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Transgenic Animals

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Abstract
Transgenesis is the process of introducing an exogenous gene called a transgene into a living organism so that the organism will exhibit a new property and transmit that property to its offspring. Transgenesis can be facilitated by liposomes, plasmid vectors, viral vectors, pronuclear injection, protoplast fusion, and ballistic DNA injection. Transgenic organisms are able to express foreign genes because the genetic code is similar for all organisms. This means that a specific DNA sequence will code for the same protein in all organisms.

Keywords: transgenesis, organisms, DNA

1. Introduction

Methods of creation of transgenic animals

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.

1.1 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. It is one of the first methods that proved to be effective in mammals (Gordon and Ruddle, 1981). 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 manipulated fertilized ovum is transferred into the oviduct of a recipient female or foster mother that has been induced to act as a recipient by mating with a vasectomized male. A major advantage of this method is its applicability to a wide variety of species.

1.2 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 that have the potential to differentiate into any type of cell (somatic and germ cells) and therefore to give rise to a complete organism. These cells are then incorporated into an embryo at the blastocyst stage of development. The result is a chimeric animal. ES cell-mediated gene transfer is the method of choice for gene inactivation, the so-called knock-out method.

This technique is of particular importance for the study of the genetic control of developmental processes. It works particularly well in mice has the advantage of allowing precise targeting of defined mutations in the gene via homologous recombination.

1.3 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.

2. Transgenic animals

2.1 Transgenic Mosquitoes

Mosquitoes are notorious for their ability to transmit diseases such as malaria and Dengue fever. In addition to traditional mosquito control strategies, researchers are developing transgenic mosquitoes with impaired competency to transmit pathogens. Efforts to control the disease are hampered by drug resistance in the Plasmodium parasites, insecticide resistance in mosquitoes, and the lack of an effective vaccine. Because mosquitoes are obligatory vectors for malaria transmission, the spread of malaria could be curtailed by rendering them incapable of transmitting parasites.

Many of the tools required for the genetic manipulation of mosquito competence for malaria transmission have been developed. Foreign genes can now be introduced into the germ line of both culicine and anopheline mosquitoes, and these transgenes can be expressed in a tissue-specific manner.

Recombinant Sindbis viruses and transposable elements are commonly used to express heterogenous genes in mosquitoes.

Oxitec which is a British bio-tech company, has created genetically modified mosquitoes, which are programmed for sudden, early death. Oxitec's technology is a variation of a proven process called "sterile insect technique" It involves irradiating male insects, causing mutations that make them sterile. When released into the wild, they mate with females passing on lethal genes which either kills the female or at least kills the young in her so then she fails to reproduce. Scientists at this British biotech company said they have evidence that their genetically modified mosquitoes can by this way for sure control the spread of dengue fever.

Although its mode of action is unknown, phospholipase A2 (PLA2) from a variety of sources significantly inhibited ookinete invasion of the mosquito midgut epithelium when mixed with an infected blood meal (Zieler *et al.* 2001) [35]. Moreira *et al*. (2002) [13, 20] have shown that transgenic mosquitoes expressing bee venom PLA2 from the carboxypeptidase promoter reduced *P. berghei* oocyst formation by 87%. Transgenic *An. stephensi* expressing PLA2 from the AgAper1 promoter inhibited oocyst formation to about the same extent (E. G. Abraham, M. Donnelly-Doman, H. Fujioka, A. Gosh, L. Moreira and M. Jacobs-Lorena, unpublished observations).

An alternative strategy is to use synthetic molecules to interfere with parasite development. Ghosh *et al*. (2001) [9]screened a phage display library for protein domains that bind to the midgut and salivary gland epithelia and identified a short peptide, named SM1, which inhibited parasite invasion. Furthermore, An. stephensi engineered with a synthetic gene expressing a SM1 tetramer under the control of the carboxypeptidase promoter were impaired in supporting *P. berghei* development. Moreover, in two out of three experiments, parasite transmission by the transgenic mosquitoes was completely blocked (Ito *et al*. 2002) [13, 20]. This inhibition is thought to occur by peptide binding to epithelial cell surface proteins (putative receptors) required for parasite invasion. The effectiveness of SM1 in inhibiting the development of parasites that cause malaria in humans remains to be demonstrated.

Wolbachia are bacteria that infect many insects, including mosquitoes. However, *Wolbachia* do not naturally infect Anopheles mosquitoes, which are the type that spreads malaria to humans. Researchers at the Johns Hopkins Bloomberg School of Public Health found that artificial infection with different *Wolbachia* strains can significantly reduce levels of the human malaria parasite, *Plasmodium falciparum*, in the mosquito, *Anopheles gambiae*. The investigators also determined that one of the *Wolbachia* strains rapidly killed the mosquito after it fed on blood. According to the researchers, *Wolbachia* could potentially be used as part of a strategy to control malaria if stable infections can be established in Anopheles.

2.2 Genetically modified pigs

Genetic modification of domestic animals can now be accomplished by a variety of methods (Robl *et al.* 2007) [25]. Genetic modification in mammals was initially via pronuclear injection. In essence, a DNA construct was injected directly into one of the pronuclei of a zygote (Brinster, 1981) [3]. This technique was initially established in mice, but later applied to a variety of mammals (Wall *et al.* 1997). The next technique is that of oocyte transduction. Mature oocytes arrested at metaphase II of meiosis are first isolated. Then a replication incompetent retrovirus is injected between the zona pellucida and the oocyte plasma membrane. After an opportunity is provided for the retrovirus to infect the oocyte and integrate into the chromosomes, then the oocyte is fertilized. This procedure is highly efficient as integration occurs only if the nuclear envelop is absent; and the oocyte is arrested in metaphase II of meiosis. Oocyte transduction was applied to cattle (Chan *et al.* 1998) [5] and then to the pig (Cabot *et al.* 2001) [4]. The third technology for creating transgenic pigs is that of sperm-mediated gene transfer (SMGT). In SMGT the DNA construct of interest is mixed with the sperm and then used for in vitro fertilization or for insemination (Lavitrano *et al.* 2006).

2.2.1 Medical reasons to genetically modify pigs

Xenotransplantation

Due to similarities in physiology, and size, pigs have been considered a source of organs for transplantation to humans. Pigs have short gestation time and large litter size, first cloned pig derived from a blastomere nucleus as a donor (Prather *et al.* 1989) [23]. Compared to non-human primates (NHPs), pigs have less chance to transmit infectious diseases to human, and have fewer ethical issues as organ donors. Unfortunately, when a pig organ or cells are transferred to a primate the cells are immediately lysed. This is due to the presence of pre-formed antibodies in the primate that recognize a specific cell surface carbohydrate; a terminal α-1, 3-galactose epitope on the cell surface. The gene in the pig that is responsible is called α-1, 3-galactosyltransferase (GGTA1). This gene is not functional in humans and Old World monkeys. It has been reported that, in humans, up to 1% of the total circulating IgG is anti-α 1, 3Gal natural antibody (Galili *et al*. 1985).

The pre-formed antibodies immediately recognize the α-1,3Gal epitope, which then activate the complement system and result in hyperacute rejection (HAR) of the transplanted cells or organs/tissues from pigs. To overcome this major obstacle in xenotransplantation, a number of strategies have been employed to reduce or eliminate α-1,3 Gal induced HAR. These methods include overexpression of α-2,3-sialyltransferase or α1,2- fucosyltransferase in pig cells to compete with α1,3GT; treatment of pig organs with α-galactosidase to remove surface α-1,3Gal epitope (Costa *et al.* 2002); expression of complement inhibitor genes, such as human decay-accelerating factor (DAF), CD59 or CD46 (membrane cofactor protein (MCP) in transgenic pig organs to suppress the complement reaction (Loveland *et al*. 2004); and temporary depletion of natural anti α-1,3Gal antibody from recipients prior to and after transplantation (Zhong *et al*. 2003). However, all these methods only partially removed the α1,3 Gal from the surface of the xenografts, or temporarily removed the anti-α-1,3 gal antibodies from recipients. The anti-α-1, 3 gal antibodies will come back as soon as the treatment stops, and the residual α1,3 Gal molecules on pig cells are still sufficient to activate the complement cascade and cause destruction of the grafts (Galili 2001).

Another possible solution to this problem of HAR is to disrupt or knock-out the gene i.e. GGTA1, that is responsible for production of α-1, 3 Gal epitopes. Once that has been accomplished (Dai *et al.* 2002) and homozygous knock-out

pigs produced (Kolber-Simonds), then the organs could be transplanted. Xenotransplantation experiments have shown that if GGTA1 is knocked out the life of the kidney and hearts could be extended without the HAR (Chen *et al.* 2005).

With a possible solution to the HAR, the focus has moved to the delayed xenograft rejection (DXR) or acute vascular rejection (AVR). The exact cause of AVR is still unknown, but may be due to antibodies against non-α-1,3Gal xeno-antigens, molecular incompatibilities of coagulation regulation between pig and NHPs, and innate cellular responses from NK cells and macrophages. Many proposals have been made to deal with AVR. Most of them are to make transgenic GGTA1 KO pigs with human genes which have specific functions. Some examples are: using human DAF, CD46 or CD59 to inhibit complement activity (van den Berg *et al.* 2000); using anti-coagulate gene tissue factor pathway inhibitor (TFPI) and CD39, or nucleoside triphosphate diphosphohydrolase I (NTPDase-1) to suppress micro thrombosis (Dwyer *et al.* 2004); using HLA-G or HLA-E to inhibit NK cells (Crew, 2007); using hemoxygenase-1 (HO-1), A20 and death decoy receptor (TR6/DcR3) genes (Akamatsu *et al.* 2004) [1]. to protect endothelial cells from inflammatory damage and apoptosis, as well as using CTLA4Ig to inhibit T cells activation (Wekerle *et al.* 2002).

Other proposals include removing potential rejection-related pig genes such as Intercellular adhesion molecular-1 (ICAM-1) or co-stimulatory molecules by directly knocking out those pig's genes or using siRNA technology to inhibit their expression. To date, several such transgenic pigs have been generated either on a wild-type pig background or GGTA1 knock-out background. GGTA1 knock-out pigs with a human DAF have been produced and available in National Swine Resource and Research Center. GGTA1 knock-out pigs with human α-1,2-fucosyltransferase gene to modify the carbohydrate has been reported (Ramsoondar *et al.* 2003). One group has added the human leukocyte antigen in transgenic pigs (Tu *et al.* 2000). A human tumor necrosis factor-alpha-related apoptosis-inducing ligand (TRAIL) transgenic pig showed the transgenic pig lymphocytes could induce apoptosis of human lymphoid cells (Klose *et al.* 2005).

Transgenic pigs with human CD39 have been generated to prevent the formation of thrombosis in transplanted organs (Dwyer *et al.* 2007) [6]. Petersen *et al.* recently reported the production of hHO-1/hDAF transgenic pigs by nuclear transfer. Kidneys from hHO-1/DAF transgenic pigs survived ex vivo perfusion for 240 min with AB-pooled human blood and exhibited no indication for xenogenic activation of the human coagulation system. On the contrary, kidneys from wild-type pigs only lasted for 60 min in ex vivo perfusion (Petersen *et al.* 2008). The same group also reported birth of seven healthy piglets carrying human A20 gene driven by a CMV-β-actin promoter (Oropeza *et al.* 2008) [21]. Many other transgenic pigs on the GGTA1 knock-out background are under development and we expect the results will be published in the near future. Successful xenotransplantation will likely require multiple modifications to overcome both innate immunity as well as the acquired immunity that will be generated against the molecules on the surface of the pig organ.

Pharmaceuticals

An interesting application of pharmaceutical production in pigs is the production of human hemoglobin in the blood of pigs (Sharma *et al.* 1994) [26]. It is thought that isolation of the human hemoglobin from the blood of the transgenic pig may provide a source for treating trauma patients. It may seem surprising, but pigs have also been used to produce pharmaceuticals in their milk. While pigs are not usually considered a dairy breed of livestock, they have been used to produce Protein C, an in-activator of coagulation factors Va and VIIIa (Van Cott *et al.* 2001), as well as human coagulation factor VIII and IX (Lindsay *et al.* 2004) in their milk. Interestingly the pig mammary epithelial cells are unique among livestock in making the complex post-translational modifications needed for FIX and FVIII biological activity (Van Cott *et al.* 2004).

Models of human disease

Pigs have also been genetically modified in attempts to create better models of human disease.

Retinitis Pigmentosa

The first model of human disease to be discussed is a line of pigs that has a mutated version of the rhodoposin gene that results in pigs getting retinitis pigmentosa (RP). This model (Banin *et al.* 1999; Petters *et al.* 1997) [2]. has a Pro347Leu mutation. These animals develop early and severe rod loss, likely resulting in subsequent degeneration of the cones in a pattern that mimics rod and cone loss in humans. The size and physiology of the pig eye is, again, similar to humans and thus these pigs will clearly be very valuable for preclinical studies (Ghosh *et al.* 2007) [10].

Cardiovascular Disease

Polyunsaturated fatty acids (PUFAs) are fatty acids with 18 or more carbon atoms and two or more double bonds. There are two major groups of PUFAs, omega-6 (n-6) and omega-3 (n-3), depending on the position of the double bond nearest the methyl end of the fatty acid. Both n-3 and n-6 PUFAs are significant structural components of the phospholipids membranes of tissues throughout the body and n-3 PUFAs are especially rich in the retina and brain (Sperling, 2006). Mammals lack the desaturases necessary to synthesize both n-6 andn-3 PUFAs. Furthermore, the n-3 and n-6 PUFAs are not inter-convertible in mammalian cells because mammals also lack the enzyme, omega-3 fatty acid desaturase. These PUFAs are essential fatty acids (EFAs) to mammals, and they must be acquired from the diet Simopoulos, 2003) [29].

There has been an enormous increase in the consumption of n-6 PUFAs due to the increased intake of vegetable oils from corn, sunflower seeds, Cotton seed, and soybeans, which all contain high levels of n-6 PUFAs but very little n-3 PUFAs. In addition, intake of n-3 PUFAs is much lower today because of the industrial production of animal feeds rich in grains containing n-6 fatty acids, leading to production of meat and eggs rich in n-6, but poor in n-3 PUFAs (Simopoulos, 2006). It has been proposed that the high n-6/n-3 PUFA ratio may contribute to the high prevalence of many modern human diseases such as CVD, diabetes, obesity, cancer and depression (Simopoulos, 2006).

The fat-1 transgenic pig is an excellent unique large animal model to study the preventive effects against atherosclerosis and the underlying mechanisms by n-3 PUFAs and balanced n-6/n-3 ratio. First, pigs have very similar physiology and lipid metabolism to humans, and pigs are a useful model for the evaluation of atherosclerosis from the perspective that lesions

develop spontaneously, their circulatory system and localization of lesions are similar to humans, and the lesions are responsive to dietary intervention by exhibiting regression after prolonged periods (Fan and Watanabe,2000) [8]; Second, the fat-1 transgenic pigs have significantly high n-3 PUFAs concentration and an ideal n-6/n-3 ratio.

Diabetes

Diabetes is a disease that is becoming more of a problem in the high fat diet and sedentary lifestyle. When pigs are active they remain lean, but when they become sedentary and on a high fat diet they have a propensity to develop type 2 diabetes (Dyson *et al.* 2006) [7]. Recently a pig has been genetically modified to create a model of type 2 diabetes. This group used a lentiviral vector to deliver a dominant negative glucose-dependent insulinotropic polypeptide receptor under the control of the rat *Ins2* promoter. These animals had a reduced insulin release and higher glucose levels as compared to non-transgenic littermates as measured in response to an oral glucose tolerance test (Renner *et al.* 2008) [24]. Genetically modified animals like these will be very useful for the study of diabetes.

Huntington's disease

Huntington's disease is associated with a CAG repeat in a gene called 'huntington'. As the length of the trinucleotide repeat increases the onset occurs at an earlier age (Macdonald *et al.* 1993; Rosenblatt *et al.* 2001). Huntington's disease is inherited as an autosomal dominant disease that gives rise to progressive neural cell death associated with choreic movements and dementia. In an attempt to make a better model to study Huntington's disease, the pig Huntington gene was cloned from a miniature pig and combined with a rat neuron-specific enolase promoter and injected into the pronuclei of pig zygotes. After embryo transfer five transgenic pigs were produced (Uchida *et al.* 2001) [31].

Cystic Fibrosis

Cystic fibrosis is the most common genetic disease in adolescents caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. This aberrant protein results in less movement of chloride ions across the membrane. When this mutation is introduced into mice they do not exhibit all of the expected phenotypes. While they do have gastrointestinal problems, they do not exhibit any airway disease (Grubb and Gabriel, 1997) either because mice have a compensatory mechanism that rescues pulmonary function, or the lifespan of the mouse is insufficient to develop the disease. Thus there is no model to study cystic fibrosis induced lung disease except for the affected children. Since the pig lung shares many physiological properties with humans, Michael Welsh's laboratory at the University of Iowa and our group (RSP) at Missouri moved forward to knockout the CFTR gene in the pig. In addition a version that has a deletion of the 508th amino acid (phenyalanine) has been created. This is the most common mutation in North American Caucasians. Heterogygote animals with the knockout mutation have been created (Rogers *et al.* 2008) and have successfully transmitted the mutation to both male and female offspring.

Alzheimer's disease

Recently a modification has been introduced to generate pigs that may exhibit symptoms of Alzheimer's disease. This was accomplished by inserting a mutated version of the "Swedish mutation" of the amyloid precursor protein gene (Kragh *et al.* 2008). While it is not yet known if the animals will develop the disease it represents a first step into this important area of human medicine.

Cell tracking

Molecular markers which permit identification of cells within a mixed population have proven to be immensely useful for a variety of studies. A number of lines of pigs transgenic for the enhanced green (Cabot *et al.* 2001; Park *et al.* 2001b) [4, 22]. red, blue (Webster *et al.* 2005) and orange (Matsunari *et al.* 2008) versions of these fluorescent proteins have been made. The utility of these cells can be illustrated. The first is a study where Multipotent Adult Progenitor (MAP) cells were isolated from the blood of a mature female pig. It was possible to differentiate these MAPs into a variety of cell types, including hollow endothelial tubules, astrocytes and glial cells, osteoblasts, adipocytes, and smooth muscle (Price *et al.* 2006) [23]. In addition, the ability of these cells to differentiate into neuronal cells and to establish connections to endogenous neurons after injection into rat brains could be easily tracked since they were fluorescent.

Another example began in 2002 when Dr. Mike Young (Harvard) and Henry Klassen (UC-Irvine) suggested using the eGFP expressing pig eye for a tracking study of ocular regeneration. The eGFP retinal progenitor cells are transplanted into the damaged eye. After up to 10 weeks the eyes are harvested where sections are cut and the contribution of eGFP expressing cells to the different cell types of the retina are determined (Klassen *et al.* 2004; Shatos *et al.* 2004) [27]. The retinal progenitor cells appear to migrate to the area of damage, integrate into the different layers of the retina, express retinal-specific markers and morphologically appear to differentiate into rods and cones.

Human/pig hybrid organs

In an attempt to produce human hepatocytes that can be transferred to humans that have impaired liver function two lines of pigs have been made that have a liver-specific promoter (albumin or alpha-fetoprotein) driving a suicide gene (thymidine kinase or cytosine deaminase; Beschorner *et al.* 2003a). The goal here was to transfer human hepatocytes to a pre-immune transgenic fetal pig. After the pig is born and reaches adult size, the pig would be treated with the appropriate precursor (ganciclovir or 5-fluorocytosine, respectively). The precursor would be taken up into all the cells in the transgenic/chimeric pig, but only in the pig hepatocytes would the precursor be converted into a toxic analog and destroy those cells. The surviving human hepatocytes would regenerate and harvested for transfer into humans that need hepatocytes. While this process is still under development it is showing promise (Beschorner *et al.* 2007).

Bioartificial liver support

One group has attempted to develop a bioartificial liver support system to treat patients with severe liver failure. Here a human albumin gene was introduced into a pig (Naruse *et al.* 2005). The gene was expressed in the pig liver. The authors suggest that pig livers producing major human hepatic proteins would be ideal to minimize xenogenic protein influx. Further development and characterization of pigs with a similar modification is warranted. Levy *et al* reported two successful extracorporeal hepatic supports with transgenic

(hDAF/hCD59) porcine livers used as a bridge to human liver transplantation (Levy *et al.* 2000) [15].

2.2.2 Agricultural reasons to genetically modify pigs

A number of genes have been inserted in pigs to either enhance growth and production traits, or to alter the product so that it is more environmentally safe, leaner or healthier for human consumption. These additions include the genes for growth hormone (GH1; Hammer, 1985), insulin-like growth factor 1 (IGF1; Pursel *et al.* 1999) [12], phytase (Golovan, 2001) [11], B-cell CLL/lymphoma 2 (BCL2; Guthrie *et al.* 2005) [12], delta 12 fatty acid desaturase (Saeki *et al.* 2004), omega-3 desaturase (hfat-1; Lai *et al.* 2006), and bovine alpha-lactalbumin (LALBA) (Bleck, 1998). Some of the modifications may improve productivity of the animal (GH1, IGF1), while others are intended to alter the composition of the carcass so that it may be more healthy for the animal, and for humans to consume (delta 12 fatty acid desaturase, hfat-1). One modification was envisioned to increase the number of viable eggs that a female would produce (BCL2), another to increase the weaning weight of piglets (LALBA), and another was to help reduce pollution caused by elimination of inorganic phosphorous (phytase).

Other modifications envisioned make the animals more or less susceptible to disease, or more tolerant to heat stress. While none of these modifications have been approved to enter the human food chain, the above modifications show that many of these ideas can be reduced to practice and result in a possible economic or health benefit to the producer and consumer.

2.3 Transgenic Cattle

A genetically altered cow that can produce cancer-fighting proteins for humans has been bred by Chinese researchers. The cow, capable of producing CD20 antibodies in its milk, was born in Beijing and a dozen more are due to be born. Beijing- Chinese scientists have genetically modified dairy cows to produce human breast milk, and hope to be selling it in supermarkets. The milk produced by the transgenic cows is identical to the human variety, with the same immune-boosting and antibacterial qualities as breast milk, scientists at China's Agricultural University in Beijing said. The transgenic herd of 300 was bred by inserting human genes into cloned cow embryos which were then implanted into surrogate cows. The technology used was similar to that used to produce Dolly the sheep, the first mammal to be cloned by scientists, in Scotland. The milk is still undergoing safety tests, but with government permission it will be sold to consumers as a more nutritious dairy drink than cow's milk.

The scientists have also produced animals that are resistant to mad cow disease, as well as beef cattle that are genetically modified to produce more nutritious meat. A model proposed by Mercier (1987) [19] has the reduction of the lactose content of milk as its objective. In the milk of transgenic sheep and cattle carrying a lactose gene, combined with an udder specific promoter, lactose is split into galactose and glucose. Such milk could then also be consumed by the large percentage of the world population suffering from lactose intolerance.

2.4 Transgenic sheep

Dolly was a female domestic sheep, and the first mammal to be cloned from an adult somatic cell, using the process of nuclear transfer (McLaren, 2000) [17]. She was cloned by Ian Wilmut, Keith Campbell and colleagues at the Roslin Institute and the biotechnology company PPL Therapeutics near Edinburgh in Scotland. She was born on 5 July 1996 and she lived until the age of six, at which point she died from a progressive lung disease (Wilmut *et al.* 1997) [33]. It was with this kind of scenario in mind that, shortly after the announcement of Dolly, the same laboratory announced the birth of Polly (Schnieke *et al.* 1997) [33]. Polly was cloned from transgenic fetal sheep fibroblasts that contained the gene for human clotting factor IX, with the replacement of the sheep collagen gene with an expression cassette designed to target expression of human factor IX to milk, a gene whose function is deficient in hereditary hemophilia (McCreath *et al*. 2000) [18]. Tracy (1990-1997) was a transgenic ewe that had been genetically modified by the Roslin Institute, near Edinburgh, Scotland, so that her milk produced a human protein called alpha antitrypsin, a potential treatment for the disease cystic fibrosis and emphysema (Wright, *et al*. 1991) [34]. The Roslin Institute is one of the world's leading centres for animal research with internationally recognized programmes on genetics, genomics, early development, reproduction, animal behaviour and welfare, and has pioneered methods for the genetic modification and cloning of farm animals.

Genetically modified sheep which contain an extra copy of a sheep growth hormone gene were produced by CSIRO researchers in Australia. The study found out that genetically modified (GM) sheep could boost the production of sheep products. The study has found that GM sheep grow faster, bigger, leaner and produce more milk and wool than their non-GM counterparts (Ward *et al.* 1986). Norm Adams, who led the CSIRO study, said that the use of GM sheep could boost production, particular sheep milk products such as yoghurts and cheeses. However, excess growth hormone could cause the GM sheep's hooves to overgrow and so require regular clipping. GM sheep were also leaner, which could result in health problems, and some are susceptible to diabetes.

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