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Transgenic farm animals: present and future

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Summary

Until recently, pronuclear microinjection of deoxyribonucleic acid (DNA) was the standard method for producing transgenic animals. This technique is now being replaced by more efficient protocols based on somatic nuclear transfer that also permit targeted genetic modifications. Lentiviral vectors and small interfering ribonucleic acid technology are also becoming important tools for transgenesis. Transgenic farm animals are important in human medicine as sources of biologically active proteins, as donors in xenotransplantation, and for research in cell and gene therapy. Typical agricultural applications include improved carcass composition, lactational performance and wool production, as well as enhanced disease resistance and reduced environmental impact. Product safety can be ensured by standardisation of procedures and monitored by polymerase chain reaction and array technology. As sequence information and genomic maps of farm animals are refined, it becomes increasingly practical to remove or modify individual genes. This approach to animal breeding will be instrumental in meeting global challenges in agricultural production in the future.

Keywords

Agricultural application – Environmental benefit – Farm animal – Gene pharming – Lentiviral vector – Microinjection – Nuclear transfer – Small interfering ribonucleic acid – Transgenic – Xenotransplantation.

Introduction: evolution of transgenic technologies

The first transgenic livestock were produced almost 20 years ago by microinjection of foreign deoxyribonucleic acid (DNA) into the pronuclei of zygotes (33). However, as microinjection has several significant shortcomings – including low efficiency, random integration and variable expression patterns related to the site of integration – research has focused on alternative methodologies for improving efficiency of generating transgenic livestock (Table I). These methodologies include:

– sperm-mediated DNA transfer (12, 54, 55)

– intracytoplasmic injection of sperm heads carrying foreign DNA (71, 72)

– injection or infection of oocytes and/or embryos by different types of viral vectors (11, 35, 37)

– ribonucleic acid (RNA) interference technology (small interfering RNA: siRNA) (14)

– the use of nuclear transfer (5, 13, 21, 53, 86).

To date, somatic nuclear transfer, which has been successful in ten species – albeit at low efficiency (47, 103) – holds the greatest promise for significant improvements in the generation of transgenic livestock (Table I). Further qualitative improvements may be derived from technologies that allow precise modifications of the genome, including targeted chromosomal integration by site-specific DNA recombinases, such as Cre or flippase (FLP), or methods that allow temporally and/or spatially controlled transgene expression (9, 45). The first genomes of farm animals (cattle, chicken) were sequenced and annotated in 2004 (2, 3). Thus, after approximately 7,000 years of domestic animal selection (16) based on the random mutations caused by radiation and oxidative injury to the genome, technology is now available to introduce or remove known genes with known functions. Despite the inherent inefficiency of microinjection technology, a broad spectrum of genetically modified large animals has been generated for applications in agriculture and biomedicine (Table II). The use of transgenic livestock for 'gene pharming' has now reached the level of commercial exploitation (47). This paper provides a brief summary of the current status of transgenic animal production and look at future implications. The authors

focus on large domesticated species and do not cover the recent developments in poultry breeding or aquaculture.

Biomedical applications of transgenic domestic animals

Pharmaceutical production in the mammary gland of transgenic animals

Gene 'pharming' entails the production of recombinant biologically active human proteins in the mammary glands of transgenic animals. This technology overcomes the limitations of conventional and recombinant production systems for pharmaceutical proteins (63, 82) and has advanced to the stage of commercial application (27, 107). The mammary gland is the preferred production site, mainly because of the quantities of protein that can be produced in this organ using mammary gland-specific promoter elements and established methods for extraction and purification of that protein (63, 82).

Guidelines developed by the Food and Drug Administration (FDA) in the United States of America (USA) require monitoring of the animals' health, sequence validation of the gene construct, characterisation of the isolated recombinant protein, and monitoring of the genetic stability of the

Table I
Evaluation of gene transfer methodology*

Methodology	Animal usage	Site of integration	Integration prescreening	Homologous recombination	Multiple transgenes	Maximum construct size limitation	Founder mosaicism	Technical difficulty
Microinjection	High	Random	No	No	Inefficient	Artificial chromosomes	Yes	😐
Somatic nuclear transfer	Low	Prescreened	Yes	Possible	Yes	Transfection limited/ artificial chromosomes	No	☹
Implantation of transgenic spermatogonia	Low	Random	Yes	Not yet	Not yet	Transfection limited	No	☹
Lentivirus injection	Low	Random	No	No	Yes	~ 5 kb	Yes	☺
Sperm-mediated gene transfer method	Low	Random	No	No	Inefficient	Not known	Not known	☺

* all grading is based on the fully matured technology

Table II
Current status of transgenesis in livestock

Area of application	Early experimental phase	Experimental stage	Advanced experimental stage	Transition experiments/ practical use	Practical use
Biomedicine					
Gene pharming	■	■	■	■	■
Antibody production	■	■	■		
Xenotransplantation					
Solid organs	■	■	■		
Cells/tissues	■	■	■	■	■
Blood replacement	■				
Disease model	■	■			
Agriculture					
Carcass composition					
Leaner meat	■	■	■		
Healthful lipids	■	■			
Lactational performance	■	■	■		
Environmental impact	■	■	■	■	
Wool production	■	■			
Disease resistance	■	■			
Fertility	■				

transgenic animals over several generations. This has necessitated, for example, the use of animals from scrapie-free countries (New Zealand) and the maintenance of production animals under strict hygienic conditions.

Products derived from the mammary gland of transgenic goats and sheep, such as antithrombin III (ATIII), α-antitrypsin or tissue plasminogen activator (tPA), have progressed to advanced clinical trials (47). Phase III trials for a recombinant human ATIII product have been completed and an application has been filed for European Market Authorisation. The protein is expected to be registered and on the market by the end of 2005. The product is employed for the treatment of heparin-resistant patients undergoing cardiopulmonary bypass procedures. At the same company that manufactures this product, 11 transgenic proteins have been expressed in the mammary gland of transgenic goats at more than one gram per litre. The enzyme α-glucosidase from the milk of transgenic rabbits has orphan drug status and has been successfully used for the treatment of Pompe's disease (94) (in the USA the term 'orphan drug' refers to a product that treats a rare disease affecting fewer than 200,000 Americans). Similarly, recombinant C1 inhibitor produced in the milk of transgenic rabbits has completed phase III trials and is expected to receive registration within the next 24 months. A topical antibiotic against *Streptococcus mutans*, for prevention and treatment of dental caries, has completed phase III trials and should be launched shortly. The global market for recombinant proteins from domestic animals is expected to exceed US$1 billion in 2008 and to reach US$18.6 billion in 2013.

Some gene constructs have failed to produce economically significant amounts in the milk of transgenic animals, indicating that the technology needs further refinements to achieve high-level expression. This is particularly true for genes that have complex regulation, such as those coding for erythropoietin or human clotting factor VIII (36, 41, 62, 67). With the advent of transgenic crops that produce pharmacologically active proteins, there is now an array of recombinant technologies that will allow selection of the most appropriate production system for each required protein (58). The production of edible vaccines in transgenic crop plants against, for example, foot and mouth disease, might become an important application for animal health (102).

Antibody production in transgenic animals

Numerous monoclonal antibodies are being produced in the mammary gland of transgenic goats (63). Cloned transgenic cattle produce a recombinant bispecific antibody in their blood (31). Purified from serum, the antibody is stable and mediates target cell-restricted T cell stimulation and tumour cell killing. An interesting new development is the generation of trans-chromosomal animals. A human artificial chromosome containing the complete sequences of the human immunoglobulin heavy and light chain loci was introduced into bovine fibroblasts, which were then used in nuclear transfer. Trans-chromosomal bovine offspring were obtained that expressed human immunoglobulin in their blood. This system could be a significant step forward in the production of human therapeutic polyclonal antibodies (51). Further studies will show whether the additional chromosome will be maintained over future generations and how stable expression will be.

Blood replacement

Functional human haemoglobin has been produced in transgenic swine. The transgenic protein could be purified from the porcine blood and showed oxygen-binding characteristics similar to natural human haemoglobin. The main obstacle was that only a small proportion of porcine red blood cells contained the human form of haemoglobin (90). Alternative approaches to produce human blood substitutes have focused on the chemical cross-linking of haemoglobin to the superoxide-dismutase system (20).

Xenotransplantation of porcine organs to human patients

Today more than 250,000 people are alive only because of the successful transplantation of an appropriate human organ (allotransplantation). However, progress in organ transplantation technology has led to an acute shortage of appropriate organs, and cadaveric or live organ donation does not meet the demand in Western societies. To close the growing gap between demand and availability of appropriate human organs, porcine xenografts from domesticated pigs are considered to be the best alternative (47, 77). Essential prerequisites for successful xenotransplantation are:

– overcoming the immunological hurdles

– preventing the transmission of pathogens from the donor animal to the human recipient

– ensuring the compatibility of the donor organs with human anatomy and physiology.

The major immunological obstacles in porcine-to-human xenotransplantation are:

– hyperacute rejection (HAR)

– acute vascular rejection (AVR)

– cellular rejection, and eventually

– chronic rejection (101).

Hyperacute rejection occurs within seconds or minutes, when, in the case of a discordant organ (e.g. in transplanting from pig to human), pre-existing antibodies react with antigenic structures on the surface of the porcine cells and activate the complement cascade; in other words, the antigen-antibody complex triggers formation of the membrane attack complex. Induced xenoreactive antibodies are thought to be responsible for AVR, which occurs within days after transplantation of a xenograft; disseminated intravascular coagulation (DIC) is a predominant feature of AVR (17, 52). Human thrombomodulin and heme-oxygenase 1 are crucially involved in the etiology of DIC and are targets for future transgenic modifications to improve the long-term survival of porcine xenografts by creating multi-transgenic pigs.

When using a discordant donor species such as the pig, overcoming HAR and AVR are the pre-eminent goals. Two main strategies have been successfully explored for long-term suppression of HAR: synthesis of human regulators of complement activity (RCAs) in transgenic pigs (18, 77) and the knockout of α-gal epitopes, the antigenic structures on the surface of the porcine cells that cause HAR (52, 74, 105). Survival rates, after the transplantation of porcine hearts or kidneys expressing transgenic RCA proteins to immunosuppressed non-human primates, reached 23 to 135 days, indicating that HAR can be overcome in a clinically acceptable manner (4). The successful xenotransplantation of porcine organs with a knockout of the 1,3-α-galactosyltransferase gene, eliminating the 1,3-α-gal-epitopes produced by the 1,3-α-galactosyltransferase enzyme, has recently been demonstrated. Upon transplantation of porcine hearts or kidneys from these α-gal-knockout pigs to baboons, survival rates reached two to six months (52, 105). A particularly promising strategy to enhance long-term graft tolerance is the induction of permanent chimerism via intraportal injection of embryonic stem (ES) cells (28) or the co-transplantation of vascularised thymic tissue (105).

Recent findings have revealed that the risk of porcine endogenous retrovirus transmission is negligible and show that this critical danger could be eliminated, paving the way for preclinical testing of xenografts (47). Despite further challenges, appropriate lines of transgenic pigs are likely to be available as organ donors within the next five to ten years. Guidelines for the clinical application of porcine xenotransplants are already available in the USA and are currently being developed in other countries. The general consensus of a worldwide debate is that the technology is ethically acceptable provided that the individual's well-being does not compromise public health. Economically, xenotransplantation will be viable, as the enormous costs of maintaining patients suffering from severe kidney disease using dialysis or supporting those suffering from chronic heart disease could be reduced by a functional kidney or heart xenograft.

Farm animals as models for human diseases

Mouse physiology, anatomy and life span differ significantly from those of humans, making the rodent model inappropriate for many human diseases. Farm animals, such as pigs, sheep or even cattle, may be more appropriate models in which to study potential therapies for human diseases that require longer observation periods than those possible in mice, e.g. atherosclerosis, non-insulin-dependent diabetes, cystic fibrosis, cancer and neuro-degenerative disorders (34, 56, 70, 92). Cardiovascular disease is an increasing health problem in aging Western societies, where coronary artery diseases account for the majority of deaths. Because genetically modified mice do not manifest myocardial infarction or stroke as a result of atherosclerosis, new animal models, such as swine that exhibit these pathologies, are needed to develop effective therapeutic strategies (32, 80). An important porcine model has been developed for the rare human eye disease retinitis pigmentosa (PR) (73). Patients with PR suffer from night blindness early in life, a condition attributed to a loss of photoreceptors. Transgenic pigs that express a mutated rhodopsin gene show great similarity to the human phenotype and effective treatments are being developed (61).

The pig could be a useful model for studying defects of growth-hormone releasing hormone (GHRH), which are implicated in a variety of conditions such as Turner syndrome, hypochrondroplasia, Crohn's disease, intrauterine growth retardation or renal insufficiency. Application of recombinant GHRH and its myogenic expression has been shown to alleviate these problems in a porcine model (26). Development of further ovine and porcine models of human diseases is underway (29).

An important aspect of nuclear-transfer-derived large animal models for human diseases and the development of regenerative therapies is that somatic cloning per se does not result in shortening of the telomeres and thus does not necessarily lead to premature ageing (85). Telomeres are highly repetitive DNA sequences at the ends of the chromosomes that are crucial for their structural integrity and function and are thought to be related to lifespan. Telomere shortening is usually correlated with severe limitation of the regenerative capacity of cells, the onset of cancer, ageing and chronic disease with significant impacts on human lifespans (85). Expression of the enzyme telomerase, which is primarily responsible for the formation and rebuilding of telomeres, is suppressed in most somatic tissues post-natally. Recent studies have revealed that telomere length is established early in pre-implantation development by a specific genetic programme and correlates with telomerase activity (84).

Transgenic animals in agriculture

Carcass composition

Transgenic pigs bearing a human metallothionein promoter/porcine growth-hormone gene construct showed significant improvements in economically important traits such as growth rate, feed conversion and body fat/muscle ratio without the pathological phenotype known from previous growth hormone constructs (69, 78). Similarly, pigs transgenic for the human insulin-like growth factor-I had ~30% larger loin mass, ~10% more carcass lean tissue and ~20% less total carcass fat (79). The commercialisation of these pigs has been postponed due to the current lack of public acceptance of genetically modified foods.

Recently, an important step towards the production of more healthful pork has been made by the creation of the first pigs transgenic for a spinach desaturase gene that produces increased amounts of non-saturated fatty acids. These pigs have a higher ratio of unsaturated to saturated fatty acids in striated muscle, which means more healthful meat since a diet rich in non-saturated fatty acids is known to be correlated with a reduced risk of stroke and coronary diseases (66, 83).

Lactation

The physicochemical properties of milk are mainly due to the ratio of casein variants, making these a prime target for the improvement of milk composition. Transgenic mice have been developed with various modifications demonstrating the feasibility of obtaining significant alterations in milk composition in larger animals, but at the same time, showing that unwanted side effects can occur (50, 89).

Dairy production is an attractive field for targeted genetic modification (44, 106). It may be possible to produce milk with a modified lipid composition by modulating the enzymes involved in lipid metabolism, or to increase curd and cheese production by enhancing expression of the casein gene family in the mammary gland. The bovine casein ratio has been altered by over-expression of beta- and kappa-casein, clearly underpinning the potential for improvements in the functional properties of bovine milk (8). Furthermore, it may be possible to create 'hypoallergenic' milk by knockout or knockdown of the β-lactoglobulin gene; to generate lactose-free milk by a knockout or knockdown of the α-lactalbumin locus, which is the key molecule in milk sugar synthesis; to produce 'infant milk' in which human lactoferrin is abundantly available; or to produce milk with a highly improved hygienic standard by increasing the amount of lysozyme. Lactose-reduced or lactose-free milk would render dairy products suitable for consumption by the large numbers of adult humans who do not possess an active intestinal lactase enzyme system. Although lactose is the main osmotically active substance in milk and a lack thereof could interfere with milk secretion, a lactase construct has been tested in the mammary gland of transgenic mice. In hemizygous mice, this reduced lactose contents by 50% to 85% without altering milk secretion (43). Unfortunately, mice with a homozygous knockout for α-lactalbumin could not nurse their offspring because of the high viscosity of their milk (89). These findings demonstrate the feasibility of obtaining significant alterations in milk composition by applying the appropriate strategy.

In the pig, transgenic expression of a bovine lactalbumin construct in sow milk has been shown to result in higher lactose contents and greater milk yields, which correlated with improved survival and development of piglets (100). Any increased survival of piglets at weaning would provide significant commercial advantages to the producer.

Wool production

Transgenic sheep carrying a keratin-IGF-I construct showed that expression in the skin and the amount of clear fleece was about 6.2% greater in transgenic than in non-transgenic animals. No adverse effects on health or reproduction were observed (22, 23). Approaches designed to alter wool production by transgenic modification of the cystein pathway have met with only limited success, although cystein is known to be the rate-limiting biochemical factor for wool growth (96).

Environmentally friendly farm animals

Phytase transgenic pigs have been developed to address the problem of manure-related environmental pollution. These pigs carry a bacterial phytase gene under the transcriptional control of a salivary-gland-specific promoter, which allows the pigs to digest plant phytate. Without the bacterial enzyme, phytate phosphorus passes undigested into manure and pollutes the environment. With the bacterial enzyme, fecal phosphorus output was reduced by up to 75% (30). Developers expect these environmentally friendly pigs to enter commercial production in Canada within the next few years.

Transgenic animals and disease resistance

Transgenic strategies to increase disease resistance

In most cases, susceptibility to pathogens originates from the interplay of numerous genes; in other words, susceptibility to pathogens is polygenic in nature. Only a

few loci are known that confer resistance against a specific disease. Transgenic strategies to enhance disease resistance include the transfer of major histocompatibility-complex genes, T-cell-receptor genes, immunoglobulin genes, genes that affect lymphokines or specific disease-resistance genes (64). A prominent example for a specific disease resistance gene is the murine Mx-gene. Production of the Mx1-protein is induced by interferon and was discovered in inbred mouse strains that are resistant to infection with influenza viruses (88). Microinjection of an interferon- and virus-inducible Mx-construct into porcine zygotes resulted in two transgenic pig lines that expressed the Mx-messenger RNA (mRNA); unfortunately, no Mx protein could be detected (65). Recently the bovine MxI gene was identified and shown to confer antiviral activity as a transgenic construct in Vero cells (6).

Transgenic constructs bearing the immunoglobulin-A (IgA) gene have been successfully introduced into pigs, sheep and mice in an attempt to increase resistance against infections (57). The murine IgA gene was expressed in two transgenic pig lines, but only the light chains were detected and the IgA-molecules showed only marginal binding to phosphorylcholine (57). High levels of monoclonal murine antibodies with a high binding affinity for their specific antigen have been produced in transgenic pigs (97).

Attempts to increase ovine resistance to Visna virus infection via transgenic production of Visna envelope protein have been reported (15). The transgenic sheep developed normally and expressed the viral gene without pathological side effects. However, the transgene was not expressed in monocytes, the target cells of the viral infection. Antibodies were detected after an artificial infection of the transgenic animals (15).

It has also proved possible to induce passive immunity against an economically important porcine disease in a transgenic mouse model (10). The transgenic mice secreted a recombinant antibody in milk that neutralised the corona virus responsible for transmissible gastroenteritis (TGEV) and conferred resistance against TGEV. Strong mammary-gland-specific expression was achieved for the entire duration of lactation. Verification of this work in transgenic pigs is anticipated in the near future.

Knockout of the prion protein is the only secure way to prevent infection and transmission of spongiform encephalopathies like scrapie or bovine spongiform encephalopathy (98). The first successful targeting of the ovine prion locus has been reported; however, the cloned lambs carrying the knockout locus died shortly after birth (24). Cloned cattle with a knockout for the prion locus have also been generated (19). Transgenic animals with modified prion genes will be an appropriate model for studying the epidemiology of spongiform encephalopathies in humans and are crucial for developing strategies to eliminate prion carriers from farm animal populations.

Transgenic approaches to increase disease resistance of the mammary gland

The levels of the anti-microbial peptides lysozyme and lactoferrin in human milk is many times higher than in bovine milk. Transgenic expression of the human lysozyme gene in mice was associated with a significant reduction of bacteria and reduced the frequency of mammary gland infections (59, 60). Lactoferrin has bactericidal and bacteriostatic effects, in addition to being the main iron source in milk. These properties make an increase in lactoferrin levels in bovine transgenics a practical way to improve milk quality. Human lactoferrin has been expressed in the milk of transgenic mice and cattle at high levels (46, 76) and is associated with an increased resistance against mammary gland diseases (93). Lycostaphin has been shown to confer specific resistance against mastitis caused by *Staphylococcus aureus*. A recent report indicates that transgenic technology has been used to produce cows that express a lycostaphin gene construct in the mammary gland, thus making them mastitis-resistant (95).

Emerging transgenic technologies

Lentiviral transfection of oocytes and zygotes

Recent research has shown that lentiviruses can overcome previous limitations of viral-mediated gene transfer, which included the silencing of the transgenic locus and low expression levels (104). Injection of lentiviruses into the perivitelline space of porcine zygotes resulted in a very high proportion of piglets that carried and expressed the transgene. Stable transgenic lines have been established by this method (36). The generation of transgenic cattle by lentiviruses requires microinjection into the perivitelline space of oocytes and has a lower efficiency than that obtained in pigs (37). Lentiviral gene transfer in livestock promises unprecedented efficiency of transgenic animal production. Whether the multiple integration of lentiviruses into the genome is associated with unwanted side-effects like oncogene activation or insertional mutagenesis remains to be investigated.

Chimera generation via injection of pluripotent cells into blastocysts

Embryonic stem cells with pluripotent characteristics have the ability to participate in organ and germ cell development after injection into blastocysts or by aggregation with morulae (81). True ES cells (that is, those

able to contribute to the germ line) are currently only available from inbred mouse strains (48). In mouse genetics, ES cells have become an important tool for generating gene knockouts, gene knockins and large chromosomal rearrangements (25). Embryonic stem-like cells and primordial germ cell cultures have been reported for several farm animal species, and chimeric animals without germ line contribution have been reported in swine (1, 87, 99) and cattle (13). Recent data indicate that somatic stem cells may have a much greater potency than previously assumed (42, 49). Whether these cells will improve the efficiency of chimera generation or somatic nuclear transfer in farm animals has yet to be shown (48).

Culture of spermatogonia and transplantation into recipient males

Transplantation of genetically altered primordial germ cells into the testes of host male animals is an alternative approach to generating transgenic animals. Initial experiments in mice showed that the depletion of endogenous spermatogenesis by treatment with busulfan prior to transplantation is effective and compatible with re-colonisation by donor cells. Recently, researchers succeeded in transmitting the donor haplotype to the next generation after germ-cell transplantation (38). The major obstacles to this strategy are the lack of efficient *in vitro* culture methods for primordial germ/prospermatogonial cells, and the limitations this imposes on gene transfer techniques for these cells.

Ribonucleic acid interference

Ribonucleic acid interference is a conserved post-transcriptional gene regulatory process in most biological systems, including fungi, plants and animals. Common mechanistic elements include siRNAs with 21 to 23 nucleotides, which specifically bind complementary sequences on their target mRNAs and shut down expression. The target mRNAs are degraded by exonucleases and no protein is translated (75). The RNA interference seems to be involved in gene regulation by controlling/suppressing the translation of mRNAs from endogenous viral elements.

The relative simplicity of active siRNAs has facilitated the adoption of this mechanism to generate transient or permanent knockdowns for specific genes. For transient gene knockdown, synthetic siRNAs can be transfected into cells or embryonic stages. For stable gene repression, the siRNA sequences must be incorporated into a gene construct (14). The conjunction of siRNA and lentiviral vector technology may soon provide a method with high gene transfer efficiency and highly specific gene knockdown for livestock.

Safety aspects and outlook

Biological products from any animal source are unique, and must be handled in a different manner from chemically synthesised drugs to assure their safety, purity and potency. Proteins derived from living systems are heat labile, subject to microbial contamination, can be damaged by shear forces and have the potential to be immunogenic and allergenic. In the USA, the FDA has developed guidelines to assure the safe commercial exploitation of recombinant biological products. A crucial consideration with animal-derived products is the prevention of transmission of pathogens from animals to humans (47). This requires sensitive and reliable diagnostic and screening methods for the various types of pathogenic organisms. Furthermore, it should be kept in mind that all transgenic applications of farm animals will require strict standards of 'genetic security' and reliable, sensitive methods for molecular characterisation of the products. A major contribution towards the goal of well-defined products will come from matrix-assisted laser desorption/ionisation time-of-flight spectometry (40, 91). Meanwhile improvements in RNA isolation and unbiased global amplification of picogram amounts of mRNA enable researchers to analyse RNA even from single embryos (7). This technology can offer insights into the entire transcriptome of a transgenic organism and thereby ensure the absence of unwanted side-effects (40, 91). Rigorous control is also required to maintain the highest possible levels of animal welfare in cases of genetic modification.

Conclusion

Throughout history, animal husbandry has made significant contributions to human health and well-being. The convergence of recent advances in reproductive technologies (*in vitro* production of embryos, sperm sexing, somatic nuclear transfer) with the tools of molecular biology (gene targeting and array analysis of gene expression) adds a new dimension to animal breeding (68). Major prerequisites for success and safety will be the continuous refinement of reproductive biotechnologies and a rapid completion of genomic sequencing projects in livestock. The authors anticipate that within the next five to seven years genetically modified animals will play a significant role in the biomedical arena, in particular via the production of valuable pharmaceutical proteins and the supply of xenografts (Table II). Agricultural applications are already being prepared (39), but general public acceptance may take as long as ten years to achieve. As the complete genomic sequences of all farm animals become available, it will be possible to refine targeted genetic modification in animal breeding and to develop strategies to cope with future challenges in global agricultural production.

Glossary

Artificial chromosome

A construction composed of the basic elements of a normal chromosome into which one or more transgene sequences may be introduced. After introduction into the nucleus, this artificial chromosome is replicated and distributed to daughter cells just like a normal chromosome. The transgene expression is more predictable as it is not influenced by integration into an unknown location.

Expression pattern

A representation of relative levels of gene expression in the various cells and tissues of a transgenic animal. This is primarily determined by the promoter, but is also dependent on the position in the genome where the construct has become integrated. As cells differentiate, certain chromosomal regions are permanently turned off.

Homologous recombination

A transgene sequence is constructed which matches the sequence of the endogenous target gene. This biases insertion to the target gene. The design of the gene construct determines whether the target gene will be rendered inactive (knocked out) or simply modified.

Inducible promoter

A promoter that can be activated by an environmental signal such as the introduction of a heavy metal or an antibiotic.

Knockout

Removal of a gene or part of a gene to eliminate a particular protein product.

Lentivirus

One of several types of virus that have been evaluated for their ability to carry genes into early-stage embryos for the purpose of introducing a transgene. The transgene is placed within the viral genome and is carried into the target embryo by the viral infection mechanism.

Microinjection

A now routine procedure in which a glass micropipette is used to deliver a small amount of DNA solution (the transgene) into the nucleus of a fertilised egg. In a small percentage of these injected eggs, the transgene becomes incorporated into one of the chromosomes and so will be present in all subsequent cell divisions and in the germ cells, so that future offspring will also carry the transgene.

Mosaicism

The situation where an individual is composed of cells of more than one genetic background. Transgene integration takes place after the first round of cell division; thus, only a portion of the cells in the resulting individual will be transgenic. It is important that the germ cells carry the transgene so that subsequent offspring will also be transgenic.

Multiple transgenic

A transgenic organism carrying more than one transgene. This can be achieved either by introduction of several transgenes at the same time, or by obtaining cells or embryos from an existing transgenic animal and using these for a second round of transgenesis. This is most efficiently achieved by nuclear-transfer-based transgenesis because the transfected cells can be screened prior to the production of an embryo.

Promoter

The control sequence that determines in which tissue or cell type and at which point in time a gene will be expressed. Some promoters are universally active, and some are active only in specific cell types or developmental stages.

Reporter gene

A sequence that codes for a detectable product such as a fluorescent protein or an enzyme which produces a visible product.

Somatic nuclear transfer

In its most common form, this involves removal of the nucleus from a fertilised egg and replacement with the nucleus of a differentiated somatic cell such as a fibroblast. The result is a cloned embryo that has the genetic composition of the nuclear donor cell.

Spermatogonia

Early developmental precursors of sperm that can be removed from the testes, transfected with a transgene, and then re-implanted to develop into mature sperm that carry the transgene.

Targeted integration

Sequences have been discovered (FLP, Cre/lox) that cause the insertion or removal of intervening gene sequences. When properly used, these systems can direct insertion to a specific location in the genome, or can at some point in the future remove portions of a transgene that are no longer needed (such as the antibiotic resistance sequences used to select transfected cells).

Transfection

A process mediated by an electrical pulse (electroporation), or by treatment with agents that make the cell membranes porous, such that DNA molecules in the culture medium are able to pass into the cell where they can be expressed or integrated into the genome.

Animaux d'élevage transgéniques : le présent et l'avenir

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Résumé

Jusqu'à une date récente, la microinjection pronucléaire d'acide désoxyribonucléique (ADN) était la méthode standard utilisée pour la production d'animaux transgéniques. Cette technique est actuellement remplacée par des protocoles plus efficaces basés sur le transfert nucléaire de cellules somatiques permettant également de réaliser des modifications génétiques ciblées. De même, les vecteurs lentiviraux et la technique de l'acide ribonucléique (ARN) interférent deviennent des outils importants de la transgenèse. Les animaux d'élevage transgéniques ont une importance en médecine humaine comme sources de protéines biologiquement actives, comme donneurs pour la xénotransplantation et à des fins de recherche en thérapie cellulaire et génique. Parmi les applications agricoles habituelles figurent l'amélioration de la composition des viandes, de la production laitière et lainière, de la résistance aux maladies, de même que la réduction de l'impact sur l'environnement. La sécurité sanitaire des produits peut être assurée par la standardisation des procédures et contrôlée par l'application de la réaction en chaîne par polymérase et de la technologie du criblage de filtres à ADN. Avec l'affinement des données relatives aux séquences et des cartes génomiques des animaux d'élevage, la suppression ou la modification de certains gènes devient de plus en plus réalisable. Cette méthode de reproduction d'animaux jouera un rôle déterminant dans la résolution des problèmes mondiaux qui se poseront demain en matière de production agricole.

Mots-clés

Animaux d'élevage – Application agricole – Avantage environnemental – « Gene pharming » – Microinjection – Séquence courte à interférence ARN – Transfert nucléaire – Transgénique – Vecteur lentiviral – Xénotransplantation.

Presente y futuro del ganado transgénico

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Resumen

Hasta hace poco tiempo, el procedimiento de rigor para obtener animales transgénicos era la microinyección pronuclear de ácido desoxirribonucleico (ADN). Ahora se empiezan a aplicar otros métodos más eficaces basados en la transferencia de núcleos de células somáticas, que además permiten inducir cambios genéticos de manera más específica. El uso de vectores lentivíricos y la técnica del ARN interferente pequeño son otros dos métodos de transgénesis cada vez más empleados. El ganado transgénico es importante en medicina humana porque de él se extraen proteínas biológicamente activas y tejidos u órganos para xenotrasplantes y porque proporciona material para investigar terapias celulares y génicas. En el terreno de la producción animal, sus aplicaciones van desde la mejora de la composición de carne, el rendimiento lechero y la producción de lana hasta el aumento de la resistencia a las enfermedades o la atenuación de los efectos ambientales de la ganadería. La estandarización de protocolos puede servir para garantizar la inocuidad de los productos obtenidos, y técnicas como la reacción en cadena de la polimerasa (PCR) o la de matrices (*arrays*) de ADN para efectuar los correspondientes controles. A medida que se perfeccionan los mapas genómicos del ganado y se conoce mejor su secuencia génica va resultando cada vez más fácil eliminar o modificar genes concretos. En el futuro, estos sistemas de selección animal serán básicos para resolver los problemas de producción agropecuaria en el plano mundial.

Palabras clave

Aplicación agropecuaria – ARN interferente pequeño – Beneficio ambiental – Ganado – "Gen pharming" – Microinyección – Transferencia nuclear – Transgénico – Vector lentivírico – Xenotrasplante.

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