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VIEWPOINT

Transgenic Animals: "Great and Small"

Technology is currently available to create transgenic livestock animals, opening up the possibility for their commercial use. One obvious direction this technology could take is to improve the quality of livestock animals. Another possibility is to consider transgenic livestock as "bioreactors," producing pharmaceutically useful proteins in the milk, blood, or urine. The use of transgenic livestock as models for human disease could also be of advantage to the biomedical community. The development of transgenic livestock technologies for commercial purposes, however, has been relatively slow. The factors responsible for this are discussed in the Prospect article by Wall et al. Limiting factors include high animal costs, the small number and low viability of embryos, the limited understanding of embryo biochemistry and physiology, low transgene integration rates, and the lack of embryonic stem cells for any of the livestock species. It appears that the potential of transgenic farm animals has not yet been explored in depth. For example, in the area of human disease models little has been done with large animals, while intensive research has been conducted with transgenic mice. Encouraging the discussion about potential uses for transgenic livestock animals can lead to increased interest and funding for this research. In this way, progress in this area can be expedited.

The Prospect article by Clark explores the potential of transgenic farm animals concerning the genetic engineering of milk. Altering the composition of livestock milk is an exciting idea with many commercial applications, and the manipulation of genes encoding major milk proteins is a realistic goal. However, many of the actual details of such manipulations remain to be developed. Today, it is not yet possible to predict with certainty the consequences of changes in the structure of milk proteins on the biophysical and biochemical properties of milk. Cell lines that could be used to test different mutations of milk protein genes are still unavailable. A variety of milk protein genes have been expressed in mice, but transgenic mice can only provide partial answers to these questions because of insufficient characterization of milk from this species.

A fundamental understanding of the regulation patterns of milk gene expression has been considered to be a requirement for predictable genetic manipulation of the mammary gland for the production of heterologous proteins. The article by Hennighausen, which will appear in a future issue, describes recent achievements in this area. Although we now better understand the hormonal and developmental regulation of mammary specific genes, practically, it is difficult to predict with any certainty the high level expression of transgenes. Analysis of hybrid genes in transgenic mice is tedious and results obtained may not reflect the expression levels in livestock animals. This has been observed in the production of recombinant human protein C in transgenic milk. The hybrid gene conferred very low expression in transgenic mice, but lead to a 100–200-fold higher level of expression in the mammary gland of the pig.

Despite our fragmented understanding of the regulation of milk gene expression, there have been several successes in the hyper-expression of recombinant proteins in transgenic livestock mammary glands. Examining goats transgenic for human tissue plasminogen activator, sheep transgenic for human α 1-antitrypsin or human factor IX and pigs transgenic for human protein C will allow us to answer a fundamental question concerning the mammary gland: is it capable of carrying out the appropriate post-translational modifications of recombinant human proteins? A number of studies report low levels of secretion of recombinant human proteins into the milk of transgenic mice. Consequently, the proteins were not purified and neither were detailed studies of post-translational modification carried out. Several human proteins with therapeutic applications, such as the human vitamin K-dependent plasma proteins, are synthesized in the form of large precursors, which undergo sequential steps of post-translational processing during their intracellular translocation. The cellular machinery required for a wide variety of post-translational steps which are nec-

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essary for the appropriate maturation and bioloical activity of a protein may be saturated as the expression of the heterologous protein increases. Ongoing characterization of human recombinant proteins purified from the milk of transgenic livestock will be critical to our understanding of the factors required at each post-translational step which may limit production of heterologous protein in the mammary gland. We already know that the glycosylation of heterologous proteins synthesized in the mammary gland of goats, sheep, pigs, and mice differ from that of the human protein. However, it is less clear whether these differences will affect pharmacological efficacy.

Requiring mammary epithelial cells to synthesize heterologous proteins is not the final step in the production of proteins for clinical use. It is also necessary to be able to separate the recombinant human proteins from animal proteins present in the milk. If it is possible to express the recombinant protein at high levels it may be feasible to use more classical methods of purification rather than depend upon immunoaffinity chromatography, usually applied to the purification of proteins found in human plasma at low concentrations. It probably will be difficult to develop a generic purification scheme for the isolation of recombinant proteins from milk. Species differences in the composition of milk proteins could pose another problem in the development of general purification methods for recombinant proteins expressed in the mammary gland. The Prospect article by Wilkins and Velander, which will appear in a future issue, addresses many of these issues in detail.

This series of Prospect articles has focused on the development of transgenic livestock. To date most heterologous genes have been expressed in the mammary gland. However, another important application of the expression of foreign genes in the mammary gland of transgenic animals has been developed. The article by Groner describes the development of transgenic animals models to study the effects of oncogene expression on mammary tumorigenesis. As discussed in this article, advances have already been made in the identification of several oncogenes whose amplification or loss of heterozygosity is associated with either metastatic disease, shortened survival periods after relapse, or very aggressive tumor development. Animals transgenic for an oncogene, or combinations of oncogenes, may prove useful in defining methods of treatment for the disease initiated and maintained by the expression of such genes.

Finally, it is obvious that the development of embryonic stem cell technology for livestock, reproducible expression of transgenes, high efficiency of transgene integration, and the predictable manipulation of transgenic livestock physiology still need significant development. Many investigators (as well as venture capitalists) do not want to wait for these developments to evolve, but instead prefer to take some calculated risks, assuming that by so doing, valuable transgenic livestock may be produced. It is fairly safe to predict that the future will see more transgenic animals on the ground, as major advances are made in our fundamental understanding and ability to generate transgenic animals.

William N. Drohan
Holland Laboratory
American Red Cross
Rockville, MD 20855