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Animal Transgenesis and its Applications

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Abstract. Nowadays, breakthroughs in molecular biology are happening at an unprecedented rate. One of them is the ability to engineer transgenic animals, i.e., animals that carry genes from other species. The technology has already produced transgenic animals such as mice, rats, rabbits, pigs, sheep, cows or microorganisms. This article presents the basics of the transgenesis technology and its applications in medicine, agriculture and industry.

Key words: animal transgenesis, applications in medicine, agriculture and industry.

INTRODUCTION

There are several definitions for the term transgenic animal. 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 *(Federation of European Laboratory Animal Associations 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 resulted animal contains the same modified genetic material. The transgenesis determines different genome modifications, whether by the insertion of a new gene, whether by the temporary or definitive inactivation of a gene already existent into the genome. The insertion of a foreign gene (transgene) into an animal is successful only if the gene is inherited by offspring so this is the reason why the success rate for transgenesis is very low.

METHODS OF CREATION OF THE TRANSGENIC ANIMALS

For practical reasons, i.e., their small size and low cost of housing then for larger vertebrates, their short generation time, and their fairly well defined genetics, mice have become the main species used in the field of transgenesis.
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. The foreign DNA (gene) must be integrated into the genome prior to the doubling of the genetic material that precedes the first cleavage in order for the animal to be born with a copy of this new information in every cell. For several hours following the entry of the sperm into the oocyte, the male and the female pronuclei can

still be seen individually under a normal light microscope and they have not fused yet into a so called zygote. The foreign DNA may be injected into either pronuclei with no difference in results; however, the DNA is typically injected into the male pronucleus because it is slightly larger and closer to the oocyte surface. These oocytes are subsequently transferred into the uterus of pseudopregnant recipient animals. 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 offspring is screened to confirm a successful integration of the gene of interest for use in further studies.

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 which are derived from the inner cell mass of blastocysts that have the potential to differentiate into any type of cell (somatic and germ cells) and therefore to give rise to a complete organism. Embryonic stem cells (ES) are used for more precise modifications of the mouse genome. This technique makes it possible to insert as well as remove or modify DNA sequences. Knock-out, knock-in and conditional mutant mice can be produced with this method. The first step is the removal of ES cells from a blastocyst. After transfection of the ES cells, selection, cloning and screening methods make it possible to detect ES cell clones that demonstrate the desired, site-specific recombination. After microinjection of the genetically modified ES cells into blastocyst-stage embryos the ES cells divide and become part of the embryo. The following chimeric animals will subsequently transmit the recombinant genotype to their offspring, if the ES cells have contributed to their germ cells.

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.

TRANSGENESIS APPLICATIONS IN MEDICINE

a) Transgenic animals as experimental models for human diseases

The creation of transgenic animals results in a shift from the use of higher order species to lower order species, and is also affecting the numbers of animals used. 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 disease 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 complex interactive processes of living mammals are not reproducible in vitro. However, transgenic animals provide 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.

In medical research the 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 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; the transgenic animals may be used also in mammalian developmental genetics and also in toxicology as responsive test animals (detection of toxicants).

However, models are not strict equivalents, so as with any other system care must be taken in drawing conclusions from the data.

b) Transplant organs may soon come from transgenic animals

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.

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.

c) Transgenic animals as drug producers

There are many researches in order to obtain transgenic animals (sheep, cows, goats) which will produce increased quantities of milk which will contain the desired protein. The researches were made in order to increase the normal milk content, such as protein (as it was already presented), casein, β-lactoglobulin, or to obtain different other proteins with pharmaceutical importance such as IX clotting factor, interleukin-2, tissue Plasminogen Activator (tPA) or urokinase. Products such as insulin, growth hormone, and blood anti-clotting factors may soon be or have already been obtained from the milk of transgenic cows, sheep, or goats.

Research is also underway to manufacture milk through transgenesis for treatment of debilitating diseases such as phenylketonuria (PKU), hereditary emphysema, and cystic fibrosis.

In 1997, the first transgenic cow, Rosie, produced human protein-enriched milk at 2.4 grams per litre. This transgenic milk is a more nutritionally balanced product than natural bovine milk and could be given to babies or the elderly with special nutritional or digestive needs. Rosie's milk contains the human gene alpha-lactalbumin.

Another important future application of transgenic animals is the 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.

Other examples of relative recently discoveries: sheep with the milk containing ά 1-anti- trypsin used for the treatment of respiratory diseases; goats producing in milk anti-thrombin III or anti-tumoral anti-bodies; cows producing GAD protein used for the treatment of the diabetes, ά-lactalbumines for the new-born; lactoferins which stimulate the immune system in humans or cows with a low content in milk of β-lactoglobulines and lactose which are not tolerated by some persons; pigs which synthesize the human hemoglobins.

TRANSGENESIS APPLICATIONS IN ANIMAL BREEDING

a) Animals with increased production

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 and, in addition, it offers the farmer an easy way to increase yields.

Nowadays transgenic cows that produce milk in large quantities compared to the normal breeds (with 20%, Watson, 1992), or with increased protein, or milk with less lactose already exist. Another important trait followed by transgenesis is the increasing of the production such as meat production in pigs, cattle, chickens (in 7 weeks they reach to 2.5 kg) or gigantic craps and salmons (11 times increased rate). There were also obtained sheep with increased wool production and also with wool of a better quality. There were also obtained turkey hens in which the hatching instinct was blocked or sterile salmons or trout, so all their energy is used just for gaining weight and is not dissipate for sexual instincts.

b) **Disease-resistant animals**

Scientists are attempting to produce disease-resistant animals, but a very limited number of genes are currently known to be responsible for resistance to diseases in farm animals.

In order to protect the farm animals against viral infectious there are transferred genes responsible for the synthesis of the viral capsidal proteins. So the scientists attempt to produce influenza-resistant pigs or avian leucose-resistant hens. There was transferred the gene Env which encodes a glycoprotein belonging to the external envelope of the avian leucose virus.

There were also obtained cows that produce β-interferon, a citokin which stimulates the resistance to the viral attack.

c) Animals with a specific desired or demonstrative trait

The transgenesis in animals was also used in order to obtain animals with a specific desired trait such as resistance in cold water (salmons in USA and Canada), or turkey hens in which the gene which determines the synthesis of prolactin was blocked, hormone responsible for the development of the hatching instinct.

The transgenesis in animals was also used for demonstrative purposes, the resulted "chimers" were made just in order to prove that transgenesis occurred. Such examples are" Umbuku lizard (a lizard with wings), dolion (a combination of a lion with a dog), lemurat scientifically named Prolos fira (a cross between a lemur and a cat), or the phosphorescent animals (such as pigs, monkeys, fish). It is maybe important to be mentioned that the phosphorescent fish (GloFish) is the first genetically modified animal to be sold as a pet.

TRANSGENESIS APPLICATIONS IN BIOTECHNOLOGIES

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.

Toxicity-sensitive transgenic animals have been produced for chemical safety testing. Microorganisms have been engineered to produce a wide variety of proteins, which in turn can produce enzymes that can speed up industrial chemical reactions.

Ananda Chakrabarty created one of the first microbes of this nature in the early 1970s. He introduced genes from several different bacteria into a strain of *Burkholderia cepacia*, giving it the ability to degrade toxic compounds found in petroleum. This microbe offered a potential alternative to skimming and absorbing spilled oil. Chakrabarty's genetically modified bacterium has never been used, however, due to public concerns about the release of genetically engineered microbes into the environment. The microbe did, on the other hand, play an important role in establishing the biotechnology industry. The U.S. Patent Office granted Chakrabarty the first patent ever for the construction and use of a genetically engineered bacterium. This established a precedent allowing biotechnology companies to protect their "inventions" in the same way chemical and pharmaceutical companies have done in the past.

Transgenic microbes have many commercial and practical applications, including the production of mammalian products. A company called Genentech was among the earliest and most successful commercial enterprises to use genetically engineered bacteria to produce human proteins. Their first product was human insulin produced by genetically engineered *Escherichia coli*. A variety of other human hormones, blood proteins, and immune modulators are now produced in a similar fashion, in addition to vaccines for such infectious agents as hepatitis B virus and measles.

Another promising application of genetically engineered microbes is in environmental cleanup, or biomediation. Scientists have discovered many naturally occurring genes that code for enzymes that degrade toxic wastes and wastewater pollutants in bacteria. Examples include genes for degrading chlorinated pesticides, chlorobenzenes, naphthalene, toluene, anilines, and various hydrocarbons. Researchers are using molecular cloning to introduce

these genes from several different microbes into a single microbe, creating "super microbes" with the ability to degrade multiple contaminants.

The obtaining and using of the transgenic animals and microorganisms have developed many ethical issues regarding their dissemination in the environment, their influence on the biodiversity, the welfare of the transgenic animals, regarding the xenotransplantation and many others.

CONCLUSIONS

- Nowadays, breakthroughs in molecular biology are happening at an unprecedented rate. One of them is the ability to engineer transgenic animals, i.e., animals that carry genes from other species.
- The technology has already produced transgenic animals such as mice, rats, rabbits, pigs, sheep, cows or microorganisms.
- There are three methods of creation of the transgenic animals, such as: DNA microinjection, the most common one, embryonic stem cell-mediated gene transfer and retrovirus-mediated gene transfer.
- Transgenic animals have multiple possible applications in medicine, agriculture and industry.
- The obtaining and using of the transgenic animals and microorganisms have developed many ethical issues.

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