Transgenic Animals

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The term transgenic animal refers to an animal in which there has been a deliberate modification of the genome - the material responsible for inherited characteristics - in contrast to spontaneous mutation (FELASA September 1992, revised February 1995). Foreign DNA is introduced into the animal, using recombinant DNA technology, and then must be transmitted through the germ line so that every cell, including germ cells, of the animal contain the same modified genetic material.

1. Historical background

Prior to the development of molecular genetics, the only way of studying the regulation and function of mammalian genes was through the observation of inherited characteristics or spontaneous mutations. Long before Mendel and any molecular genetic knowledge, selective breeding was a common practice among farmers for the enhancement of chosen traits, e.g., increased milk production.

During the 1970s, the first chimeric mice were produced (Brinster, 1974). The cells of two different embryos of different strains were combined together at an early stage of development (eight cells) to form a single embryo that subsequently developed into a chimeric adult, exhibiting characteristics of each strain.

The mutual contributions of developmental biology and genetic engineering permitted rapid development of the techniques for the creation of transgenic animals. DNA microinjection, the first technique to prove successful in mammals, was first applied to mice (Gordon and Ruddle, 1981) and then to various other species such as rats, rabbits, sheep, pigs, birds, and fish. Two other main techniques were then developed: those of retrovirus-mediated transgenesis (Jaenisch, 1976) and embryonic stem (ES) cell-mediated gene transfer (Gossler et al., 1986).

Since 1981, when the term transgenic was first used by J.W. Gordon and F.H. Ruddle (1981), there has been rapid development in the use of genetically engineered animals as investigators have found an increasing number of applications for the technology.

2. Methods of creation of transgenic animals

For practical reasons, i.e., their small size and low cost of housing in comparison to that for larger vertebrates, their short generation time, and their fairly well defined genetics, mice have become the main species used in the field of transgenics.

The three principal methods used for the creation of transgenic animals are DNA microinjection, embryonic stem cell-mediated gene transfer and retrovirus-mediated gene transfer.

a) DNA microinjection.

This method involves the direct microinjection of a chosen gene construct (a single gene or a combination of genes) from another member of the same species or from a different species, into the pronucleus of a fertilized ovum. It is one of the first methods that proved to be effective in mammals (Gordon and Ruddle, 1981). The introduced DNA may lead to the over- or under-expression of certain genes or to the expression of genes entirely new to the animal species. The insertion of DNA is, however, a random process, and there is a high probability that the introduced gene will not insert itself into a site on the host DNA that will permit its expression. The manipulated fertilized ovum is transferred into the oviduct of a recipient female, or foster mother that has been induced to act as a recipient by mating with a vasectomized male.

A major advantage of this method is its applicability to a wide variety of species.

b) Embryonic stem cell-mediated gene transfer.

This method involves prior insertion of the desired DNA sequence by homologous recombination into an in vitro culture of embryonic stem (ES) cells. Stem cells are undifferentiated cells that have the potential to differentiate into any type of cell (somatic and germ cells) and therefore to give rise to a complete organism. These cells are then incorporated into an embryo at the blastocyst stage of development. The result is a chimeric animal. ES cell-mediated gene transfer is the method of choice for gene inactivation, the so-called knock-out method.

This technique is of particular importance for the study of the genetic control of developmental processes. This technique works particularly well in mice. It has the advantage of allowing precise targeting of defined mutations in the gene via homologous recombination.

c) Retrovirus-mediated gene transfer.

To increase the probability of expression, gene transfer is mediated by means of a carrier or vector, generally a virus or a plasmid. Retroviruses are commonly used as vectors to transfer genetic material into the cell, taking advantage of their ability to infect host cells in this way. Offspring derived from this method are chimeric, i.e., not all cells carry the retrovirus. Transmission of the transgene is possible only if the retrovirus integrates into some of the germ cells.

For any of these techniques the success rate in terms of live birth of animals containing the transgene is extremely low. Providing that the genetic manipulation does not lead to abortion, the result is a first generation (F1) of animals that need to be tested for the

expression of the transgene. Depending on the technique used, the F1 generation may result in chimeras. When the transgene has integrated into the germ cells, the so-called germ line chimeras are then inbred for 10 to 20 generations until homozygous transgenic animals are obtained and the transgene is present in every cell. At this stage embryos carrying the transgene can be frozen and stored for subsequent implantation.

Transgenic Animals as Biotechnology

Transgenic animals are just one in a series of developments in the area of biotechnology. Biotechnology has transformed the way in which we understand processes such as engineering and manufacturing. These terms now include the use of living organisms or their parts to make or modify products, to change the characteristics of plants or animals, or to develop micro-organisms for specific uses. The novel uses of biological techniques such as recombinant DNA techniques, cell fusion techniques, mono and polyclonal antibody technology and biological processes for commercial production have altered traditional distinctions and methods (US Congress, Office of Technology Assessment, 1989). Genetic manipulations at the level of DNA have also changed long held views as to what is considered to be animal, plant and human. In turn, these changes have made it more difficult to evaluate the ways in which animals are used and have obscured distinctions between pure and applied research.

Consideration of the acceptability of creating specific transgenic animal strains or genetic manipulation involving interchanging DNA between species and kingdoms could be a simple animal care issue or a societal decision. The following is an attempt to show what the ability to create transgenic animals or engage in other forms of DNA manipulation means in terms of traditional ACC functions, not forgetting that this impacts on wider considerations of human responsibility for the welfare of other life forms.

The creation of transgenic animals is resulting in a shift from the use of higher order species to lower order species, and is also affecting the numbers of animals used. This shift in the patterns of animal use is being monitored by the CCAC through the use of the Animal Use Data Form.

An example of the replacement of higher species by lower species is the possibility to develop disease models in mice rather than using dogs or non-human primates.

In the long term, a reduction in the number of animals used, for example to study human diseases, is possible due to a greater specificity of the transgenic models developed. On the other hand, the success of the method has led to using its potential for investigating a wider range of diseases and conditions. The actual use of some species may be increased, in addition to the numbers of animals which are sacrificed as donors during the creation process. The potential of the technology has also made it possible to consider employing cattle, swine, sheep and goats as processing units to manufacture proteins or as organ donors.

The complex interactive processes of living mammals are not reproducible in vitro. However, transgenic animals provide a means of evaluating genetic modifications in terms of anatomical and physiological changes in a complex system. Transgenic models are more precise in comparison to traditional animal models, for example the oncomouse with its increased susceptibility to tumor development enables results for carcinogenicity studies to be obtained within a shorter time-frame, thus reducing the course of tumor development in experimentally affected animals. However, models are not strict equivalents, so as with any other system care must be taken in drawing conclusions from the data.

A representative, but non-inclusive, list of purposes for which transgenic animals have been used indicates the wide ranging application of this biotechnology:

- in medical research, transgenic animals are used to identify the functions of specific factors in complex homeostatic systems through over- or under-expression of a modified gene (the inserted transgene);
- in toxicology: as responsive test animals (detection of toxicants);
- in mammalian developmental genetics;
- in molecular biology, the analysis of the regulation of gene expression makes use of the evaluation of a specific genetic change at the level of the whole animal;
- in the pharmaceutical industry, targeted production of pharmaceutical proteins, drug production and product efficacy testing;
- in biotechnology: as producers of specific proteins;
- genetically engineered hormones to increase milk yield, meat production; genetic engineering of livestock and in aquaculture affecting modification of animal physiology and/or anatomy; cloning procedures to reproduce specific blood lines; and
- developing animals specially created for use in xenografting.

Important general considerations include the extent to which experience acquired in the laboratory with regard to husbandry should influence industry standards for keeping animals created specifically as living machines for the production of proteins, antibodies, etc. What words are appropriate to describe and evaluate the condition of animals now used as production units? The successful cloning of Dolly underlines the fact that innovative developments in animal science are part of the mainstream of biotechnology. In addition, the use of xenografts, at least at the public health level makes animal and human welfare inseparable.