production of a new gene product from the animal. This article focuses on the techniques of recombinant DNA for changing the genome of domestic animals, and thereby improving their productive value to the farmer.

RECOMBINANT DNA TECHNIQUES

The ability to isolate, modify and re-introduce segments of DNA (genes) in animals originates from basic scientific research a little over a decade ago. The discovery of enzymes able to cut DNA (restriction endonucleases) and the development of methods for the reconstruction of cut DNA segments are two examples. Next, the utilization of modified bacterial DNA able to replicate rapidly within micro-organisms made possible the isolation of many copies of a gene or if desired its protein product. One example of the use of recombinant DNA techniques is the production of human growth hormone, for the treatment of pituitary dwarfism in humans or to increase milk production in cows.

EMBRYO MANIPULATION FOR GENE TRANSFER

Once genes have been obtained, the next step is the integration of these genes into the germline of recipient animals where they may be expressed and subsequently propagated as a new character in the offspring. Recent developments in reproductive biology (reviewed by Church 1987) describe techniques which enable the stable integration of recombinant DNA into an animal's genome. The techniques were developed using mice and have been applied with a lower success rate in domestic livestock (Hammer *et al.* 1985). Animals are given a course of hormones to induce superovulation. Following fertilisation eggs are removed and several hundred copies of recombinant DNA are microinjected into the pronucleus (usually

male) of the fertilised one-cell egg. Injected eggs are then transferred to the oviduct of foster mothers and allowed to develop. In mice only 1 0-20% of the eggs survive microinjection and reimplantation, of which approximately 20% are found to carry one or more copies of the recombinant DNA. The animals containing foreign DNA within their genome are known as "transgenic" animals.

Other techniques for producing transgenic animals are currently being developed. The most promising of these being the use of embryonic stem cells, which can be grown outside the body and then reintroduced into the early embryo to participate in the development of a complete animal (Bradley et *a/.* 1984).

GENE TRANSFER AND THE DEVELOPMENT OF TRANSGENIC ANIMALS

The most successful and striking experiments to date have been with mice inserted with growth hormone genes from either rat or man (Palmiter 1982 and 1983). The transgenic mice had elevated levels of growth hormone, grew more rapidly and for longer periods. This exciting result raised the possibility of using similar techniques to stimulate rapid growth of commercially valuable animals. Transgenic rabbits, pigs, sheep and cattle containing recombinant growth hormone gene constructs have been reported (Hammer et *al.* 1985). However, the relatively low success rate in production and reproduction of transgenic livestock, to date, means the commercial production of such animals is still in the future.

The lack of success in large animals appears due to several factors including the limited knowledge of embryo development at the molecular level, inadequate handling of fertilised eggs in culture, the use of inappropriate recombinant DNA constructs for the specific animal and limits on the reproductive ability of transgenic animals. What regulates the expression of specific recombinant genes is not well understood, but it appears that sequences immediately flanking the coding portions of genes may be important, as for example in the correct expression of the chicken transferrin gene in the liver of transgenic mice (McKnight et al. 1983). However not all recombinant genes are expressed appropriately (Lacy et al. 1983) nor is their integration into the host animal's genome without side effects (Wagner *et al 1983).*

APPLICATION OF GENE TRANSFER AND EXPRESSION TO LIVESTOCK PRODUCTIVITY

While the success rate is lower than hoped for, transgenic livestock are a reality (Palmiter et al. 1983; Church 1987). The pig has proven most successful due to the relative ease in gathering sufficient fertilised eggs for microinjection. To date a number of transgenic pigs have been reported expressing elevated levels of growth hormone Hammer *et al. 1985).* The transgenic animals had 1-10 copies of a human gene promoter spliced to a pig growth hormone gene integrated into their genome. Some but not all of the transgenic pigs grew 20% larger and reached market weight three weeks earlier than non-transgenic litter mates. Moreover as the transgenic animals were fertile, copies of the gene were passed on to the progeny. The ultimate aim is to produce pigs which carry the inactive growth hormone gene which can be switched on only when required, stably integrated within the animals genome.

In addition to modifying the expression of existing genes, as is the case for growth hormone, gene transfer may also be able to increase animal productivity by overcoming limitations in production or by creating new products. For example the concentration of the amino acid cysteine in blood limits wool production (Reis and Schinkel 1963). Cysteine is an essential amino acid supplied only through dietary intake, which when supplemented can produce a 20% increase in wool growth. Cysteine biosynthesis genes taken from yeast are able to be expressed in cultured sheep cells. These same cysteine constructs are now being injected into sheep embryos with the hope of giving this productive advantage to the animal.

TARGET AREAS FOR FUTURE APPLICATION IN DOMESTIC ANIMALS

The prospect for the production of pharmaceuticals from transgenic animals or microflora, so-called "molecular farming" has aroused recent interest (reviewed by Clark et al. 1987). Transgenic animals are envisaged as having a recombinant gene construct comprising a gene for a biologically and/or commercially important protein coupled to regulatory sequences of a milk protein gene; allowing the correct processing and secretion of the useful protein into the milk from which it could then be purified.

CONCLUSION

Biotechnology offers agriculture potential to produce both entirely new products as well as enhance the efficiency of producing existing products. The aim of recombinant DNA gene transfer technology for livestock production is to develop genetically superior or novel animals that will form the nucleus for future animal breeding strategies and will give the producer new economic opportunities.

References

Bradley A., Evans M.. Kaufman M.K., Robertson E. 1984. Formation of germ-line chimeras from embryo-derived teratocarcinoma cell lines. *Nature* 309: 255-257.

Church R.B. 1987. Embryo manipulation and gene transfer in domestic animals. *Tibtech* 5: 13-19.

Clark A.J.. Simons P., Wilmut I., Lathe R. 1987. Pharmaceuticals from transgenic livestock. *Tibtech 5: 21-25.*

Hammer R., Pursel V.G., Rexroad C.E.. Wall R.J., Bolt D.J.. Ebert K.M., Palmiter RD., Brinster R.L. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. *Nature 315:* 680-683.

Lacy E., Roberts S., Evan E.P., Burtenshaw M.D., Constantini F.D. 1983. A foreign β-globin gene in transgenic mice: integration at abnormal chromosomal positions and expression in inappropriate tissues. *Cell* 34: 343.358.

McKnight G.S., Hammer R.E.. Kuenzal E.A.. Brinstj R.L. 1983. Expression of the chicken transferrin gene in transgenic mice. Cell 34: 335-341.

Palmiter R.D., Brinster R.L., Hammer R.E., *Trumbauer* M.E., Rosenfeld M.G., Birnberg W.C., Evans R.M. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. Nature *300*: 611-615.

Palmiter R.O., Norstedt G., Gelinas R.E., Hammer R.E., Brinster R.L. 1983. Metallothioneine-human GH fusion genes stimulate growth of mice. Science 222: 807-814.

Reis P.J., Schinkel P.G. 1963. Some effects of sulphur containing amino acids on the growth and composition of wool *Aust. J. Biol. Sci. 16:* 218-230.

Wagner E.F., Cavarrubias L.. Stewart T.A.. Mintz B. 1983. Prenatal lethalities in mice homozygous for human growth hormone gene sequences integrated in the germ line. Cell 35: 647-655.