

LASA POSITION PAPER

Transgenics

Introduction

Organisms whose genome has been modified by the artificial introduction of foreign DNA are termed “transgenic”. Transgenic mammals have been produced by either of two methods. Firstly, and most commonly, foreign DNA is injected into one of the two pronuclei of fertilised eggs. Alternatively, and with increasing frequency, DNA is introduced into cultured stem cells, so-called “embryonic stem” (ES) cells, derived from the early embryo. So far, ES cells that are able to form sperm or eggs (when introduced into an embryo) following in vitro genetic modification have only been obtained from mice, despite considerable effort to obtain them in other species. However, there is now the further prospect, in other mammals, of using transplantation into enucleated eggs of nuclei from other types of cell that have taken up foreign DNA in culture (“cloning”).

An important advantage of introducing foreign DNA into organisms via cultured cells rather than directly via pronuclei of fertilised eggs is that it is possible to verify whether the desired genetic change has been achieved before any animals are produced. Pronuclear injection affords no control over whether or where the introduced DNA integrates into that of the host so that many offspring may have to be produced in order to obtain one that exhibits the desired genetic change. Hence, the ES cell (or other cultured cell) approach to transgenesis is inherently more economical in terms of animal use and should therefore, wherever practicable, be the method of choice. Additional routes whereby foreign DNA can be introduced into mammals are also being explored, but none has yet reached a stage where evaluation is possible.

Potential Costs of Transgenesis

The production and use of transgenic animals can compromise animal welfare in several ways:

1. *Suffering due to phenotype* - Transgenic animals are used to simulate a wide range of human genetic diseases or developmental or functional gene abnormalities. Some of these animal models may suffer pain and distress
2. *Suffering during production* - Animal welfare can be compromised due to the techniques (vasectomisation, superovulation, embryo harvesting and embryo transfer) used in genetic manipulation
3. *Random integration* - Random integration of genes can compromise welfare of foetuses and adults:
 - a) Foetal and post-natal death - The random integration of a transgene can result in a significant level of foetal and post-natal deaths. It is uncertain at what stage in development foetuses can experience pain and distress, or how far the welfare of the mother is compromised by foetal death. However post-natal death is of greater concern.

- b) Unpredictable results in adults - In some cases, insertion of the gene into an inappropriate place can lead to animals suffering due to deformities, disease and organ failure.
- 4. *Increased production and use of transgenic animals* - The number of animals used for transgenesis has increased significantly over recent years. It seems highly likely that this trend will continue. As with all experiments involving the use of animals, it is important that, from the very beginning, each transgenic experiment has a definable benefit.

Potential Benefits of Transgenesis

1. *Increased specificity* - Traditional methods (e.g. radiation or chemical) of genetic mutagenesis are low in specificity and random in nature. Transgenic technology can induce specific genetic modifications that overexpress or inhibit the activities of single genes. Use of selected techniques may provide very high specificity, including the ability to influence tissue and temporal aspects of gene expression through use of targeting vectors, promoters and inducers.
2. *Opportunities for Reduction* - Genetically modified characteristics may enhance the response of animals in some experimental procedures, such as carcinogenicity screening. Such increased sensitivity in detecting biological effects has the potential for reductions in numbers of animals used and in the time to complete studies.
3. *Opportunities for Refinement* - Genetically manipulated animals can provide disease models characterised by specific and relevant mechanisms to the process under investigation (e.g. cystic fibrosis, hypertension). Transgenic disease models offer more similarity and relevance than many classical models (which often involve surgical modification of animals). The expression of the human poliovirus receptor in transgenic mice has permitted the replacement of primates as the susceptible laboratory animal model for vaccine testing.
4. *Biotechnology* - The ability to express foreign proteins, especially complex structures of high molecular weight, allows valuable pharmaceutical materials to be produced by higher mammals that are beyond the synthetic capacity of yeasts or bacteria. Genetic modification also offers tissues for xenotransplantation that may benefit patients where organs from human donation are in very short supply.

Recommendations regarding Good Practice for Transgenesis

1. *Experimental considerations* - Attention should be paid to the design of constructs for transgenesis so as to maximise the likelihood of obtaining the desired outcome before embarking on the production of transgenic animals. For gene-targeting studies via ES cells, where the aim is to replace a native gene by an altered version, this entails making use of available knowledge relating to the structure of the relevant gene and its relatives, and any structure-function analyses of its protein product. In addition, information on the normal temporal and spatial pattern of expression of genes may indicate when in development the adverse effects of their disruption might be anticipated.

Where the aim is to obtain expression of foreign genes, design of constructs should take into account inclusion of features that have proved beneficial in previous studies. Where possible, DNA constructs for use in transgenesis should be validated in cultured cells before being introduced into developing organisms. When breeding transgenic animals, the possible dependence on genetic background of the occurrence or severity of a mutant phenotype, and whether it is manifest in heterozygotes, must be considered.

2. *Laboratory animal science considerations -*

- a) Home Office Standard Section 19b's. Based on protocols written by a major transgenic Institute, the Home Office has now produced standard 19b's for each stage of the transgenic process. Project Licence applicants should be encouraged to use these when they are preparing their applications.
- b) Well-Designed Humane Endpoints. One way of minimising animal welfare problems is to design experimental endpoints that are clear and objectively measurable and which can be recognised well in advance of significant deterioration in an animal's condition. It is not always possible to identify such so-called "humane endpoints" but, wherever possible, they should be specified in paragraph (vi) of Section 19b.
- c) Procedural and Identification Methodologies. Donor females should be the maximum age that is compatible with a good physiological response to the superovulation protocol. Small, young donor females should not be paired with large and aggressive stud males. Consideration should always be given, in the case of procedures that involve surgical intervention, to whether there is a requirement for post-operative analgesia. Transgenic animals should be identified using a method that causes minimum discomfort.
- d) Tissue Typing Methodology. In the mouse, the standard tissue to be biopsied (under anaesthesia) for DNA analysis is the cartilaginous tip of the tail. The quantity taken can be minimised if PCR is the analytical methodology used. It is advisable to freeze part of the sample in order to be able to repeat the DNA analysis without having to take another biopsy. Alternative sources of biological material for DNA analysis include tissue from ear punches, blood and cells from the lining of the mouth.
- e) Need for Closer Observation. The unpredictable nature of transgenesis means that newly generated animals should always be observed closely for unexpected phenotypes.
- f) Embryo and Gamete Cryopreservation. The need to maintain small tick over colonies of transgenic animals can be avoided through the cryopreservation of embryos and gametes (such as sperm).

Need for Further Research and Development

There are five main areas where further research and development into the methodologies used in transgenesis would be of great value:

1. *Improvements in the efficacy of pronuclear microinjection and gene targeting*
2. *The transfer of ES technology to species other than the mouse*
3. *Alternative techniques for the incorporation of foreign DNA into the host animal's genome- e.g. electroporation of mammalian embryos*
4. *Further refinement of the cloning technologies*
5. *Improvements to in vitro fertilisation and artificial insemination in the mouse.*

Summary

Transgenesis is a powerful scientific research technology that has enabled considerable progress to be made, in many challenging areas of biomedical research, relatively quickly. It is, by its very nature, heavily dependent on the use of animals. This means that good laboratory animal science has a key role in ensuring that the cost benefit balance is as it should be.

All those involved in this research methodology should make every effort to comply with the principles of good practice that have been outlined above. These principles will require updating as existing techniques are changed in the light of experience and as new techniques are developed.

