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Genesis of transgenic animals

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Abstract
Genetically modified organisms are created in the laboratory to amplify desired characteristics which are beneficial to mankind. Work on transgenic expression systems using animals began in the early 1980s for improving the genetic characteristics of livestock. Transgenic animals acquire genetic material (from another species) through human intervention rather than through normal sexual reproduction. Transgenic animals are used to increase production efficiency of farm animals in a short duration, to produce medicines, nutraceuticals and tissues for transplant into human, to introduce a desired property as animal model for human diseases and to use as xeno-transplantates. The genetic material can be inserted to gonads or germ cells or fertilized ovum or somatic cells. Different methods like DNA microinjection, embryonic stem cell method, retrovirus mediated transfer etc are used to produce transgenic animals.

Keywords: Cell, genetic, livestock, transgenic

Introduction
Over the last three decades biotechnology has advanced to a level where it is generally feasible to make particular changes to the genome, so that the expressed characteristics of living organisms changes. The product of such a change is called a transgenic or a genetically modified organism (GMO). A transgenic organism is one into which a gene from some other species has been transferred through human intervention. Transgenic animals include animals that result from the molecular manipulation of endogenous genomic DNA, including all techniques from DNA microinjection to embryonic stem (ES) cell transfer and "knock-out" mouse production. The transferred gene is called a transgene. The better knowledge of gene structure and function allows the preparation of recombinant genes having a more predictable expression in transgenic animals.
There are several types of transgenic animals like transgenic sheep, birds, chickens, pigs, insects etc. Transgenic animals produced with the purpose of producing better and good quality breed, increased in milk yield, as well as to produce organs to meet the demand for organ transplantation. Genetically modified animals are proving ever more vital in the development of new treatments and cures for many serious diseases.

Different methods for production of transgenic animals
a) Transgenic animal creation through the gonads
In this method the possibility of transfecting spermatogonia *in situ* via infusion of transgenes into seminiferous tubulesor transfection of germ cell precursors *in vitro* followed by transplantation into host testis was tested. It was demonstrated that testis-derived cells transplanted into the testis of infertile males could populate the host testis, generate sperm, and produce offspring. The next step is to transfect the testis-derived cells before transplantation.

b) Transgenic animal creation through the germ cells
In germline gene transfer, the parents' egg and sperm cells are changed with the goal of passing on the changes to their offspring. Germline gene transfer is not being actively investigated generally in larger animals and humans. Since 1989, a new method for the production of transgenic animals has been available known as sperm-mediated gene transfer (SMGT). The use of SMGT alone or in combination with other methods to facilitate the introduction of exogenous DNA through the membrane of the sperm resulted in the production of transgenic animals (Wang *et al*., 2001) [14]. It is based on the intrinsic ability of sperm cells to bind and internalise exogenous DNA molecules and to transfer them into the oocyte at

fertilisation. The major benefits of the SMGT technique were found to be its high efficiency, low cost and ease of use than other methods. Furthermore, SMGT does not require embryo handling or expensive equipment. Sperm mediated gene transfer could also be used to generate multigene transgenic pigs that would be of benefit as large animal models for medical research, for agricultural and pharmaceutical applications. For xenotransplantation, which requires extensive genetic manipulation of donor pigs to make them suitable for grafting to humans.

c) Methods of transgenic animal creation throughfertilized eggs or embryos

There are several principal methods used for the creation of transgenic animals.

i) DNA single microinjection

The direct DNA microinjection into the pronuclei of embryos was the first technique which led to regular and relatively easy success in mammals (Gordon *et al*., 1990) [1]. Male and female pronuclei are microscopically visible after several hours of the entry of the sperm into the oocyte. The transgene may be microinjected into either of these pronuclei. By the method of pronucleus microinjection Big Blue animals and Muta Mousehave been generated. Transgene integration into the genome of founder animals is low in this technique i.e. only 0.5-3% of the microinjected embryos producing transgenic offspring. All the transfection techniques are applicable to cultured animal cells, but due to the tediousness of the technique and the limited number of cells that can be handled, microinjection is not used generally. The number of embryos generated by superovulation is low and the success of microinjection is possible only when embryos were prepared in vitro after oocyte maturation and fertilization followed by in vitro development of the microinjected to the blastocyst stage (Krimpenfort *et al*., 1991) [7]. It is proved efficient in several fish species and mainly in salmonids. In insects (Drosophila) and worms (*Caenorhabditis elegans*) (Thierry-Mieg *et al*., 1997) [12], foreign DNA is injected into gonad syncytium.

DNA double microinjection

Here the microinjection of a foreign DNA into the pronucleus facilitates the transfer of a foreign gene into both pronuclei of fertilized egg, and was successfully used for production of transgenic mice (Kupriyanov *et al*., 1998) [8]. This approach was extrapolated (Chrenek *et al*., 2005, 2007) [6, 5] in a larger species and increasing the transgene integration efficiency upon microinjection of DNA into both pronuclei of rabbit embryos. In the case of double microinjection, degradation of embryos (lysis, fragmentation, irregular division) takes place. Double microinjection doesn't cause significantly higher percentage of embryo degeneration. Success of the integration of a foreign gene through this method was at least 30% more than DNA single microinjection.

ii) Cytoplasmic injection

Cytoplasmic injection is based on the established method of DNAligand- polylysine conjugate to transfect mammalian cells/DNA-transferrin-polylysine transfecting avain cells. This method allows the injection of the gene into the zygote even if the pronuclei are not visible (sheep, pigs, cattle). There is also the unexplored avenue that injection of DNA/ polylysine mixtures anywhere in the zygote (including the pronucleus) may yield transgenic animals. To overcome some of the difficulties of pronuclear microinjection some laboratory developed another passive technique for the production of transgenic mice called Intracytoplasmic Sperm Injection-mediated transgenesis (ICSI-Tr).

iii) Embryonic stem cell-mediated gene transfer

The term embryonic stem cells (ES cells) were used to denote a cell line isolated directly from mouse embryos. 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. With the use of ES cells, a single copy of the transgene is integrated into a determined locus by homologous recombination (HR) allowing the generation of knock-outs, knock-ins or the exchange of genes or large chromosomal regions (Capecchi, 2005; Laible and Alonso-Gonzalez, 2009; Ohtsuka *et al*., 2012) [2, 9, 11].

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. Prepare a pseudo-pregnant mouse. 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 (not 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.

iv) Retrovirus (Adenovirus)-mediated gene transfer

To increase the probability of an expression, the 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. 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 socalled 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.

v) Nuclear transfer

Nuclear transfer involves the transfer of each nucleus (genetically modified) from a multicellular embryo into an enucleated metaphase II oocyte. Nuclear transfer has become an active field of research over the last decade, culminating in

reports over the past two years of live offspring from differentiated cells of sheep, cattle, and mice. Dozens of laboratories are producing calves and lambs whose genetic parents are transgenic somatic cells transfected via electroporation. In spite of the fact that research orients towards modification of somatic cell genome through homologous recombination with the aim of producing so called "knock-out" (targeted gene is blocked) or "knock-in" (which refers to the integration of the transgene to specific site in the cell). After selecting the positive cells ("knock-out" or "knock-in"), these may serve as a donor of nucleus mainly for the purpose of cloning. As the production of cloned (or transgenic) animals is demanding, combination of the techniques of transgenesis, cloning allows achieving up to 30% better results. The principle depends on the utilization of transgenic somatic cells (or nucleus) for the purpose of cloning. Blastomeres isolated from cloned together with early transgenic embryos are subsequently used for the purpose of chimera. Then chimeric cloned transgenic animals can be obtained.

d) Methods of transgenic animal creation through somatic cells

In somatic gene transfer the recipient's genome is changed, but the change is not passed on to the next generation. Matsumoto *et al*. (2001) [10] developed a non-viral and plasmid-based method for arterial gene transfer by *in vivo* electronic pulse, using a newly designed T-shaped electrode. Using rabbit carotid arteries they first optimized gene transfer efficiency, and firefly luciferase gene transfer via electronic pulse under 20 V voltage (the pulse length: Pon time 20 mins, the pulse interval: Poff time 80 mins, number of pulse: 10 times) showed the highest gene expression. Electroporationmediated gene transfer of *E. coli* lacZ with nuclear localizing signal revealed successful gene transfer to luminal endothelial cells and to medial cells. They concluded that *in vivo* electroporation mediated arterial gene transfer is readily facilitated, safe and may prove to be an alternative form of gene transfer to the vasculature (Wall, 1996) [13].

Ethical issues related to transgenic animals

The social opinion on transgenic animal research is divided almost in the middle. Use of animals in biotechnological research causes great suffering to the animals. But most people seem to accept some animal suffering to serve the basic interest and welfare of mankind. It is felt that by using animals for the production of pharmaceutical proteins we made them as factories. This seems not to recognize that animals also are living beings which feel pleasure and pain just as we do. Some people feel that animals should be regarded as equal to humans in that they have the same basic rights as human beings. However, in most societies animals are relegated to a position several steps below that of man. An argument attempts to focus on integrity of species in that each biological species has a right to exist as a separate identifiable entity. But biologists do not regard a species as a fixed, water-tight entity. They are regarded as dynamic, constantly evolving groups. Finally, the introduction of human genes into animals, and vice-versa, may be seen by many as clouding the definition of "humanness". But most of the known human genes are not unique, and comparable genes do occur in animals. In addition, many retroviruses have integrated into the human genome without any recognizable devaluation of our humanness.

Conclusion

Throughout history, transgenic animal has made significant contributions to human health and well-being. The recent advances in reproductive technologies (in vitro production of embryos, sperm sexing, somatic nuclear transfer, Lentiviral transfer of oocytes and zygotes, Chimera generation by injecting the pluripotent cells) adds a new dimension to animal breeding. The application of transgenic animals showed in future years genetically modified animals will play a significant and important role in the biomedical field, in particular via the production of valuable pharmaceutical proteins and the supply of xenografts. New and exciting techniques being developed will continue to expand this important and useful area of experimentation.

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