Lecture 35

Transgenic Animals

Introduction

Transgenic animals are animals (most commonly mice) that have had a foreign gene deliberately inserted into their genome. Such animals are most commonly created by the micro-injection of DNA into the pronuclei of a fertilized egg which is subsequently implanted into the oviduct of a pseudopregnant surrogate mother. This results in the recipient animal giving birth to genetically modified offspring. The progeny are then bred with other transgenic offspring to establish a transgenic line. Transgenic animals can also be created by inserting DNA into embryonic stem cells which are then micro-injected into an embryo which has developed for five or six days after fertilization, or infecting an embryo with viruses that carry a DNA of interest. This final method is commonly used to manipulate a single gene; in most cases this involves removing or 'knocking out' a target gene. The end result is what is known as a ‘knockout’ animal.

Transgenic Techniques

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.

1. Embryonic stem cell-mediated gene transfer

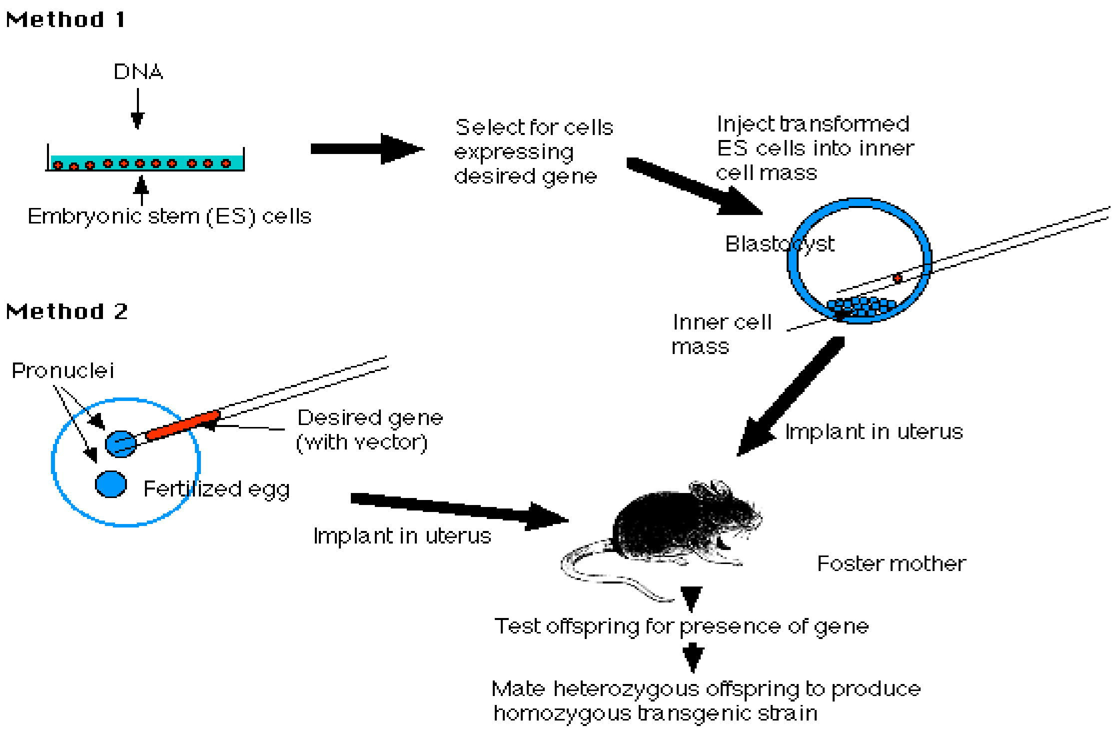
in this technique, the foreign DNA is inserted into the embryonic stem cells culture in in vitro environment by homologous recombination. Stem cells are the cells other than the sex cells which have the ability to differentiate into different types of cells giving rise to a complete organism. If the study of the genetic control is concerned then this technique is of significance and it is best used mice. It is also able to target the mutations in the genes by homologous recombination.

2. DNA Microinjection

DNA microinjection is concerned with the insertion of a single gene or group of gene into the pronucleus of a fertilized ovum. The genes of interest are taken either from the same species or from different species. This method is best used in mammals where it introduces new DNA. The introduction of new DNA may lead to the expression of either certain genes within the mammal or the expression of those genes which are newly added into the animal's genome. There is also this possibility that the gene inserted may not reach the desired site in the genome. The genetically modified ovum is then transferred into the recipient female. This method can be applied in various species.

3. Retrovirus-mediated gene transfer

Sometimes the new genes are added into the genome of the animals to increase the expression. To insert new genes, retroviruses are used as vectors which are responsible for taking the genetic material into the organism's genome. The genes inserted through this method are not expressed in all the cells. If the retrovirus succeeds to enter the germ cells of the organism, it is possible that the transgenes will be expressed in all the cells in the next generation.



Applications of Transgenic Animals

1) Can use gene transfer to improve the productivity of livestock.
2) Introduce genes for faster growth rates or leaner growth patterns.
3) A model substituting the normal gene for hemoglobin to replace sickle cell anemia gene.

Genetic Engineering

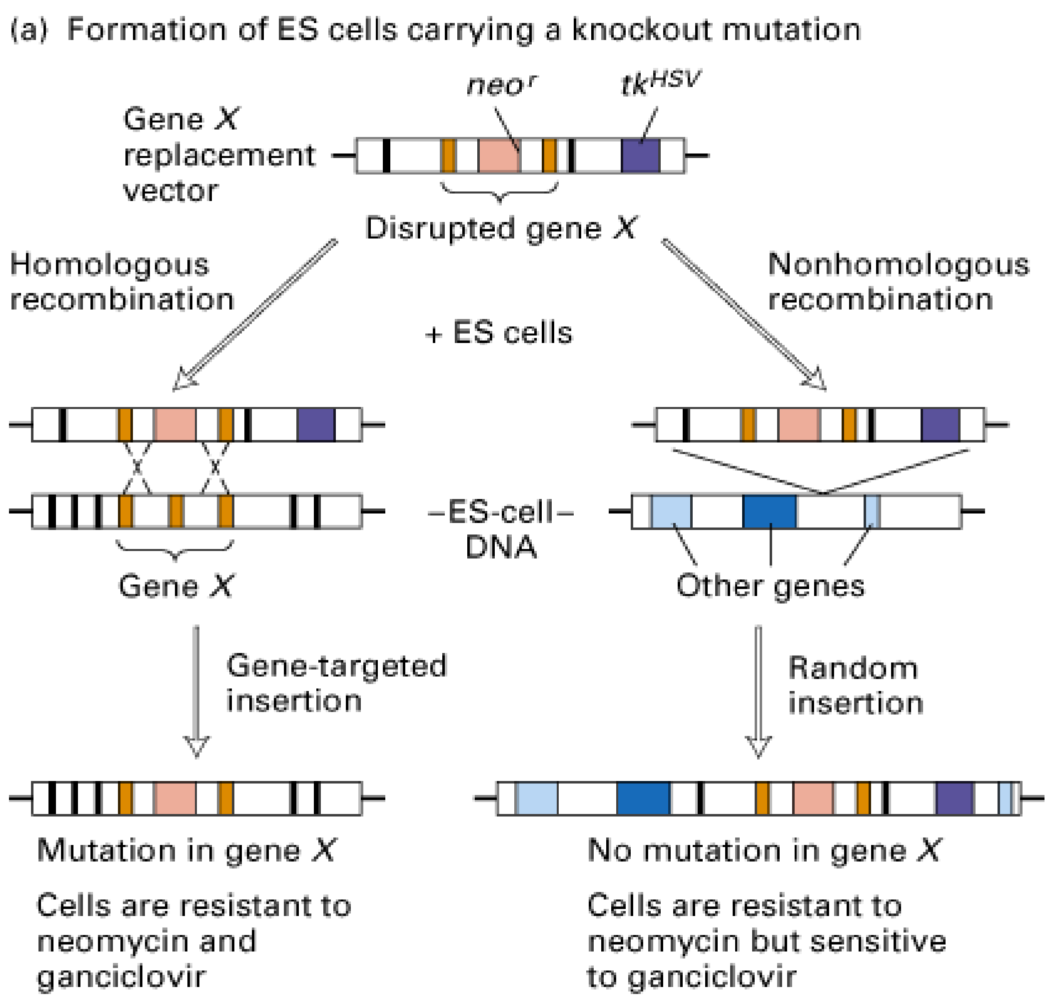
Genetic engineering alters the genetic make-up of an organism using techniques that remove heritable material or that introduces DNA prepared outside the organism either directly into the host or into a cell that is then fused or hybridized with the host.

Process

1) The first step is to choose and isolate the gene that will be inserted into the genetically modified organism. The gene can be isolated using restriction enzymes to cut DNA into fragments and gel electrophoresis to separate them out according to length. Polymerase chain reaction (PCR) can also be used to amplify up a gene segment, which can then be isolated through gel electrophoresis. If the chosen gene or the donor organism's genome has been well studied it may be present in a genetic library. If the DNA sequence is known, but no copies of the gene are available, it can be artificially synthesized.

2) The gene to be inserted into the genetically modified organism must be combined with other genetic elements in order for it to work properly. The gene can also be modified at this stage for better expression or effectiveness. As well as the gene to be inserted most constructs contain a promoter and terminator region as well as a selectable marker gene. The promoter region initiates transcription of the gene and can be used to control the location and level of gene expression, while the terminator region ends transcription.

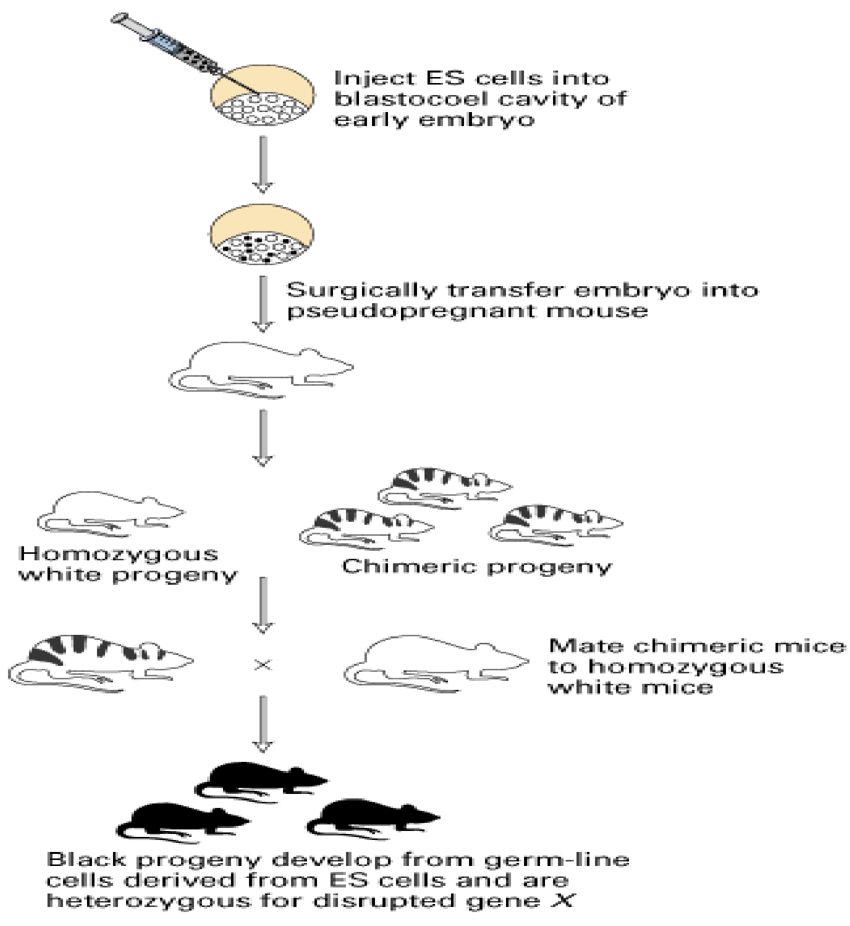
3) The most common form of genetic engineering involves inserting new genetic material randomly within the host genome. Other techniques allow new genetic material to be inserted at a specific location in the host genome or generate mutations at desired genomic loci capable of knocking out endogenous genes. The technique of gene targeting uses homologous recombination to target desired changes to a specific endogenous gene. This tends to occur at a relatively low frequency in plants and animals and generally requires the use of selectable markers. In addition to enhancing gene targeting, engineered nucleases can also be used to introduce mutations at endogenous genes that generate a gene knockout.



Transformation

1) The first step is to choose and isolate the gene that will be inserted into the genetically modified organism. The gene can be isolated using restriction enzymes to cut DNA into fragments and gel electrophoreses to separate them out according to length. Polymerase chain reaction (PCR) can also be used to amplify up a gene segment, which can then be isolated through gel electrophoresis. If the chosen gene or the donor organism's genome has been well studied it may be present in a genetic library. If the DNA sequence is known, but no copies of the gene are available, it can be artificially synthesized.

2) The gene to be inserted into the genetically modified organism must be combined with other genetic elements in order for it to work properly. The gene can also be modified at this stage for better expression or effectiveness. As well as the gene to be inserted most constructs contain a promoter and terminator\ region as well as a selectable marker gene. The promoter region initiates transcription of the gene and can be used to control the location and level of gene expression, while the terminator region ends transcription. The selectable marker, which in most cases confers antibiotic resistance to the organism it is expressed in, is needed to determine which cells are transformed with the new gene. The constructs are made using recombinant DNA techniques, such as restriction digests, ligations and molecular cloning The manipulation of the DNA generally occurs within a plasmid.



Benefits of Transgenic Animals

The benefits of these animals to human welfare can be grouped into areas:

1. Agriculture

2. Medicine

3. Industry

1. Agricultural Applications

a) Breeding

Farmers have always used selective breeding to produce animals that exhibit desired traits (e.g., increased milk production, high growth rate) Traditional breeding is a time-consuming, difficult task. When technology using molecular biology was developed, it became possible to develop traits in animals in a shorter time and with more precision. In addition, it offers the farmer an easy way to increase yields.

b) Quality

Transgenic cows exist that produce more milk or milk with less lactose or cholesterol, pigs and cattle that have more meat on them, and sheep that grow more wool. In the past, farmers used growth hormones to spur the development of animals but this technique was problematic, especially since residue of the hormones remained in the animal product.

2. Medical Application

a) Xenotransplantation

Patients die every year for lack of a replacement heart, liver, or kidney. For example, about 5,000 organs are needed each year in the United Kingdom alone. Transgenic pigs may provide the transplant organs needed to alleviate the shortfall currently, xenotransplantation is hampered by a pig protein that can cause donor rejection but research is underway to remove the pig protein and replace it with a human protein.

b) Human gene therapy

Human gene therapy involves adding a normal copy of a gene (transgene) to the genome of a person carrying defective copies of the gene. The potential for treatments for the 5,000 named genetic diseases is huge and transgenic animals could play a role. For example, the A. I. Virtanen Institute in Finland produced a calf with a gene that makes the substance that promotes the growth of red cells in humans.

3. Industrial Applications

In 2001, two scientists at Nexia Biotechnologies in Canada spliced spider genes into the cells of lactating goats. The goats began to manufacture silk along with their milk and secrete tiny silk strands from their body by the bucketful. By extracting polymer strands from the milk and weaving them into thread, the scientists can create a light, tough, flexible material that could be used in such applications as military uniforms, medical microsutures, and tennis racket strings.

References
http://www.whatisbiotechnology.org/science/transgenic
http://people.ucalgary.ca/~browder/transgenic.html
http://www.biotecharticles.com/Genetics-Article/Transgenic-Animals
htmlhttp://www.actionbioscience.org/biotechnology/margawati.html
https://en.wikipedia.org/wiki/Genetic_engineering