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Transgenesis: Catalyst to Improve livestock

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Abstract

For improvement of livestock production and human health care products, the advent of DNA recombinant technology and the possibility of gene transfer between organisms of distinct species, or even distinct phylogenic kingdoms, has opened a wide range of possibilities. Transgenic animals are routinely used in the laboratory as models in biomedical research. Over 95 per cent of those used are genetically modified rodents, predominantly mice. They are important tools for researching human disease, being used to understand gene function in the context of disease susceptibility, progression and to determine responses to a therapeutic intervention.

Introduction

Over centuries animal breeding practices were performed to improve the genetic potential of animals and to introduce new traits, through genetic selection. But the number of gene combinations achieved through this process has limitations, since breeding is only possible between animals of same or closely related species. Transgenesis is a revolutionary technology which introduces new genes to a species, which belong to an entirely different species. A **transgene** is a gene that has been transferred by any genetic engineering techniques, from one organism to another. The introduction of a transgene, in a process known as transgenesis, has the potential to change the phenotype of an organism. *Transgene* describes a segment of DNA containing a gene sequence that has been isolated from one organism and is introduced into a different organism. This non-native segment of DNA may either retain the ability to produce RNA or protein in the transgenic organism or alter the normal function of the transgenic organism's genetic code. In general, the DNA is incorporated into the organism's germ line. For example, in higher vertebrates this can be accomplished by injecting the foreign DNA into the nucleus of a fertilized ovum. This technique is routinely used to

introduce human disease genes or other genes of interest into strains of laboratory mice to study the function or pathology involved with that particular gene.

History:

- First transgenic animal was a "Supermouse" created by Ralph Brinster (U Pennsylvania) and Richard Palmiter (University of Washington) in 1982. Created by inserting a human growth hormone gene in mouse genome, offspring was much larger than the parents.
- In 1997, the first transgenic cow, Rosie, produced human alpha-lactalbumin -enriched milk at 2.4 grams per litre.
- A New Zealand research group developed a genetically modified dairy herd capable of producing 'medicinal milk' containing recombinant human lactoferrin (rhLF) by transgenic technology.
- In 2000, AgResearch generated their first transgenic cows. These cows produced modified or 'designer' milk. AgResearch's first transgenic cows had extra bovine (cow) kappa casein genes inserted in their genome. This resulted in increased kappa casein in their milk as the transgenic cow had their 2 naturally occurring kappa casein genes along with the inserted kappa casein gene(s). This research was the first proof that transgenic technology could be used to modify milk composition in cows.
- In 2001, two scientists 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 micro sutures, and tennis racket strings.

Different steps in transgenic animal production:

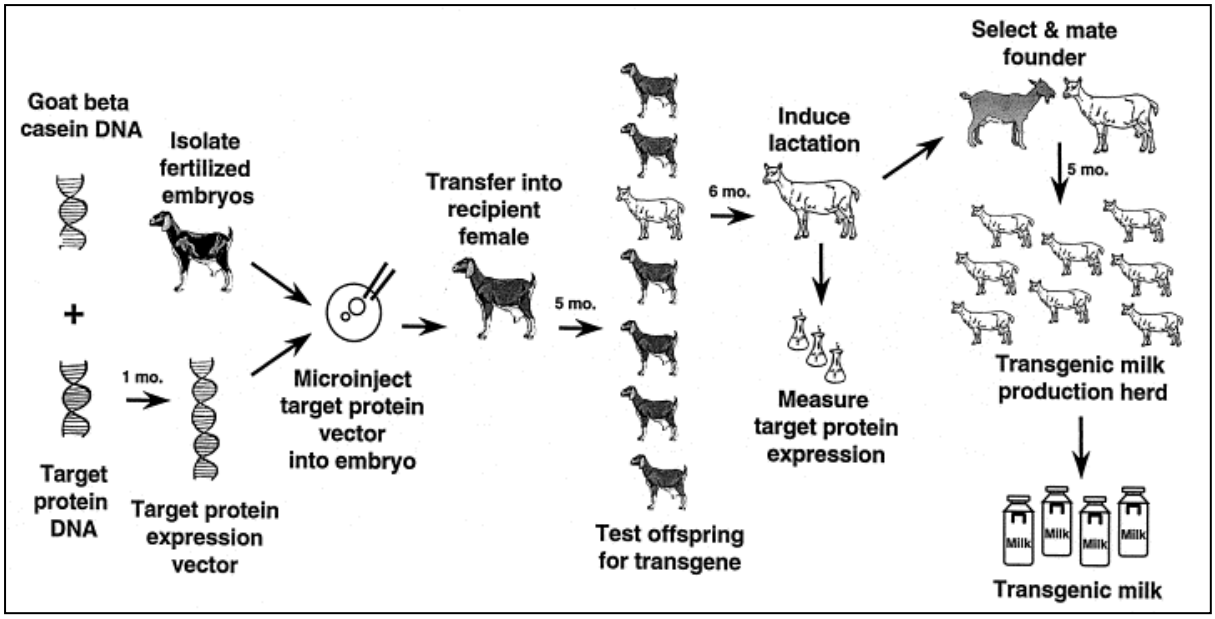
1. Gene of interest is isolated in a strand of DNA.

2. DNA is cut specific points by restriction enzymes. The enzymes recognize certain sequences of bases on the DNA strand and cut where the sequences appear.

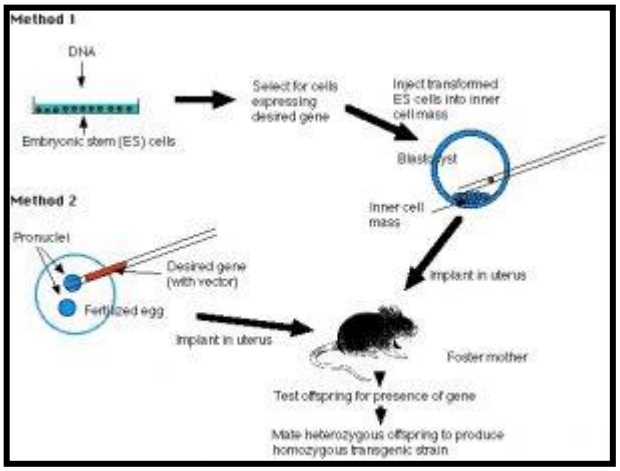
3. The cut DNA is jointed with a vector, which may be a virus (e.g.Retro viral vector) or a plasmid. The vector carries the gene of interest into organisms that will produce the protein.

4. When the genes are transferred in this way they get expressed in the desired organ of animals.

5. In addition to vector method, direct microinjection of nuclear material into invitro fertilized (IVF) embryos, and genetically modified embryonic stem cell transfer are effective techniques for transgenic animal production. Among these methods, transgenic animal production through stem cell transfer is very specific in locating the organ of desired action.



- **Embryonic Stem Cell-Mediated Gene Transfer:** In 1981, the term embryonic stem cells (ES cells) were used to denote a cell line isolated directly from mouse embryos while, the term embryonal carcinoma cells (EC) were derived from teratocarcinomas. Embryonic stem cells (ES cells) are harvested from the inner cell mass (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal, including its gametes as shown:



- Using recombinant DNA methods, build molecules of DNA containing the structural gene you desire (e.g, the insulin gene), vector DNA to enable the molecules to be inserted into host DNA molecules, promoter and enhancer sequences to enable the gene to be expressed by host cells.
- Transform ES cells in culture to expose cultured cells to the DNA so that some will incorporate it.
- Select for successfully transformed cells.
- Inject these cells into the inner cell mass (ICM) of mouse blastocysts.
- Embryo transfer.

- Prepare a pseudopregnant the stimulus of mating elicits the hormonal changes needed to make her uterus receptive.
- Transfer the embryos into her uterus.
- Hope that they implant successfully and develop into healthy pups (no more than one-third will).
- Test her offspring.
- Remove a small piece of tissue from the tail and examine its DNA for the desired gene. No more than 10- 20% will have it, and they will be heterozygous for the gene.
- Establish a transgenic strain
- Mate two heterozygous mice and screen their offspring for the 1:4 that will be homozygous for the transgene.
- Mating these will found the transgenic strain.

- **Retrovirus mediated gene transfer**

Transgenic mice produced by retroviral transduction of male germ line stem cells. Male germ line stem cells have ability to self-renew and genetic modification of these cells would help to study the biology of their complex self-renewal and differentiation processes and to generate wide range of transgenic animal species. A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. Retroviruses used as vectors to transfer genetic material into the host cell, resulting into a generation of chimera (an organism consisting of tissues or parts of diverse genetic constitution). Chimeras are inbred for as many as 20 generations until homozygous (carrying the desired transgene in every cell) transgenic offspring are born. The method was successfully used in 1974 when a simian virus was inserted into mice embryos, resulting in mice carrying this DNA.

- **Nuclear Transfer Method**

In this method, the transgenic goats were produced by nuclear transfer of fetal somatic cells. Donor karyoplasts were obtained from a primary fetal somatic cell line derived from a 40-day transgenic female fetus produced by artificial insemination of a non-transgenic adult female with semen from a transgenic male. Live offspring were produced with two nuclear transfer procedures.

Oocytes at the arrested metaphase II stage were enucleated, electro fused with donor somatic cells, and simultaneously activated.

In the second procedure, activated in vivo oocytes were enucleated at the telophase II stage, electro fused with donor somatic cells, and simultaneously activated a second time to induce genome reactivation.

There was generation of three healthy identical female offspring. Genotypic analyses confirmed that all cloned offspring were derived from the donor cell line. Analysis of the milk of one of the transgenic cloned animals showed high-level production of human antithrombin

III. The nuclear transfer application may be more useful and beneficial for agricultural is the ability to efficiently produce a large number of identical offspring derived from a particular mating. Therefore, nuclear transfer using a embryonic cell lines derived from that mating maybe more attractive.

- **Transfection of Gametes**

The first transfection procedures occurred in the early 1960s and experiments with different cell types and tissues has now become widespread. Different transfection methods have been employed:

1. The in vitro procedure when foreign genes are introduced into cultured cells or tissues.
2. The in vivo method, when genes are directly introduced into the tissue (by injection, aerosol, etc).
3. The ex-vivo system, in which cells are transfected in vitro and then introduced into a living organism.

Gametes are incubated during short time periods in a solution containing the gene constructions and then they are checked for transfection, used for inseminations or for in vitro fertilization procedures. In several cases naked DNA was employed successfully, but DNA-Liposome complexes or electroporation procedures have been also used. In the case of the female gamete in vitro transfections using liposomes or retroviruses have been applied successfully. As well as, electroporation, high velocity microprojectiles or particle gun methods have been also employed. The localization of the foreign gene in spermatozoa has been done using fluorescent in situ hybridization, autoradiography or immunocytochemistry. After using the in vitro or in vivo transfection procedures high percentages (80%) of spermatozoa appeared transfected. These results usually showed that the foreign gene appeared into the nucleus of spermatozoa and molecular procedures (Slot-Blot, PCR, Southern Blot and gene sequences) have shown the presence of the transgene in the DNA of the gametes.

- **Artificial Chromosome Mediated Gene Transfer**

A group of nuclei injected with transgene DNA, the eggs are transferred in medium of incubation and visual evaluation within next few hours. An individual animal develops after receiving the transgene DNA is referred as founder of a new transgenic lineage. Also, Yeast Artificial Chromosomes (YACs) transgenic mice are generated by using pronuclear microinjection and represents latest generation of vectors which have the great advantage of large insert size. This method succeeded in mice and rabbits.

- **Testis cell transplantation method:** Testis cell transplantation method is shown in figure 2 and its steps are as follows:

(A) A single-cell suspension is produced from a fertile donor testis.

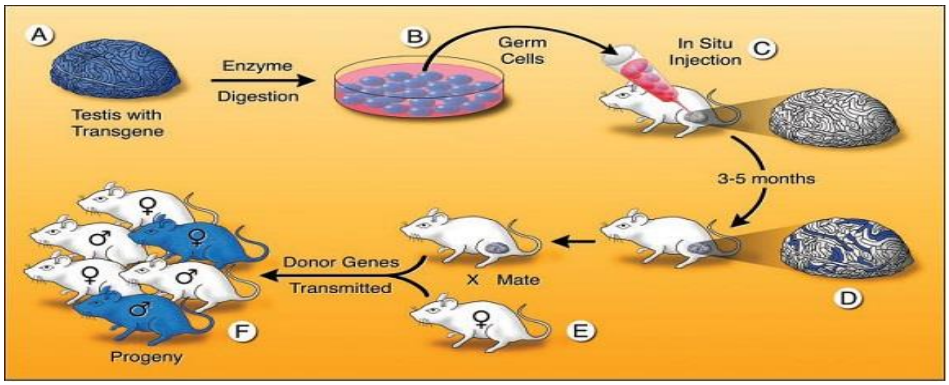
(B) The cells can be cultured

(C) Microinjected into the lumen of seminiferous tubules of an infertile recipient mouse.

(D) Only a spermatogonia stem cell can generate a colony of spermatogenesis in the recipient testis. When testis cells carry a reporter transgene that allows the cells to be stained blue, colonies of donor cell-derived spermatogenesis are identified easily in recipient testes as blue stretches of tubule.

(E) Mating the recipient male to a wild-type female

(F) Produces progeny, which carry donor genes.



Recent methods for production of Transgenic Animals

Lentiviral Transfer of Oocytes and Zygotes

This method is used to overcome previous limitations of viral mediated gene transfer, containing the silencing of the transgenic locus and low expression levels. Example including, generation of transgenic cattle by lentiviruses requires microinjection into the oocytes. Recently H. M. Sang from Roslin Institute has reported a different approach to overcome the problem associated with retroviral vectors. This study employed lentiviral based vectors. These vectors have several advantages compared to the conventional retrovectors in that they can infect non-diving cells, can carry large amounts of transgene ~ 10kb, and can show stable expression in the tissue where they are introduced. The technique was successful in showing about 100 fold increases in the level of transgenesis.

Chimera Generation by injecting the Pluripotent Cells

Embryonic stem cells with pluripotent cells have ability to participate in organ and germ cell production after injection into the blastocysts. Embryonic stem cells are important one for generating the gene knockins, large chromosomal rearrangements as well as gene knockouts. As like embryonic stem cell, the another type of cells such as primordial germ cells are used for production of no. of farm animals and chimeric animals without germ line contribution have been reported in swine.

Conclusion

Different methods are being used to produce transgenic animals which not only help in study of human disease but also to produce products from transgenic animal eg milk. Other than breeding, transgenesis is a revolutionary tool which form a totally different strain. It holds a great potential in many different fields like agriculture, medicine and food industry.

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