The application of transgenic techniques to common domestic animals and fish

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Introduction

In the early 1980's with the development of pioneering microinjection techniques in mammalian eggs (*Gordon et al.* 1980), the production of transgenic animals became possible. Since that time, there has been dramatic development in research leading to the manipulation of phenotype, genotype, and the reproductive features of animals. Without doubt, the possibility of introducing genes into the germ lines of various mammalian species has been one of the major scientific developments of the recent past.

The term, transgenic animal, is used to describe animals whose genome has been altered to include genes from foreign sources by methods than those used in traditional breeding. Usually the foreign gene is injected directly into the fertilized egg, is taken up by the egg, and is incorporated into one of the chromosomes in a random fashion.

The potential possibilities of this new technology on the improvement of conventional livestock have led to speculations about creating farm animals which will be better sources of food, give leaner meat, have enhanced growth, be resistant to diseases, and express favorable alterations in the quality and quantity of milk, wool, and egg composition. Such speculations have not, however, been realized thus far. In fact, it is now ten years after the first successful injection of the herpes virus containing the gene which codes for the enzyme, thymidine kinase, into a mouse egg (*Gordon et al.* 1980) and there are still monumental difficulties facing the development of useful transgenic livestock.

Published in "Food and New Biotechnology". Nordic report 18, 1991. (Nordic Council of Ministers and The Nordic Council).

Methods for developing transgenic animals

Until now, there are three reliable methods for producing transgenic animals. The most widely used method is the microinjection of a few hundred copies of the desired gene into the pronucleus of the fertilized egg (see review by *Jaenish* 1988). This method works very well in mice because the pronuclei are clearly visible and easily handled. Although only about 2.5 % of mouse eggs survive this treatment, this is not a major problem because of the availability of eggs. It is much more difficult in common farm animals because their fertilized eggs are opaque, making the pronuclei invisible. Less than 1 % of the eggs of farm animals survive microinjection (*Prusel et al.* 1989). Another disadvantage of this method is that it cannot be used to introduce genes into cells at later developmental stages.

Another method for the introduction of foreign genes into eggs employs retroviral vectors. These vectors use a retrovirus that has been made defective through the delection of critical genes. The foreign gene of choice is spliced into the vector, and this chimeric molecule is packaged as a viral particle and used to infect the egg. As such, the use of retroviral vectors provides the most efficient mode of introducing genes into the germline, since the chimeric retroviral genome integrates itself into the host genome through a precisely defined mechanism. Moreover, the use of these vectors is technically easy, and since the virus can infect a variety of cell types, it permits the introduction of the gene at various stages of development. It is also possible that one can combine the retroviral method with microinjection (*Wilmut et al.* 1988).

Table 1. Advantage and disadvantage of methods for the introduction of foreign genes into embryos (from ILAR News vol. 30, No. 3, 1988).

Method	Advantages	Disadvantages
Microinjection	Technical easy, high transgene expression. The combination of technical simplicity and expression makes it the most widely used technique.	Significant host DNA rearrangements, integration of large fragments.
Retrovirus infection	Technically easiest, single proviral DNA makes it well suited to cloning insertional mutations.	Low expression, limit to size of foreign gene insert.
Embryonic stem cells	Can select for expression or cellular mutation prior to production of animal.	Technically most difficult, germline transmission may may not occur.

The third method for the production of transgenic animals is through the use of embryonic stem cell injection. Embryonic stem cells from the blastocysts of a host are first established in culture during which time they retain their karyotype. For the production of transgenic animals, it is relatively easy to introduce genes of interest into the cultured stem cells, either by microinjection or by retroviral infection. Once the stem cell has taken up the foreign gene, they can then be injected into the host blastocyst where they colonize the embryo and contribute to the germ line of the resulting chimeric animal. Thus far, only a few laboratories have reported a successful germline contribution of such stem cells, but this technique is receiving increasing attention (*Church* 1987).
The various advantages and drawbacks of the three systems are summarized in Table 1.

Current status
Initial experiments involving the expression of rat and human growth hormone in transgenic mice were extremely successful. The genes were expressed at high levels, resulting in very large mice, and, in some instances, the expression was tissue-specific and under the control of an inducible promoter (*Hammer* 1985). The success in mice has not yet been repeated in other domestic animal species.

In the classic experiment on mice, high expression of a transgene coding for rat or human growth hormone could be obtained if the promotor sequence from a metallothionein gene was linked to the growth hormone coding sequence (*Palmiter et al.* 1982). The metallothionein promoter caused growth hormone expression when the animals were fed on a diet which contained zinc, an inducer of metallothionein. Large amounts of growth hormone were produced in the liver of the transgenic mice, since the metallothionein promoter is liver-specific, whereas growth hormone is normally produced in the pituitary. Due to the excessive amounts of growth hormone, the transgenic mice were much larger than normal. Moreover, they were able to pass the gene to their progeny through the germline.
Since this work in 1982, there has been a dramatic increase in experimentation which attempted to transfer genes into animals other than rodents (*Van Brunt* 1988).

Molecular farming
Transgenic techniques aim to alter the animal's genotype in an economically and biomedically advantageous way. There are already transgenic animals which produce pharmaceuticals (e.g. tissue plasminogen activator, coagulation factor IX and interleukin-2). It is possible to direct the synthesis of the new/foreign protein to the mammary

gland, and get it secreted into milk. For example, ten milking cows would be enough to cover the need of coagulation factor IX in the world. There is much promise in this area. However, several technical problems have to be solved before the promises become a reality.

A number of commercial interprises have a long-term goal to use eggs of transgenic chickens as production "factories" for human pharmaceuticals (see review *Van Brunt* 1988).

Transgenic pigs

Gene transfer experiments with pigs have been directed at developing transgenic pigs which give leaner meat and have increased body weight (*Prusel et al.* 1989). The microinjection of pig eggs is a difficult task due to the opacity of the fertilized egg. Therefore, the efficiency of microinjection is very low as evidenced by three years of studies during which only 8 % of 7.000 injected eggs developed to birth, and of those, only 7 % carried the transgene (*Prusel et al.* 1989). This integration efficiency of approximately 0.6 % does not compare favorably with an efficiency of 2.5–5.0 % seen in mice. The same low efficiency was reported for the introduction of a Moloney murine leukemia virus (MLV) which carried the growth hormone gene (*Vize* 1988). The transgenic pigs in these experiments survived embryogenesis and continued to grow and develop, and the levels of growth hormone increased as expected. However, this did not lead to a dramatic gain in body weight, and rather, the animals exhibited severe health problems such as peptic ulcers, pericarditis, and infertility which has made propagation of transgenic pigs impossible. Other pathological conditions included glomerulonephritis and degenerative joint diseases. The transgenic pigs grew faster in the early stages of their life and they were much leaner, but they died prematurely as a result of their numerous illnesses. So, although it was possible to stimulate the pigs' growth and enhance the conversion of food to protein, there are several health and economic and ethical problems related to the continuation of this research. Within the next decade, more basic research is needed in order to understand the complicated process of mammalian growth which is regulated by several hormones which act on the background of genes that are, as yet, unknown. Practical applications of transgenic technology to swine, such as improved meat and resistance to infectious diseases and parasites, are still several years in the future.

Transgenic chickens

The introduction of genes into the chicken is of interest both for research purposes and for use in the poultry industry where improved muscle growth, egg production, and disease resistance are the major interests. The production of transgenic chickens is hampered by the necessity of penetrating the egg shell and by the very small, and almost invisible, pronuclei which are the targets for the microinjection.

One group has succeeded in introducing foreign genes into the nuclei and obtained stable integration into the germline by the use of viral vectors injected through holes in the egg shell (*Bosselman et al.* 1989). A number of commercial enterprises devote their attention to the use of chicken eggs as production "factories" for human pharmaceuticals (see review *Van Brunt* 1988). However, these are admittedly long germ goals.

Transgenic cattle

As with pigs, there was initially a great deal of enthusiasm surrounding the first attempt to introduce the growth hormone gene into the fertilized egg of a cow (*Church* 1987). Unfortunately, only a few of these have survived beyond the embryonic stage, and as with pigs, there have been problems with understanding the molecular physiology of growth and with experiments leading to early death and adverse pathological effects.

Transgenic fish

The area of transgenic fish is one that has developed rapidly and has been met with success. There is much interest in transgenic fish due to the excellent nutritional value of fish, and several laboratories are actively involved in this type of research. The microinjection of foreign genes into fish egg nuclei is relatively straightforward and results thus far seem to be very promising. The rat growth hormone gene has been introduced with success into trout, salmon, and carp (*Maclean* 1987). The fish had incorporated the gene, and the mammalian growth hormone was expressed in the liver. The manipulation of the fish is much easier than in mammals (*J. E. Disney et al.* 1988), and since there seem to be no major ethical concerns, making transgenic fish is even more appealing. The production goals for making transgenic fish are: improved growth rate, altered temperature requirements, oxygen and salinity ranges, altered flavour and improved disease resistance. Since 1990, scientists from Auburn University have been conducting a promising study on genetically engineered carp raised in outdoor ponds (Dunham 1990). These carp contain a growth hormone gene from the trout. This additional growth hormone gene should make the carp grow faster, thereby reducing production costs. The experiments being conducted by the Auburn group will determine whether the new gene affects the reproductive capacity of brood carp and how it affects their survival, growth rate and behaviour. In laboratory experiments the trout growth hormone gene resulted in carp that were 20–40 % bigger. These experiments in carp are preliminary to experiments using catfish which are a multi-million dollar industry in the US. Since carp mature faster than catfish, it was reasonable to do the transgenic experiments on carp first. However, there seems to be no major difficulties in producing transgenic catfish that contain a trout growth hormone gene. Thus, it appears that within a few years, many transgenic fish species will make a significant contribution to the food market because they are easy to breed, have very desirable nutritional characteristics, and their development will be economical (*Powell et al.* 1990).

Concluding comments

The potentially powerful techniques of introducing foreign genes into animals in order to obtain beneficial traits have so far been less feasible than selective breeding. The capability to engineer complex multi-genic traits, such as milk production and meat composition does not exist yet. Preliminary results with single genes are promising, but it is clear that many difficulties must be overcome before multigenic traits can be introduced into farm animals. The promising results with mice induced a kind of frantic enthusiasm, and, although research in this area proceeds at a rapid pace, it is not realistic to assume the use of transgenic farm animals as a food source in the near future. As far as production of high quality protein the development of transgenic fish is of great interest. Lack of major technical and ethical obstacles in generating transgenic fish is a stimulating factor in this development. It will soon be likely that fish can be genetically engineered so that they are produced and used as a source of high quality food. They can also represent a way of supplying valuable protein to the developing countries who experience wet and dry seasons. Without doubt transgenic fish are the greatest hope for the future in regard to biotechnology of food.

While transgenic farm animals will not find a place in the marketplace within the next few years, their real value will be realized in the basic research laboratory. They are, and will continue to be, used in a wide variety of projects to study important fundamental questions regarding the regulation of gene expression and the regulation of development. They are being used to study problems that could only be dreamt of previously.

Thus far, the most notable achievements from the genetic engineering of animals is a

better understanding of critcal aspects of genetic control and physiology. There has been a stimulation of basic research due, in part; to the commercial promise of the techniques. As a tool for basic research, transgenic animals have been indispensible. There exist literally thousands of transgenic animals carrying a wide assortment of genes under the control of diverse genetic control elements, both natural and synthetic. Indeed, the first patent on an animal was awarded for a mouse carrying a foreign oncogene for the purpose of carcinogenicity testing (Nature: see Editorial *336:* 293, 1988).

The application of transgenic techniques to common domestic animals is, however, still in the early stages of research. Technical obstacles include the difficulties in retrieving embryos at a defined stage and their microinjection. Another obstacle is the expense in constructing transgenic livestock. The cost of generating a small number of transgenic sheep carrying a desired hybrid gene is approximately $ 3,000,000 over three years (*Clark et al.* 1987). With time, these costs will probably decrease substantially, but for the near future, they will not be competitive with the cost and succeess of selective breeding.

Transgenic animal production and patenting also introduces several ethical problems. There is strong opposition from organizations which believe that through the creation of transgenic animals scientists are expanding a type of "genetic parasitism" on animals. These organisations find that these types of research are inacceptable and demand a cessation.

Thus, although the preliminary data and ideas for the future seem to be attractive in the area of transgenic animals, it is likely that it will take several years, possibly even decades, before transgenic animals – with the probable exception of fish – can be regarded as a routine source of commercial food.

At this stage in the development and utilization of transgenic animals in the food supply, assessments can be only made on a case by case basis.

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