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Transgenic Animals-Review Paper

Renald Blundell
Department of Physiology and Biochemistry,
Biomedical Science Building, Faculty of Medicine and Surgery,
University of Malta, Msida MSD06, Malta

Developments in the biological sciences in the last years have changed mankind's ability to manipulate the genetics, cell biology and physiology of biological organisms. These techniques, collectively termed biotechnology, create the opportunity for modifying domestic animals in ways that markedly increase the efficiency of production. Among the procedures being developed for animal production systems are marker-assisted selection of specific alleles of a gene that are associated with high production, production of transgenic animals, production of large amounts of previously-rare proteins through use of genetically-engineered bacteria or other cells and identification of new biologically-active molecules as potential regulators of animals function.

Transgenic animals are animals in which part of a foreign gene (i.e., transgene) is inserted in their genome. A typical strategy for producing a transgenic animal is illustrated in Fig. 1. The vast majority of transgenic animals (mice) have been produced to answer basic research questions. Molecular biologists have used this technology to characterise genetic regulatory elements. In some systems such as mammary glands that lack good cell culture models, transgenic animals are one of the few approaches available to researchers to identify which genetic sequences confer tissue specificity, developmental gene regulation and feedback control of gene expression.

Transgenic technology has been used to perturb homeostasis of various systems to study immunology, neurology, development, thryroid function, circulatory and cardiac function, intermediary metabolism, muscle development, bone growth, haemoglobin switching and reproduction[1].

Transgenic animals have also been used to generate a wide array of disease models, such as those for sickle cell disease, prostatic hyperplasia, atherosclerosis, retinoblastoma, diabetes mellitus, learning impairment and cystic fibrosis[3]. In all studies mentioned, the mouse served as the animal model. For many of these studies a

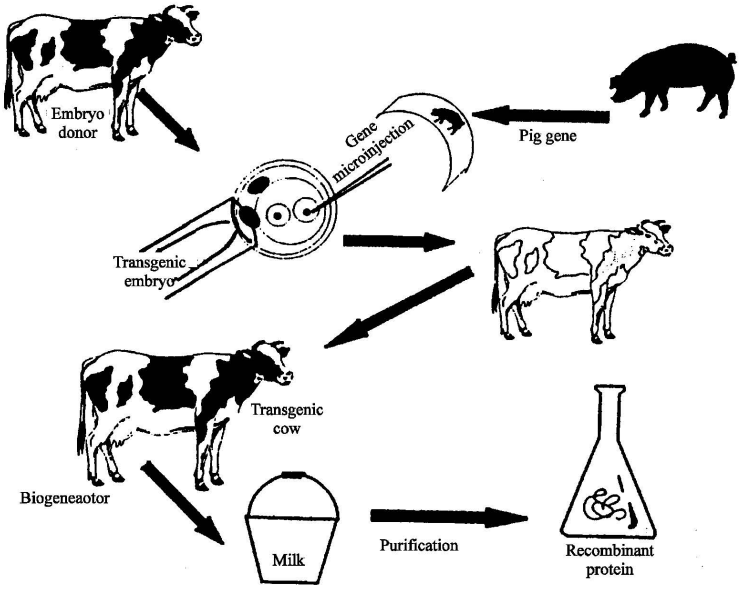

Fig. 1: Typical strategy for producing a transgenic animal. In the study illustrated, the goal is to produce a transgenic cow that secretes a pig protein into milk[2]

larger animal model would be desirable[1]. Examples of diseases studied in farm animal models are shown in Table 1.

Table 1: Animal models of human disease[4]

Animal group	Biomedical problem	Specific disease
Cattle	Genetic/development defect	Chediak-Higashi syndrome
		Hereditary parakeratosis
		Hereditary syndactyly
		Hereditary thymic hypoplasia, zinc deficiency, lethal trait A 46
		Hydrocephalus Tibial hemimelia
	Neoplastic disease	Lymphosarcoma
		Ultimobranchial thyroid tumors
	Metabolic/nutritional disease	Glycogenosis, Type II
		GM1 gangliosidosis
		Mannosidosis, induced
		Mannosidosis, spontaneous
		Osteopetrosis
	Degenerative disease	Pancreatolithiasis
	Infectious disease	Ostertagiasis rotaviral enteritis venereal vibriosis
Sheep	Genetic/developmental defect	Muscular dystrophy
		Dubin-johnson syndrome
		Ilberts syndrome
	Neoplastic disease	Adenocarcinoma, intestine
		Lymphosarcoma
		Pulmonary carcinoma
	Metabolic/nutritional disease	Congenital goiter
		Copper poisoning, chronic
		Glucose-6-phosphate dehydrogenase deficiency
		Glutathione deficiency
		Mannosidosis, induced
		Photosensitivity
	Degenerative disease	Prosthetic cardiac valves
		Ceroid-lipofuscinosis
		Anti-BM glomerulonephritis
	Infectious disease	Bluetongue Jaagziekte
Goat		Hereditary myotonia
Horse		Agammaglobulinemia, X-linked Anti-GMB nephritis
		Combined immunodeficiency (severe)
		Exotosis, multiple, hereditary
		Infectious anaemia
		Lymphosarcoma
		Mannosidosis, induced
		Selective IgM deficiency
		Thrombocytopenia purpura
		Vitiligo
Pig		ArthritisCerebrospinal lipodystrophy GM_2 Gangliosidosis
		Hypervitaminosis A
		Lactational osteoporosis
		Lymphosarcoma
		Malignant hyperthermia
		Melanoma Ochratoxicosis
		Vitiligo von Willebrand's disease

Table 2: Examples of human protein under development in milk of transgenic animals[7]

Protein	Use	Species
α-1-anti-protease inhibitor	Inherited α-1-antitrypsin deficiency	goat
α-1-antitrypsin	anti-inflammatory	goat, sheep
anti-thrombin III	sepsis and disseminated intravascular coagulation resulting from genetic or acquired deficiency	goat
collagen	burns, bone fraction, urinary incontinence	cow
Factor IX	haemophilia	sheep, pig
Factor VIII	haemophilia	pig
fibrinogen	fibrin glue, burns, surgery, localised chemo-therapeutic drug delivery	pig, sheep
human fertility hormones	infertility, contraceptive vaccines	goat, cow
human haemoglobin	blood replacement for transfusion	pig
human serum albumin	surgery, burns, shock, trauma	goat, cow
lactoferrin	bacterial gastro-intestinal infection	cow
LatPA	venous stasis ulcers	goat
monoclonal antibodies	anti-colon cancer	goat
protein C	protein C deficiency, adjunct tPA therapy to prevent clot formation	pig, sheep
tissue plasminogen activator	heart attacks, deep vein thrombosis, pulmonary embolism	goat

Another field of transgenic technology, is to support the potential value of transgenic animals in livestock production systems. Most transgenic livestock projects have focused on enhancing growth in swine by over-expression of growth hormone, IGF-I, or estrogen receptor[5]. A smaller number of projects have been designed to enhance disease resistance in pigs and sheep and recently, transgenic sheep with enhanced wool production have been produced.

In the last 6 years a new industry, the transgenic animal bioreactor industry, has developed. The goal of this industry is to produce pharmaceuticals and nutraceuticals (food with therapeutic value) primarily in the milk of farm animals[6]. Examples of some human proteins developed in milk of transgenic animals are shown in Table 2.

A number of pharmaceutically active human proteins have been successfully produced in the milk of transgenic animals at commercially viable levels Table 3. The estimated annual US requirements of some transgenic proteins are shown in Table 4.

Which mammal to use? Mammals vary quite differently in size and several of them have been chosen to produce recombinant proteins in their milk. Ruminants,

Table 3: A comparison of pharmaceutically important human proteins expressed in the mammary gland of transgenic animals compared with expression levels in alternate systems[8]

Protein	Biological function/ clinical utility	Expression levels*	World-wide sales ($US)
Factor IX	Blood clotting factor/ haemophilia B treatment	25 ng mL^{-1} (100 μg mL^{-1}, only 2% active)	$ 25,000/g
α-1-antitrypsin	Neutrophil elastase inhibitor/emphysema	7 mg mL^{-1} (60 ng mL^{-6} cells day^{-1})	$ 100 m
Interleukin-2	Cancer, AIDS and leprosy therapy	430 ng mL^{-1} (10 μg mL^{-1} day^{-1})	$ 20 m
t-PA	Thrombolytic agent/ myocardial infarction	3 mg mL^{-1} (460 μg mL^{-1})	$ 230 m
Growth hormone	Hypopituitary dwarfism/ chronic renal insufficiency	11 mg mL^{-1} (200 μg mL^{-1})	$ 575 m
Protein C	Haemostasis regulator / stroke, septic shock	1.0 mg mL^{-1} (<0.4 μg mL^{-1} h^{-1})	$ 960 m

*Expression levels obtained in the mammary gland of transgenic animals. Values in brackets are production levels achieved by microbial fermentation or mammalian cell culture

Table 4: Estimate annual US requirements and costs of some potential bioreactor products[1]

Item	Estimated quantity needed kg	Current cost per gram $	Annualmarket $ x 10^6
F VIII[1]	0.3	2,900,000	882
F IX[2]	4	40,000	160
Protein C	10	10,000	100
AT III[3]	21	7000	150
Fibrinogen	150	1000	150
Albumin[4]	315x10^3	3.56	1120

[1]Blood coagulation factor 8, [2]Blood coagulation factor 9, [3]Antithrombin 3, [4]Human serum albumin

Table 5: The estimated number of transgenic animals needed to satisfy the annual US market (Table 4) for selected pharmaceuticals[1]

Species	F VIII[1]	F IX[2]	Protein C	AT III[3]	Fibrinogen	Albumin[4]
Rabbit	54	714	1785	3750	27x10^3	56x10^6
Pig	1	1	25	53	380	800x10^3
Sheep	1	13	33	70	500	1050x10^3
Goat	1	7	17	35	250	525x10^3
Cow	1	1	2	3	17	35x10^3

[1]Blood coagulation factor 8, [2]Blood coagulation factor 9, [3]Antithrombin 3, [4]Human serum albumin

namely goat and sheep, appear to be the best candidates to produce proteins up to several tons per year[9,10]. The pig is considered as a possible living fermentor, although milk cannot be collected as easily as from ruminants[11].

The rabbit produces up to 200-250 mL of milk per day. Its milk is particularly rich in protein and a significant proportion of milk can be obtained. Transgenic rabbits can be easily obtained at a relative low cost. This species is also highly prolific and it is therefore a good candidate for the production of recombinant proteins not exceeding 1 kg per year[12].

The cow is probably the only mammalian species potentially capable of synthesising 400 tons of human albumin, which are needed each year Table 5.

On first inspection, it seems unreasonable to think that an organisation would consider generating 27,000 rabbits necessary to produce 150 kg of fibrinogen. The labour that is required to maintain and milk those animals would be enormous, especially in light of the fact that 17 cows might be capable of producing all of the fibrinogen required to satisfy current US needs.

However, the required number of rabbits could be produced in 3 to 4 years by using homologous males and Artificial Insemination (AI), but 7 to 8 years would be needed to produce the 17 cows. The efficiency of producing transgenic animals should also be considered. From about 40 mouse eggs injected only one transgenic mouse was produced. The efficiency from sheep, goats and cattle is much lower, requiring approximately 110, 90 and 1600 eggs injections respectively per transgenic animal[13]. Furthermore, only 50% of transgenic offspring express their transgene. Producing a transgenic sheep or goat can easily cost $60,000 and producing a transgenic cow or bull can exceed $300,000[14].

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