TRANSGENIC ANIMALS APPLIED TO LIVESTOCK PRODUCTION

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SUMMARY

Gene transfer techniques were established in domestic livestock during the latter half of the 1980's but transgenic animals have yet to be fully exploited in agriculture. The work during the last five years has served to focus attention on a number of technical and scientific problems that will form the basis for future research.

As early as 1974, Jaenisch and Mintz demonstrated the feasibility of producing transgenic animals by the injection of SV40 DNA into the blastocoel cavity of early mouse embryos. During the 1980's the generation of transgenic mice by direct microinjection of DNA into a pronucleus of the fertilised egg became a routine procedure in many laboratories (for review see Jaenisch, 1988). Transgenic mice have enhanced our understanding of many basic areas of biology including gene expression, development, immunology and cancer. These advances stemmed from the ability to introduce new genes into the germline; more recently, techniques enabling the specific manipulation of endogenous genes by the use of embryonal stem (ES) cells (Evans and Kaufman, 1981) and homologous recombination (Capecchi, 1989) have been developed, and will provide a further impetus to these studies.

Success in mice prompted similar experiments in farm animals; the first paper describing the production of transgenic livestock by pronuclear injection was published in 1985 by Hammer *et al*. Since then there have been relatively few reports describing transgenic livestock. By the end of the 1980's the introduction of only sixteen genes had been reported for the three major livestock species (Pursel *et al*. 1989). This slow progress is partly a result of the time-scale and expense of carrying out such experiments on large animals. Furthermore, the resources required are far in excess of those needed to make transgenic mice and this limits the number of laboratories with the necessary capabilities. Gene transfer in domestic livestock is also complicated by the relative opacity and fragility of the eggs, necessitating particular patience and care in handling. It is perhaps not surprising that the efficiency of producing transgenic animals by microinjection remains relatively low (on average about 1-2 % of the injected and transferred eggs will develop into transgenic animals).

Many of the gene transfer experiments carried out with farm animals during the 1980's attempted to manipulate growth and related characteristics such as feed efficiency. Constructs encoding growth regulating genes such as growth hormone or growth hormone releasing factor were introduced. This direction came from earlier experiments with transgenic mice published by Richard Palmiter and Ralph Brinster and their colleagues (e.g. Palmiter *et al*. 1982). These workers described transgenic mice carrying metallothionein-growth hormone constructs that expressed high levels of foreign growth hormone in their blood. They had an accelerated growth rate and, in some cases, grew to nearly double the size of their non-transgenic littermates. However, these dramatic effects on growth have not been realised with transgenic livestock. For example, only small increase in growth rate (~15%) were observed in transgenic pigs carrying

bovine growth hormone and then only when the protein content of the diet was enhanced (Pursel et al. 1989). In fact, the chronic expression of growth hormone in these animals gave rise to a number of disease conditions including lameness, uncoordinated gait, exopthalamus and infertility. Similar results have been observed with transgenic sheep carrying a growth hormone gene (Nancarrow et al. 1988).

Other approaches to improving animal production traits have been taken. The normally-essential amino acid cysteine is limiting in wool production and research groups are actively engaged in producing transgenic sheep that carry the two bacterial genes which would enable the conversion of serine to cysteine (Ward et al. 1986). The project will ultimately require targeting of the expression of these genes to the rumen where both serine and sulphur are readily available.

Genetically engineering farm animals to withstand infectious diseases or parasites is an attractive option, and has a clear parallel to work that has been extremely successful in transgenic plants. Nevertheless, it is still not clear what the best strategies are for accomplishing this goal. Producing animals that express a specific immunoglobulin for a particular antigen has been accomplished in mice (Grosschedl et al. 1984). However, it remains uncertain whether this approach would be effective in the field, considering that immunoglobulin transgenes inhibit the functioning of endogenous immunoglobulin genes and, also, that a pathogen would, sooner or later, adapt to evade a single immunoglobulin. Other approaches are being tried, including the introduction of genes encoding anti-viral agents such as interferons or Mx (Brem et al. 1988)

Another application of transgenic livestock, developed during the 1980's, is for the production of valuable biomedical proteins (Clark et al. 1987). There is, to date, one report of transgenic farm animals expressing such proteins. (Clark et al. 1989). In this work the expression of human factor IX and human α_1-antitrypsin were targeted to the mammary gland using sequences derived a milk protein gene, β-lactoglobulin. The level of expression of the foreign protein in milk was low and insufficient for commercial exploitation. However, additional work using transgenic mice as a model system has demonstrated the feasibility of the approach and transgenic animals expressing very high levels of human α_1-antitrypsin in the milk have been produced (Archibald et al. submitted).

The latter half of the 1980's saw transgenic techniques established in livestock. However, there is clearly some way to go before genetically engineered farm animals are exploited fully in agriculture and biotechnology. The work over the last five years has served to focus attention on a number of technical and scientific limitations which will be the subject of research well into the 1990's.

A major technical limitation in working with both sheep and cattle is the limited supply of eggs for gene transfer. Recently, procedures have been described for the maturation and fertilisation of sheep and cattle oocytes recovered from the ovaries of slaughtered animals (Lu et al. 1987). While this procedure has the potential to provide large numbers of fertilised eggs, considerable research is required to maximise the number of eggs that have the potential for normal development.

The efficiency of producing transgenic animals would be dramatically increased if injected eggs could be screened for their transgenic status before implantation. The polymerase chain reaction will allow the detection of a gene present as a single copy in a single cell biopsied from an embryo (Saiki et al. 1988). The success of this approach will depend on the proportion of

injected eggs which develop in culture, the survival rate following biopsy and the ability to discriminate between integrated and non-integrated DNA.

The isolation of ES cells from farm animals would provide an alternative means of achieving gene transfer. Although the subject of active research in many laboratories they have yet to be described for livestock species. A major advantage of ES cells is that clones of cells can be selected for their transgenic status in culture, before they are used to colonise a recipient embryo into which they are injected. However, the transgenic young that are born will be chimeric, and, in farm animals, this would cause a delay of several years before introduction into the germline. A solution to this delay may be direct nuclear transfer from ES cells in culture to enucleated eggs or oocytes (Wilmut et al. 1990). In this way several genetically identical transgenic animals could be made and the chimeric generation avoided. Finally, the development of ES cells and homologous recombination will enable precise, targeted changes, such as specific gene deletion or gene replacement to be accomplished.

Our understanding of the control of gene expression in animals advanced dramatically during the 1980's. The interaction of *cis* and *trans* regulatory elements has been described for the promoter regions of many genes. A variety of transcription factors and, in some cases, the genes that encode them have now been isolated and characterised. However, these advances have not provided all the information that is necessary for making effective constructs for gene transfer. For example, a problem routinely encountered with transgenic animals is the variability of expression of the same transgene when compared from one transgenic line to the next - the "position effect", thought to be due to the effect of sequences at the site of integration of the transgene. It has been shown that some genes, (most notably the globin genes) are linked to DNA sequences which overcome the position effect and, if these regions of DNA are incorporated into the transferred DNA, then the transgene will work in a reliable, position independent, manner (Grosveld et al. 1987). It is not entirely clear, yet, how such dominant control regions function. They may mediate interactions between the gene and structural elements within the nucleus ensuring that the relevant promoters and enhancers are accessible to *trans* acting factors. Certainly, such possible interactions with nuclear structures have important implications for transgene design (Stief et al. 1989).

Much of the transgenic research in the 1980's exploited regulatory elements derived from animal genes to target the expression of transgenes. In the future, transgenic approaches may make use of entirely heterologous regulatory elements so that a particular transgene can be regulated externally and independently of the host genes. It is tempting to speculate that elements from insects, plants or even bacteria could be used to generate specific "on/off" genetic switches to regulate genes in transgenic animals.

At present, the genetic engineering of domestic livestock is limited to the addition of extra genes to the germline. In terms of animal improvement, there are a number of examples where a genetic gain could be accomplished by the selective inactivation of a specific gene. The development of ES cells and gene targeting to accomplish gene deletion or gene replacement is an obvious research goal for the 1990's. There are, however, other strategies that may be appropriate in certain circumstances. In the 1980's the use anti-sense RNA transcripts was advanced as a possible means for specifically inactivating gene expression. However, this approach, so far, has failed to live up to its earlier promise and there are very few reports describing its successful application in transgenic animals. In the 1990's 'ribozymes' (Haseloff and Gerlach, 1988), RNAs which are capable of catalytically cleaving other RNA molecules at predicted sites may succeed where anti-sense RNA has failed. The approach may be particularly useful for targeting the inactivation of gene function of infective agents such as viruses, conferring a form of "molecular immunity".

Clearly, a goal for the 1990's is to increase the efficiency and the precision of germline manipulation. However, for many production traits it is still far from clear how a particular genetic gain could be accomplished, even with an increasingly sophisticated transgenic technology. For example, the adverse effects seen in transgenic livestock carrying growth hormone genes are due to chronic high levels of expression throughout the life of the animals. But, exactly what pattern of growth hormone expression would enhance growth rate and feed efficiency without adverse consequences; should (say) transgene expression be pulsatile and limited to a particular stage(s) of the animals' life? There are large gaps in basic scientific knowledge, particularly at the interface between molecular biology and physiology which research in the 1990's will address and which will provide essential information in the choice and design of transgenes.

In conclusion, although gene transfer has been established in livestock, progress has been limited by a number of technical and scientific constraints. Many of these limitations have been identified and they are the subject of active research which will enable the full exploitation of this very powerful technology within the forseeable future.

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