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Transgenic Animals

An Interactive Qualifying Project Report

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By

Jan-Eric Ahlfors
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Approved:

David S. Adams

Prof. David S. Adams
Project Advisor

ABSTRACT

The purpose of this Interactive Qualifying Project was to investigate the topic of transgenic animals, and to determine the impact of this complex new technology on society. Information is presented on how such animals are created, examples of which ones have been created to date, their importance to society, and the ethics surrounding their construction and use. The knowledge gained in this project instilled a pro-transgenic perspective in the author, with some cautions regarding rare cases of animal suffering.

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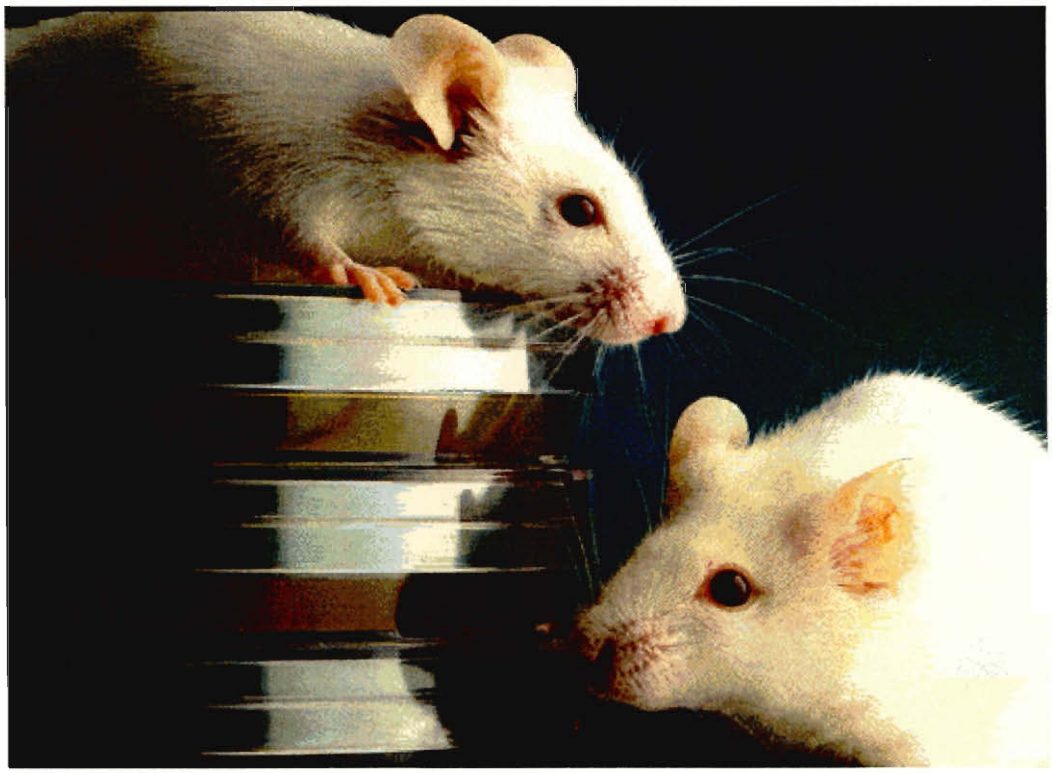
Executive Summary

The purpose of this Interactive Qualifying Project was to investigate transgenic animals, a new technology within the field of biology, and to examine the complex impacts of this new technology on society. Transgenic animals (TA) are animals in which a foreign gene(s) has been inserted. They are useful tools for the study of biological functions of proteins and secondary gene products synthesized by the action of protein catalysts. Technologies for the production of food, nutritional products, and ingredients from transgenic animals are maturing and yielding exciting results in experimental and farm animals. However, some ethical issues surrounding transgenic animals need to be solved and the enormous amounts of resources needed for transgenic livestock production cause the costs for making transgenic animals to be extraordinarily high.

Altogether, transgenic animals are poised to dramatically increase the well-being of countries. Farm animals producing complex biopharmaceuticals means that former and more expensive methods of harming and sacrificing organisms can be abandoned. Farm animal production can be made more efficient and has the potential to produce the same amount of food using fewer animals. Disease models such as the PDAPP transgenic mouse model help to create cures for diseases such as Alzheimer's which would end the suffering of millions of people and billions of dollars in temporary treatments that could be used more beneficially elsewhere, including environmental protection. Committees and agencies that deal with the ethical issues and any possible problems that may result from transgenic technology need to be set up and maintained. The benefits of transgenic animals to society, however, are overwhelming and would benefit from additional government and public support.

Project Objective

The purpose of this Interactive Qualifying Project was to investigate transgenic animals, a new technology within the field of biology, and to examine the complex impacts of this new technology on society. The objective was accomplished by researching primary biological literature in order to understand how, and what kinds of, transgenic animals have been made to date. The middle chapters explored transgenic animals' benefits to society, and ethical issues concerning their use. The final chapter presents the author's conclusions about this new beneficial technology. Throughout the report, all attempts were made to explain the information in layman's term to facilitate public understanding of this complex, but powerful, technology.



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Introduction

Transgenic animals (TA) are animals in which a foreign gene(s) has been inserted. They are useful tools for the study of biological functions of proteins and secondary gene products synthesized by the action of protein catalysts. Research in nutrition and allied fields is benefiting from their use as models to contrast normal and altered metabolism. Although food, nutritional products, and ingredients from transgenic animals have not yet reached consumers, the technologies for their production are maturing and yielding exciting results in experimental and farm animals. Transgenic mice, in the form of gene knockouts by the homologous recombination technique or random insertion of wildtype or mutant transgenes, are important tools that provide insight into the function of a gene in vivo and can provide models of disease states to test hypotheses for potential therapeutic intervention.

Page et al. (1992) define TA as "a result of the incorporation of a foreign gene such that it becomes an integral part of the natural chromosomal makeup of the animal." Kopchick et al. (1996) propose a definition in which the key elements are the incorporation of exogenous DNA into the germ line of animals and the preservation of such genetic material in subsequent generations. However, exogenous DNA can also be incorporated transiently into specific tissues,

thus producing TA that express heterologous proteins without incorporating foreign DNA into their germ lines. For example, Archer *et al.* (1994) used retroviruses for the direct transfer of the human growth hormone (hGH) gene into the mammary glands of goats causing the secretion of hGH in their milk. In addition, homologous proteins that are expressed in certain tissues of a given species can be transgenically expressed in tissues in which they are not normally found. An example of this case is the work of Kelder *et al.* (1998) in which a murine enzyme (α-galactosyltransferase), which is normally expressed in liver, is transgenically expressed in lactating mammary glands of mice.

Transgenic animals (TA) express, or may express if properly induced, proteins encoded by cDNA or genes usually appended to heterologous promoters or transcription regulatory elements (TRE). These fusion genes are commonly referred to as transgenes. It is important to note that the expression of proteins, which are primary gene products, may or may not be the only acquired features of a given TA. If the transgene-encoded protein is an enzyme and if its substrates are present within the cell, then secondary gene products also will be synthesized. TA may accumulate these products in tissues or biological fluids in which they are not normally present. (Prieto *et al.*, 1999)

Mice have become the transgenic animals of choice as biomedical models for certain diseases and for understanding the roles of different genes. Several factors have contributed to this boom, including the mouse's spectacular fecundity and relatively low maintanance costs. Some profilic pairs, for instance, can produce more than 250 descendants in just a year on a little more than grain and water. But scientists also like mice because they are physiologically and genetically similar to humans. Millions of mice are used to screen drugs and potentially dangerous compounds for safety. Most human genes appear to have a related mouse version, making it possible to gain insight into human diseases using gene-altered mouse models that suffer from similar ills but aren't subject to the same ethical concerns as human patients. Technologies that have made it easier than ever to tinker with the mouse's genome have only enhanced the rodent's value. (Marshall, 2000)

The relevance of TA to the field of nutrition may be better illustrated by a few examples, including pigs and mice that express human proteins in their milk (Archibald *et al.*, 1990; Clark, 1998) or in their urine (Meade and Ziomek 1998); pigs with unique fat/muscle ratios due to the expression of peptide hormones (Wieghart *et al.*, 1990); sheep with altered carcass composition due to transgenic expression of hormones (Wise *et al.*, 1988); mice that have altered milk oligosaccharides and glycoproteins (Prieto *et al.*, 1995); the production of lactose-free milk or milk with reduced lactose content due to elimination of α-lactalbumin (Karatzas and Turner,

1997; Stacey *et al.*, 1994; Stacey *et al.*, 1995); and tilapia overexpressing GH (Martinez *et al.*, 1996). Although transgenic animal products are not as yet available to consumers, products obtained from transgenic plants are already in the marketplace, and many vegetables and fruits are being targeted for modifications and improvements through the use of transgenic technologies. These are not necessarily focused on improving the nutritional quality of food products, but often on increasing efficiencies in the production of cultivars, improving resistance to insects and pathogens, and changing or enhancing organoleptic characteristics. Because of the fast pace at which TA technologies are evolving, it is conceivable that a significant portion of food products on supermarket shelves will become targets for functional, compositional, or nutritional improvements.

Economically the enormous amounts of resources needed for transgenic livestock production, the costs for one expressing transgenic animal are extraordinary high. It has been calculated that one expressing transgenic mouse requires average expenses of US$120 whereas one expressing transgenic pig would amount to US$25,000, one transgenic sheep US$60,000 and one transgenic cow US$546,000 when in vivo derived zygotes are used (Wall *et al.*, 1992). Thus for cattle, transgenic production can only be practical through in vitro production of embryos as it reduces costs by 50-60%. (Niemann, 2000)

Numerous disease models of transgenic animals have been produced in a matter of years showing the importance of transgenic animals in research. As an example, below are 61 current listings in the Strain Category of 'Breast Cancer' in the Jackson Laboratory Induced Mutant Resource of genetically engineered mice search form. This search form is available at the following URL: http://www.jax.org/pub-cgi/imrpub.sh?objtype=rptquery.

Table 1.

Symbol	Name	Mutation	Mutation Made By	Stock#	Strain
Atm	ataxia telangiectasia mutated	*Atm*^{tm1Awb}	Dr. Anthony Wynshaw-Boris	002753	129S6/SvEvTac-*Atm*^{tm1Awb}
Bax	Bcl2-associated X protein	*Bax*^{tm1Sjk}	Dr. Stanley J. Korsmeyer	002994	B6.129X1-*Bax*^{tm1Sjk}
Ccnd1	cyclin D1	*Ccnd1*^{tm1Wbg}	Robert Weinberg	002537	B6;129S-*Ccnd1*^{tm1Wbg}
Ccnd1	cyclin D1	*Ccnd1*^{tm1Wbg}	Robert Weinberg	002935	FVB.129S2(B6)-*Ccnd1*^{tm1Wbg}
Cdc37	cell division cycle 37 homolog (S. cerevisiae)	TgN(MMTV-Cdc37)1Stp	Lilia Stepanova	003690	STOCK TgN(MMTV-Cdc37)1Stp
Cdkn1b	cyclin-dependent kinase inhibitor 1B (P27)	*Cdkn1b*^{tm1Mlf}	Dr. Matthew Fero	003122	129/Sv-*Cdkn1b*^{tm1Mlf}

Cdkn1b	cyclin-dependent kinase inhibitor 1B (P27)	*Cdkn1b*^{tm1Mlf}	Dr. Matthew Fero	002781	B6.129-*Cdkn1b*^{tm1Mlf}
Cre	Cre recombinase	TgN(WapCre)11738Mam	Dr. Kay-Uwe Wagner	003552	B6129-TgN(WapCre)11738Mam
Cre	Cre recombinase	Tgn(MMTV-Cre)1Mam	Dr. Kay-Uwe Wagner	003551	B6129-Tgn(MMTV-Cre)1Mam
Cre	Cre recombinase	Tgn(MMTV-Cre)4Mam	Dr. Kay-Uwe Wagner	003553	B6129-Tgn(MMTV-Cre)4Mam
E2f1	E2F transcription factor 1	*E2f1*^{tm1Meg}	Dr. Seth Field	002785	B6;129S-*E2f1*^{tm1Meg}
Erbb2	avian erythroblastosis oncogene B-2	TgN(MMTVneu)202Mul	Dr. William Muller	002376	FVB/N-TgN(MMTVneu)202Mul
Erbb2 Trp53	avian erythroblastosis oncogene B-2, transformation-related protein 53	TgN(MMTVneu)202MulTgN(Trp53R172H)8512Jmr	IMR Colony	003181	FVB-TgN(MMTVneu)202MulTgN(Trp53R172H)8512Jmr
Erbb2TGFA	avian erythroblastosis oncogene B-2,transforming growth factor-alpha (Human)	TgN(MMTVneu)202MulTgN(MMTVTGFA)29Rjc	IMR Colony	003182	FVB-TgN(MMTVneu)202MulTgN(MMTVTGFA)29Rjc
HRAS	Harvey RAS (Human)	TgN(WapHRAS)69Lln	Dr. Loretta L. Nielsen	002409	B6;SJL-TgN(WapHRAS)69LlnY^{SJL}
HRAS	Harvey RAS (Human)	TgN(WapHRAS)69Lln	Dr. Loretta L. Nielsen	002410	FVB/N-TgN(WapHRAS)69LlnY^{SJL}
Igf1	insulin-like growth factor 1 (Rat)	TgN(WapIgf1)39Dlr	Dr. Derek LeRoith	002500	C57BL/6J-TgN(WapIgf1)39Dlr
Igf1	insulin-like growth factor 1 (Rat)	TgN(WapIgf1)39Dlr	Dr. Derek LeRoith	003078	FVB-TgN(WapIgf1)39Dlr
IGFBP3	insulin-like growth factor binding protein-3 (Human)	TgN(WapIGFBP3)67Dlr	Dr. Derek LeRoith	002499	C57BL/6J-TgN(WapIGFBP3)67Dlr
lacZ	beta-galactosidase	TgN(TIE2LacZ)182Sato	Dr. Thomas Sato	002856	FVB/N-TgN(TIE2LacZ)182Sato
Mdm2	transformed mouse double 3T3 cell minute 2	*Mdm2*^{tm1Bay}	Dr. Stephen N. Jones	002968	B6,129S-*Mdm2*^{tm1Bay}
MET	met proto-oncogene (hepatocyte growth factor receptor) (Human)	TgN(MtTPRMET)243	Dr. Timothy Wang	002675	FVB/N-TgN(MtTPRMET)243
MET	met proto-oncogene (hepatocyte growth factor receptor) (Human)	TgN(MtTPRMET)773	Dr. Timothy Wang	002775	FVB/N-TgN(MtTPRMET)773
Myc	myelocytomatosis oncogene	TgN(WapMyc)212Bri	Dr. Ralph Brinster	002677	FVB/N-TgN(WapMyc)212Bri
Notch4	Notch gene homolog 4, (Drosophila)	TgN(MMTVInt3)3Rnc	Dr. Robert Callahan	002437	FVB/N-TgN(MMTVInt3)3Rnc
Notch4	Notch gene homolog 4, (Drosophila)	TgN(WapInt3)10Rnc	Dr. Robert Callahan	002755	FVB/N-TgN(WapInt3)10Rnc
Oxt	oxytocin	*Oxt*^{tm1Wsy}	Dr. W. Scott Young, III	002713	B6;129S-*Oxt*^{tm1Wsy}

PIP	prolactin induced protein, gross-cystic-disease fluid protein-15	TgN(MMTVPIP)1Shu	Dr. Robert Shiu	003337	STOCK TgN(MMTVPIP)1Shu
Plg	plasminogen	*Plg*^{tm1}	Dr. Thomas Bugge	002830	B6.129P2-*Plg*^{tm1}
Prlr	prolactin receptor	*Prlr*^{tm1Cnp}	Dr. Paul A. Kelly	003141	129X1/SvJ-*Prlr*^{tm1Cnp}
Prlr	prolactin receptor	*Prlr*^{tm1Cnp}	Dr. Paul A. Kelly	003142	B6.129P2-*Prlr*^{tm1Cnp}
PyVT	Polyoma virus middle T antigen	TgN(MMTVPyVT)634Mul	Dr. William Muller	002374	FVB/N-TgN(MMTVPyVT)634Mul
Rb1	retinoblastoma-1	*Rb1*^{tm1Tyj}	Dr. Tyler Jacks	002548	C.129S2(B6)-*Rb1*^{tm1Tyj}
Rb1	retinoblastoma-1	*Rb1*^{tm1Tyj}	Dr. Tyler Jacks	002546	C3Ou.129S2-*Rb1*^{tm1Tyj}
Rb1	retinoblastoma-1	*Rb1*^{tm1Tyj}	Dr. Tyler Jacks	002900	FVB.129S2(B6)-*Rb1*^{tm1Tyj}
Src	Rous sarcoma oncogene	*Src*^{tm1Sor}	Dr. Philippe Soriano	002381	B6;129S-*Src*^{tm1Sor}
Stat5a	signal transducer and activator of transcription 5A	*Stat5a*^{tm1Mam}	Dr. Lothar Hennighausen	002833	B6;129S-*Stat5a*^{tm1Mam}
TAg	SV40 large T-antigen	TgN(C3-1-TAg)cJeg	Dr. Jeff Green	003382	B10.D2-TgN(C3-1-TAg)cJeg
TAg	SV40 large T-antigen	TgN(C3-1-TAg)cJeg	Dr. Jeff Green	003380	C57BL/6J-TgN(C3-1-TAg)cJeg
TAg	SV40 large T-antigen	TgN(C3-1-TAg)cJeg	Dr. Jeff Green	003381	FVB-TgN(C3-1-TAg)cJeg
TAg	SV40 large T-antigen	TgN(WapTAg)1Knw	Dr. Barbara Knowles	003188	C57BL/6J-TgN(WapTAg)1Knw
TAg	SV40 large T-antigen	TgN(WapTAg)3Knw	Dr. Barbara Knowles	003189	C57BL/6J-TgN(WapTAg)3Knw
Tcfap2a	transcription factor AP-2, alpha	*Tcfap2a*^{tm1Jae}	Dr. Rudolf Jaenisch	002794	C.129S4-*Tcfap2a*^{tm1Jae}
TGFA	transforming growth factor-alpha (Human)	TgN(MMTVTGFA)254Rjc	Dr. Robert Coffey	002459	B6D2-TgN(MMTVTGFA)254Rjc
TGFA	transforming growth factor-alpha (Human)	TgN(MMTVTGFA)254Rjc	Dr. Robert Coffey	002953	FVB/NJ-TgN(MMTVTGFA)254Rjc
TGFA	transforming growth factor-alpha (Human)	TgN(MMTVTGFA)29Rjc	Dr. Robert Coffey	002373	B6D2-TgN(MMTVTGFA)29Rjc
TGFA	transforming growth factor-alpha (Human)	TgN(MMTVTGFA)29Rjc	Dr. Robert Coffey	003016	FVB/NJ-TgN(MMTVTGFA)29Rjc
TGFA	transforming growth factor-alpha (Human)	TgN(MtTGFA)100Lmb	Dr. Glenn T. Merlino	002421	FVB/N-TgN(MtTGFA)100Lmb
TGFA	transforming growth factor-alpha (Human)	TgN(MtTGFA)42Lmb	Dr. Glenn T. Merlino	002422	STOCK-TgN(MtTGFA)42Lmb
Tgfa	transforming growth factor, alpha (Rat)	TgN(WapTgfa)215Bri	Dr. Ralph	002	FVB/N-

			Brinster	678	TgN(WapTgfa)215Bri
TGFB1	transforming growth factor-beta 1 (Simian)	TgN(MMTVTGFB1)46Hlm	Dr. Harold Moses	002375	B6,D2-TgN(MMTVTGFB1)46Hlm
TGFB1	transforming growth factor-beta 1 (Simian)	TgN(MMTVTGFB1)46Hlm	Dr. Harold Moses	002933	FVB/NJ-TgN(MMTVTGFB1)46Hlm
Trp53	transformation-related protein 53	TgN(Trp53R172H)8512Jmr	Dr. Jeffrey Rosen	002659	FVB/N-TgN(Trp53R172H)8512Jmr
Trp53	transformation-related protein 53	TgN(Trp53R172L)4491Jmr	Dr. Jeffrey Rosen	002660	FVB/N-TgN(Trp53R172L)4491Jmr
Trp53	transformation-related protein 53	*Trp53*^{tm1Tyj}	Dr. Tyler Jacks	002101	B6.129S2-*Trp53*^{tm1Tyj}
Trp53	transformation-related protein 53	*Trp53*^{tm1Tyj}	Dr. Tyler Jacks	002526	C.129S2(B6)-*Trp53*^{tm1Tyj}
Trp53	transformation-related protein 53	*Trp53*^{tm1Tyj}	Dr. Tyler Jacks	002547	C3Ou.129S2(B6)-*Trp53*^{tm1Tyj}
Trp53	transformation-related protein 53	*Trp53*^{tm1Tyj}	Dr. Tyler Jacks	002899	FVB.129S2(B6)-*Trp53*^{tm1Tyj}
tTA	tetracycline regulated transactivator	TgN(MMTVtTA)1Mam	Dr. Lothar Hennighausen	002618	C57BL/6J-TgN(MMTVtTA)1Mam
Wnt1	wingless-related MMTV integration site-1	TgN(Wnt1)1Hev	Dr. Harold E. Varmus	002870	B6SJL-TgN(Wnt1)1Hev
Wnt1	wingless-related MMTV integration site-1	TgN(Wnt1)1Hev	Dr. Harold E. Varmus	002934	FVB/NJ-TgN(Wnt1)1Hev

Chapter 1:

Methods of Generating Transgenic Animals

Transgene Architecture

Figure 1 is a schematic representation of transgene architecture. Transgenes contain trans-response elements (TRE) (a synonym for 'promoter'), which control the expression of the protein-encoding DNA sequences. Some TRE's are tissue specific and thus the expression of the adjacent DNA is targeted to certain tissues or organs. Examples of these are the lactogenic TRE, which enhance DNA transcription during late pregnancy and lactation, and target gene expression to the epithelial cells of the lactating mammary gland (Wall *et al.*,1991; Mercier and Vilotte, 1993). Other TRE are inducible; that is, they can be 'activated' by applying external stimuli. One example is the metallothionein TRE (Busch *et al.*, 1994), which is induced to promote transcription by the addition of heavy metals to the animal's diet. Another is the phosphoenolpyruvate carboxykinase TRE, which is not active during fetal development, but is turned on after birth and can be regulated by the amount of dietary carbohydrate (McGrane *et al.*, 1988). Other TRE direct expression to many tissues simultaneously (Wong *et al.*, 1997). Most transgenic experiments result in insertion of the transgene into chromosomal DNA. For this reason, transgene expression may also be affected by the transcriptional state of surrounding genomic DNA. Dominant control sequences known as locus control regions (LCR) are used to "shield" the transgene from such effects (Fujiwara *et al.*, 1997). Transgenes can also be protected from position effects by other types of boundary elements known as matrix attachment regions (MAR) or scaffold attachment regions (McKnight, *et al.*, 1996). (Prieto *et al.*, 1999)

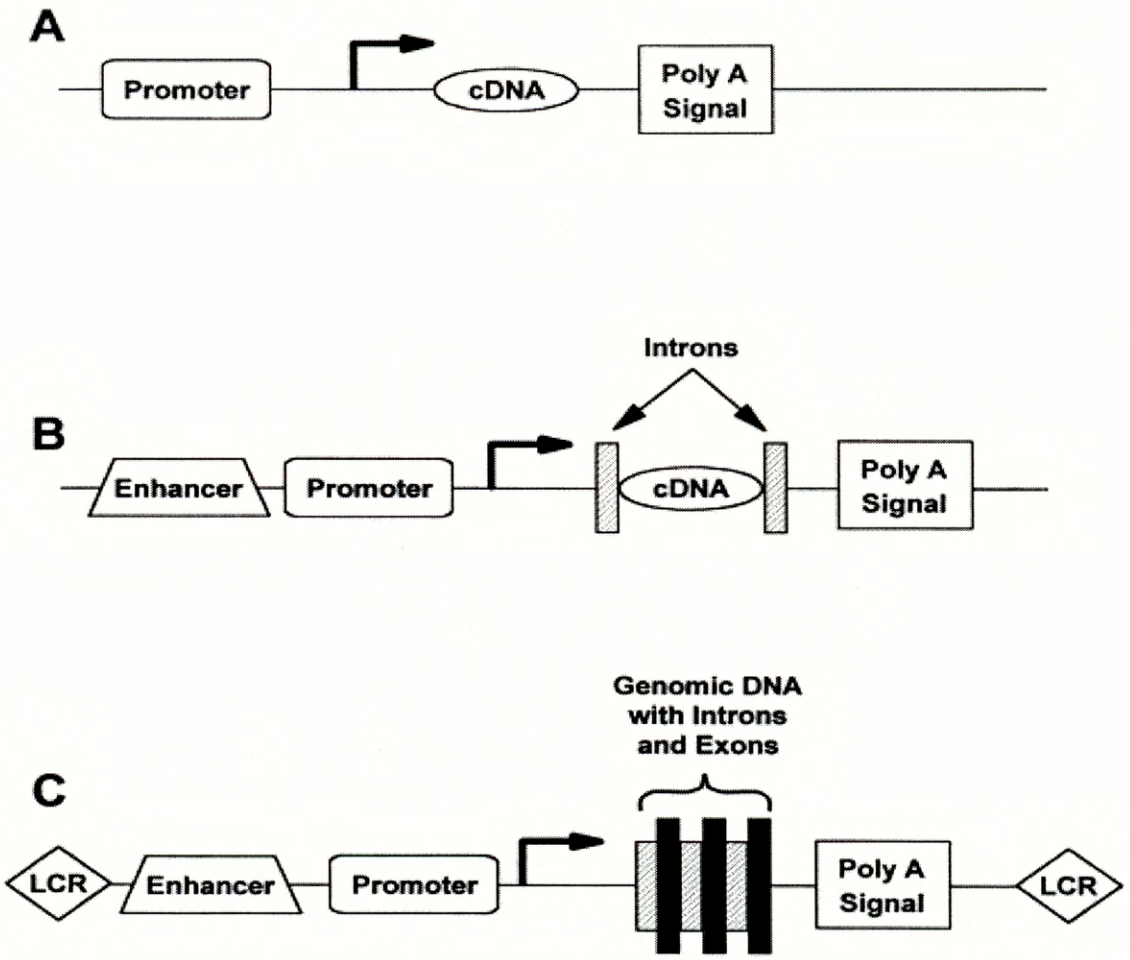


Figure 1. Schematic representations of different fusion genes for transgenic expression. *(A)* Simple construct containing a short transcription regulatory element, a cDNA, and polyadenylation encoding sequence. *(B)* More complex construct showing a larger regulatory element including an enhancer and strategically placed introns. *(C)* A construct with "shielding" elements, in this case, locus control regions (LCR), which contain a full genomic sequence. On occasion, the full genomic sequence including transcriptional regulatory elements have been successfully used.

The core fragment of the transgene is the DNA sequences that encode protein. Sometimes the only available DNA is a cDNA, which is generated from mRNA libraries by reverse transcription. In contrast, most genomic DNAs contain intervening sequences called introns, which are not translated. Introns tend to stabilize and promote protein expression and can be engineered into a fusion gene. Alternatively, genomic DNA with all its introns is preferred. On the other hand, there are practical limitations to the size of the transgene. In general as the transgene becomes larger (i.e., 20 kb) the rate of production of TA is decreased. This is thought to be due to fragmentation of DNA during the microinjection procedure. (Prieto *et al.*, 1999)

It has been shown that the control of transgene expression can be improved by increasing the length of the flanking genomic DNA sequences. Artificial chromosomes are able to carry extremely large DNA fragments of more than one megabase (Mb). Artificial chromosomes have been derived from yeast; they include centromeres, telomeres, and origins of replication as essential components. Microinjection of a 450-kb genomic YAC harbouring the murine tyrosinase gene resulted in transgenic mice, which showed a position-independent and copy-number-dependent expression of the transgene, and caused albinism to be rescued in transgenic mice and rabbits (Schedl *et al.*, 1992; Schedl *et al.*, 1993; Brem *et al.*, 1996). A 210-kb YAC construct has been microinjected into rat pronuclei and α-lactoglobulin and human growth factor were expressed in the mammary gland of transgenic rats (Fujiwara *et al.*, 1997 & 1999). Artificial chromosomes can also be constructed in bacteria (BACs), which can easily be genetically modified to allow homologous recombination. Transgenic mice were generated via pronuclear injection of BACs and germline transmission and proper expression of the transgene was achieved (Yang *et al.*, 1997). Recently, also mammalian artificial chromosomes (MAC) have been engineered by employing endogenous chromosomal elements from YACs or extra chromosomal elements from viruses or BACs and P1 artificial chromosomes (PACs) (Vos, 1997). (Niemann, 2000)

Transgene Delivery

The most commonly employed technique to introduce transgenes into animals is microinjection of the fusion gene into the male pronucleus of embryos as reviewed by Jänne et al. (1992) and Velander et al. (1997). These embryos are then implanted in pseudopregnant females and the resulting offspring are assessed after birth for the presence of the transgene. It is not possible to regulate the number of copies or the sites in which the transgene will be inserted into the chromosomal DNA of the recipient. Likewise, the influence of the surrounding chromatin cannot be predicted. That is the reason why shielding elements such as the LCR and MAR described above are used in transgene construction. The number of copies of the transgene may or may not affect the final level of transgene-encoded protein produced.

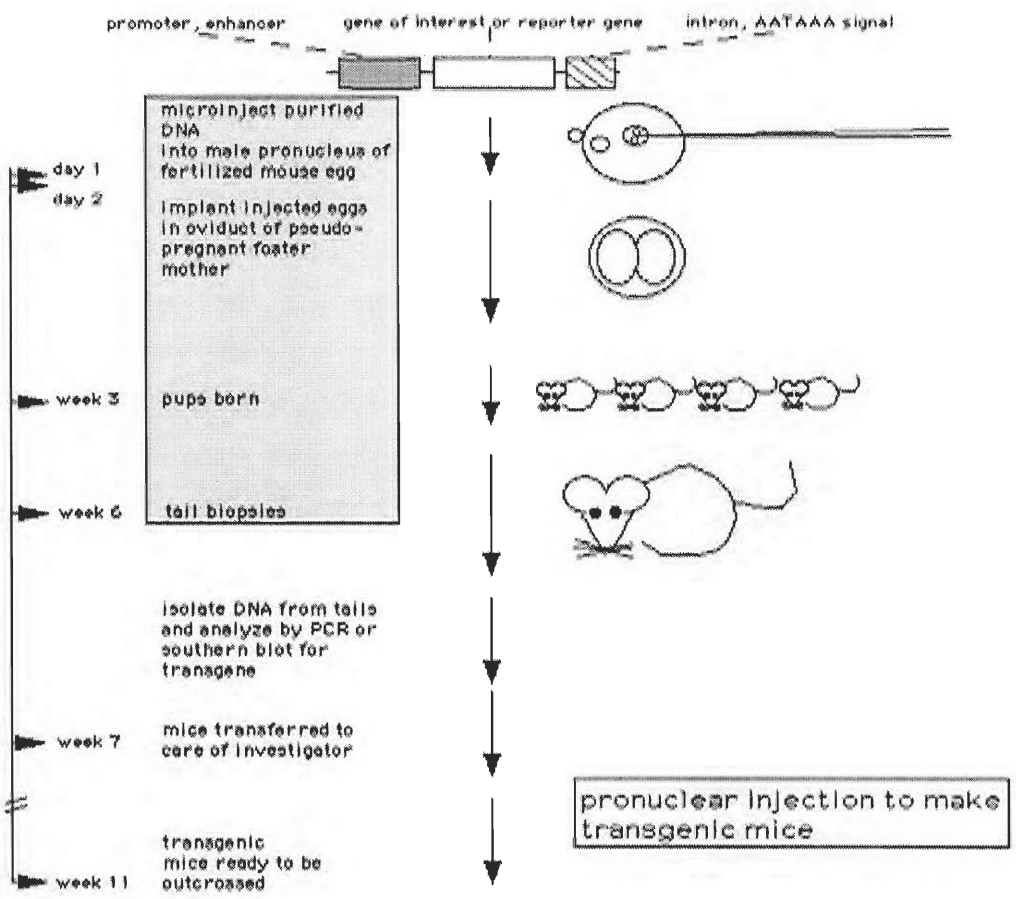


Figure 2. © U. of Virginia Transgenic Mouse Core Facility, Sonia Pearson-White, Director

Because of their short generation times, mice have been frequently produced using embryo microinjection. The use of this technique for the preparation of larger transgenic farm animals with significantly longer gestation periods requires long waiting periods before the results of an experiment can be evaluated. For this reason, techniques have been developed to determine if microinjected embryos at advanced stages of development have incorporated the transgene prior to implantation into surrogate mothers (Hyttinen *et al.*, 1994; Jura *et al.*, 1994; Saberivand and Outteridge, 1996).

A second technique currently used in freshwater fish and marine organisms is electroporation. Eggs are incubated in the presence of the transgene and electrical pulses are applied (Ono *et al.*, 1997). Alternatively, sperm can be electroporated, thus acquiring the transgene (Tsai *et al.*, 1997). This sperm is then used to fertilize eggs, which results in transgenic organisms. Electroporation is also used to introduce transgenes into somatic mammalian cells and

pluripotent embryonic stem cells (Notarianni *et al.*, 1997). These cells can be cultured and tested for transgene incorporation. Cells that carry the transgene can then be introduced into host morulae, which are then implanted into recipient mothers. The resulting TA are chimeric; that is, the transgene is present in some, but not all, of the cells of the progeny including gametes. Homozygous animals for the transgene can be produced by crossbreeding selected animals. Embryonic stem cells are frequently used to generate targeted gene mutations. (Prieto *et al.*, 1999)

TA can also be generated by infection with retroviruses and retroviral vectors. Embryos or pluripotent cells can be transfected using this technique. An interesting application of retroviral transfection described by Archer et al. (1994) involved the direct transfection of animals through the teat canal using such vectors. In this case, the exogenous DNA was incorporated only into the genome of mammary gland cells and was not transmitted through the germ line. Transfection takes place during hormone-induced mammary gland differentiation and transgene-encoded proteins can be found in the milk. The transgene is transcribed at least for the duration of lactation. (Prieto *et al.*, 1999)

Finally, once cells containing transgenes or targeted mutations are available, it is possible to obtain derived TA or TM by nuclear transfection. In this technique nuclei are obtained from cell cultures of transfected cell lines such as fibroblasts and are transferred into enucleated oocytes. These oocytes are then implanted into pseudopregnant animals. This demonstrates that somatic cells can be used to generate TA (in the sense that they still contain a human-made fusion gene). This technology can expedite the development of a productive herd that would be comprised of clones from an original TA (Schnieke *et al.*, 1997). The recent generation of transgenic rats and mice by testis-mediated gene transfer may not only provide a more efficient means of TA production but also allow TA production in currently "resistant" species (Chang *et al.*, 1999). This method generates transfected sperm by direct injection of DNA-liposome complexes into the male testis. These animals are then mated to normal females to generate the TA. (Prieto *et al.*, 1999)

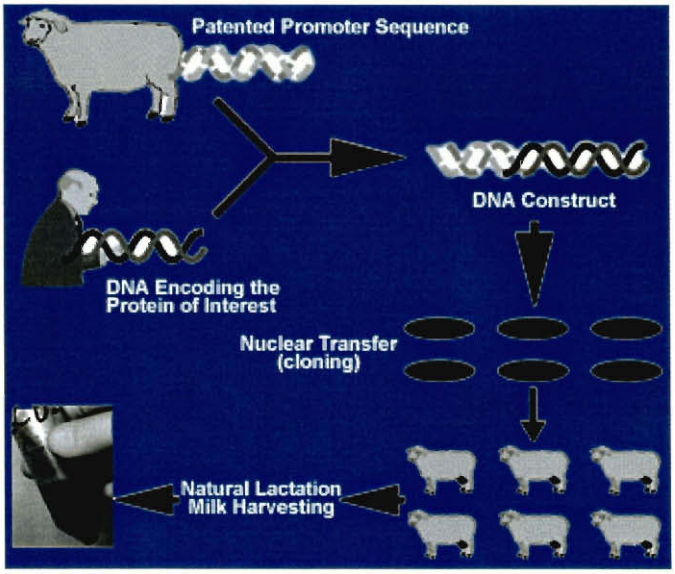


Figure 3. ©2000 PPL Therapeutics

Chapter 2:

Breakthroughs in Transgenic Animals

1976 Rudolf Jaenisch uses a virus to transfer DNA to mouse embryos.

1979 Liu *et al.* used microinjection to insert mRNA into mouse and human cells. (Liu *et al.*, 1979)

1981 G.R. Martin creates the first knock out mouse (Martin, 1981).

1982 Palmiter *et al.* create the first transgenic animals by inserting rat growth hormone genes into mice causing the mice to grow to almost twice their normal size. (Palmiter *et al.*, 1982)

1983 Bosma *et al.* create the SCID mouse which lack an immune system and later becomes a valuable tool for studying human tumors transplanted in mice (Bosma *et al.*, 1983)

1985 Hammer *et al.* create the first transgenic farm animals (Hammer *et al.*, 1985).

1985 Brian Sauer introduces the Cre-loxP system for temporal control of transgenic gene expression (Sauer and Henderson, 1988).

1988 A. Anderson creates 'OncoMouse,' the first tumor-prone mouse, which is patented by Harvard the same year (Anderson, 1988).

1995 David Adams and coworkers create the first successful Alzheimer's mouse (Games *et al.*, 1995).

1997 Schnieke *et al.* create Polly and five other lambs bearing the gene for human factor IX (Schnieke *et al.*, 1997).

1998 Wakayama *et al.* clone mice from somatic cells by using nuclear transfer (Wakayama *et al.*, 1998).

1999 McCreath *et al.* create Cupid and Diana, two sheep clones, one containing a marker gene as a control, and one containing both the marker and a gene for α-1 antitrypsin. (McCreath *et al.*, 2000)

1999 Tang *et al.* create 'smart mouse' by overexpressing the NR2B subunit postnatally in the mouse forebrain (Tang *et al.*, 1999).

2000 Nexia Biotechnologies, Inc. of Montreal, Canada, create Webster and Peter, two goats that carry a gene from arachnids that codes for the spider silk protein.

Doctors Ron James, left, and Keith Cambell, center, of PPL Therapeutics, with Dr. Ian Wilmut of the Roslin Institute and Dolly and Polly.

Goats Peter and Webster will sire a herd of which the females will produce the spider silk protein, BioSteel, in their milk. Scientists will extract the protein to make BioSteel fibers for bulletproof vests, aerospace and medical supplies. (©2000 Discovery Communications Inc., July 30, 2000)

Table 2. Examples of mouse models produced through transgenic or targeted mutagenesis technologies. (Prieto *et al.*, 1999)

Primary gene product	Application	Reference (year)
Brown adipocyte uncoupling protein	Source of transfected brown fat tumors	Ross et al. (1992)57
Expression of human triglyceride lipase	Lowering HDL cholesterol levels	Busch et al. (1994)29
Mutant thyroid hormone receptor	Resistance to thyroid hormone	Wong et al. (1997)31
Apoliprotein E	Diet sensitive atherosclerosis	Plump et al. (1992)58
Bovine growth hormone	Diet (carbohydrate) sensitive growth hormone in plasma	McGrane (1988)30
A2 Adenosine receptor	Thyroid hyperplasia, hyperthyroidism	Ledent et al.(1992)59
Alpha 1B adrenergic receptor	Growth stimulation, malignancy induction, other	Ledent et al. (1997)60
Cholesteryl ester transfer protein and apolipoprotein B	Cholesterol feedback regulation, LDL induction	Liu et al. (1997)61
Breast cancer oncogenes	Effect of diet in cancer onset	Rao et al. (1997)62
Human renin and angiotensinogen	Atherosclerosis	Sugiyama et al. (1997)63
α1-3/4 Fucosyltransferase	Proliferative state of epithelial small intestine cells	Bry et al. (1996)56
α-Galactosidase	Fabry's disease	Ohshima et al. (1997)64

HDL–high density lipoprotein. LDL–low density lipoprotein.

Table 3. Transgenic expression of growth hormone (GH) and related proteins. (Prieto *et al.*, 1999)

Primary gene product	Transgenic animal	Promoter	Highlight	Reference (year)
Bovine GH	Mice	hMTA-IIA	Increase in growth rate	Palmiter et al. (1982)75
Human GH	Mice	hMTA-IIA	Increase in growth rate	Palmiter et al. (1983)41
Porcine GH	Pig	hMT-IIA	Increased weight gain	Vize et al. (1988)76
Human IGF-I	Mice	MT-I	Somatic growth gain	Mathews et al. (1988)77
Ovine GH	Lamb	oMT-IA	Body fat as low as 1/5 of controls	Ward et al. (1989)78
Human GH	Pig	MT-I	Decrease in carcass fat	Pursel et al. (1989)67
Bovine GH	Pig	MT-I	Decrease in carcass fat	Pursel et al. (1989)67
Human IGF-I	Pig	MT-I	Elevated IGF-I	Pursel et al. (1989)67
Bovine GH	Pig	PEPCK	41% reduction in backfat depth	Wieghart et al. (1990)8
Tilapia GH	Tilapia	hCMV	F1 82% larger than control	Martinez et al. (1986)13
Rainbow trout GH	Carp	RSV	20–40% faster growth	Chen et al. (1993)79

hMT-IIA–human metallothionein IIA. oMT-IA–ovine metallothionein IA. MT-I–mouse metallothionein IA. PEPCK–rat phosphoenolpyruvate carboxykinase. hCMV–human cytomegalovirus enhancer-promoter. RSV–rous sarcoma virus.

Table 4. Examples of human milk proteins expressed in transgenic animals. (Prieto *et al.*, 1999)

Human milk protein	Animal	Reference (year)
Lactoferrin	Cow	Krimpenfort (1993)99
Lactoferrin	Mouse	Krimpenfort (1993)99
Bile salt stimulated lipase	Mouse	Strömqvist et al. (1996)100
Lysozyme	Mouse	Maga et al. (1994)101
Fucosyltransferase α1-3/4 FT	Mouse	Prieto et al. unpublished data
Fucosyltransferase α1-2FT I*	Mouse, rabbit	Prieto et al. (1995)1

α1-2FT I Is not found in human milk. The isozyme α1-2FT II is expressed in human milk

Chapter 3:

Transgenic Animals as Disease Models

Alzheimer's Mouse Models

Alzheimer's disease (AD) was named after the physician Alois Alzheimer who in 1907 reported the case of an elderly female patient who had severe cognitive impairments and a characteristic pathology within the brain. Currently, AD and associated dementias affect approximately 10% of over 65 year olds and 30% of over 80 year olds and are the fourth leading cause of death among the elderly. Morphologically AD is characterised by the deposition of amyloid plaques and neurofibrillary tangles in the cortex and hippocampus followed by neuronal and synaptic loss. The neuritic plaques are extracellular lesions that are composed of the 40 to 42/3 amino acid long peptide Aβ fragments derived from amyloid precursor protein (APP), whereas the neurofibrillary tangles are intracellular lesions composed of twisted filaments of tau protein. Two to three percent of early onset cases are linked to single point mutations in the gene which encodes amyloid precursor protein (Goate *et al.*, 1991). These include the Swedish (APP670/671) and London (APP717) familial mutations. A larger proportion of early onset AD cases (70-80%) are linked to loci on chromosome 14, which correspond to presenilin-1 (PS-1; Sherrington *et al.*, 1995). A structurally related protein, presenilin-2 (PS-2), encoded on chromosome 1, has also recently been identified and linked to AD (Rogaev *et al.*, 1995). The precise function of these proteins is not known, however, it has been hypothesised that presenilins regulate APP processing and that the missense mutations in PS-1 (25 to date) affect the formation of amyloid-β (Aβ). (Seabrook and Rosahl, 1999)

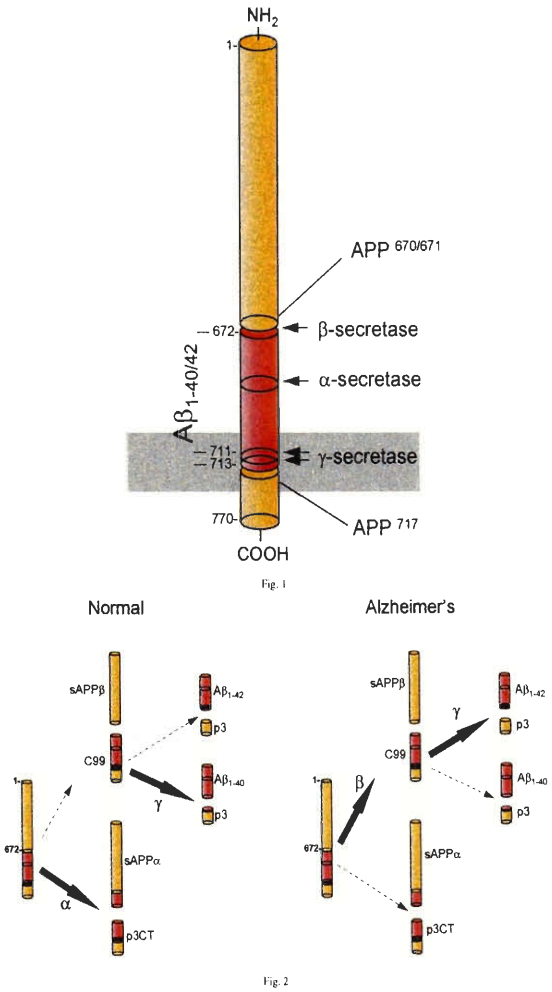


Fig. 4. (Top) Schematic diagram of amyloid precursor protein and sites at which it is preferentially cleaved by proteases, including that of the Aβ1-40 and Aβ1-42/43 fragments (in red). (Bottom) Proposed metabolism of APP under normal conditions compared to that in early-onset Alzheimer's disease. In Alzheimer's disease it has been hypothesised that APP is preferentially metabolised via β secretase activity leading to the generation of soluble C99 peptide fragments. However, it remains to be determined whether it is the generation of the C99 fragment or the subsequent production of Aβ1-42 by γ-secretase activity that is the primary factor leading to the common pathology seen in early-onset and sporadic late-onset AD. A disruption of the normal function of APP may also contribute to the cognitive deficits. (Seabrook and Rosahl, 1999)

Conventional gene targeting by the homologous recombination technique in embryonic stem cells has also been successfully applied to investigate the physiological role of genes involved in the predisposition to AD. A complete inactivation of the mouse APP gene was achieved by deleting a DNA sequence encoding the APP promoter and its first exon including the AUG translation initiation codon and the signal peptide of APP (Zheng *et al.*, 1995). Similar approaches to either interrupt or delete an exon(s) were chosen to generate gene knockouts of the presenilin 1 gene resulting in complete ablation of the PS-1 protein (Wong *et al.*, 1997; Shen *et al.*, 1997; De Strooper *et al.*, 1998). Transgenic mice harboring mutant forms of the APP and/or PS-1 gene associated with AD in humans are a valid tool to study the pathophysiological role of those genes in AD. Due to the relatively short life span of mice, of just 1-2 years, a high

overexpression of the transgene is considered to be necessary to achieve the development of AD-like symptoms in these animals. Therefore, a strong, brain specific promoter is typically chosen to drive the transgene(s) of interest. Currently, those transgenic lines which are most successful were established using the platelet-derived growth factor (PDGF)-β promoter (Games *et al.*, 1995), the promoter and regulatory regions of the PrP gene (Hsiao *et al.*, 1996), and the murine Thy-1 promoter and exons (Sturchler-Pierrat *et al.*, 1997). In all three lines a 7-10-fold overexpression of the transgenes was needed to develop features of AD-like pathology. Transgenic lines overexpressing human PS-1 transgenes carrying FAD mutations were established by using the PDGF promoter (Duff *et al.*, 1996), the mouse PrP promoter (Borchelt *et al.*, 1996) the hamster PrP promoter (Citron *et al.*, 1997) and the human Thy-1 promoter (Qian *et al.*, 1998).

Table 5. Genetic parameters of TgAPP mice with amyloid deposits. (Janus *et al.*, 2000)

Tg line	APP cDNA	Mutation	Transgene promoter	Strain background	APP overexpressi
PDAPP, South San Francisco [28,31]	695+751+770 (see text)	V717F	PDGF-β	Swiss Webster, C57BL6, DBA/2	10×
Tg2576, Minnesota [36,37]	695	Swedish	Hamster PrP	C57BL6×SJL	5–6×
TgAPP22, Basel [39]	751 with optimized Kozak sequence	Swedish plus V717F	Human Thy-1	C57	2×
TgAPP23, Basel [39]	751 with optimized Kozak sequence	Swedish	Murine Thy-1	C57	7×
TgAPP/Ld/2, Leuven [40]	695	V717I	Murine Thy-1	FVB/N and FVB/N×C57BL6	25× (RNA)
TgAPP/Sw/1, Leuven [40]	695	Swedish	Murine Thy-1	FVB/N	7× (RNA)
Tg2576 plus mutant presenilin 1 [54]	695	Swedish	Hamster PrP	C57BL6×SJL	5–6× (protein)
Tg Hu/MoAPP plus presenilin 1 [55]	695 (mouse/human hybrid)	Swedish	Mouse PrP [111]	C57BL6J plus C3H/HeJ mixture	~2× (protein)

APP overexpression refers to protein unless noted otherwise.

Overall the most successful of the above transgenic lines, the PDAPP transgenic mouse, was developed at David Adams laboratory at Worcester Polytechnic Institute (Games *et al.*, 1995) and consists of a mutant minigene containing all exons plus some essential introns covering all three alternative splice forms of APP. Human APP exhibits a number of splice variants of 695, 751 and 775 amino acid residues, reflecting the presence or absence of exons 7 and 8 encompassing the so-called Kunitz inhibitor domain. These mice were cleverly constructed so as to encompass all of these spliced forms. They contain an APP cDNA interposed with a genomic DNA fragment encompassing introns 6-8, and driven by the platelet-derived growth factor β-chain (PDGFβ) promoter, hence the PDAPP nomenclature. The APP cDNA contains the `Indiana' mutation, V717F, which is associated with enhanced cleavage at the γ-secretase site to generate a preponderance of $A\beta_{1-42}$ (Suzuki *et al,* 1994). These mice exhibit florid amyloid deposition commencing at ca. 6-9 months. Neuroanatomic distribution of the plaques, whose major component is $A\beta_{1-42}$, is detailed in Table 6. Very detailed neuropathological analysis has defined thioflavin-S positive Aβ deposits, neuritic plaques, synaptic loss, astrocytosis, and microgliosis. Overt NFTs are absent, as they are for other APP mice, though hyperphosphorylated

forms of tau are detectable. Neuronal loss is not evident (Irizarry *et al.*, 1997). Unlike some APP overexpressed mice, premature death is not reported to be associated with this Tg line. (Janus *et al.*, 2000)

Table 6. Neuropathology in APP Tg mice. (Janus *et al.*, 2000)

Tg line[a]	Age at onset of amyloid deposition	Plaque morphology	Plaque location	As-trocy-tosis	Mi-croglio-sis	Neuronal loss	NFT elements?
PDAPP	from 6 months onwards	diffuse and compacted plaques	hippocampus, Corp Call, CC	yes	yes	not significant at 18 months	AT8 positive
Tg2576	at 9–12 months	deposits stain with Congo Red	hippocampus, CC, amygdala	yes	yes	none at 16 months	AT8 positive
Tg2576 plus mutant PS1	6 months	as above	as above	yes	yes	N.D.	(as above)
TgAPP22	18 months	mostly diffuse	hippocampus	yes	yes	N.D.	AT8 staining around Congo Red staining plaques, no NFTs
TgAPP23	6 months	congophilic and diffuse	hippocampus, neocortex	yes	yes	up to 25% in CA1 at 14–18 months	as above
TgAPP/Ld/2	13–18 months	diffuse, composed in major part of $A\beta_{1-42}$	hippocampus, CC	yes	yes	no overt neuronal loss or degeneration	AT8 staining
TgAPP/Sw/1	18–25 months	diffuse, composed in major part of $A\beta_{1-40}$	hippocampus, CC	yes	yes	as above	AT8 staining
Tg Hu/MoAPP plus PS1	10 months	plaques composed of both $A\beta_{1-40}$ and $A\beta_{1-42}$	hippocampus, CC	yes	yes	N.D.	N.D.

N.D., not done; CC, cerebral cortex.

Since amyloid-β peptide (Aβ) seems to have a central role in the neuropathology of AD and familial forms of the disease have been linked to mutations in the amyloid precursor protein (APP), the PDAPP transgenic mouse model has been used to screen possible drugs and methods for curing AD. Recent research has shown that immunization with amyloid-β works like a vaccine in the PDAPP transgenic mouse by preventing the development of β-amyloid-plaque formation in young mice as well as reducing the extent and progression of these AD-like neuropathologies in older mice where the amyloid-β deposition is already well established (see figs 5&6)(Schenk *et al.*, 1999). Recent human clinical trials of this vaccine has not only been shown to be safe but also not to cause any side effects in the patients.

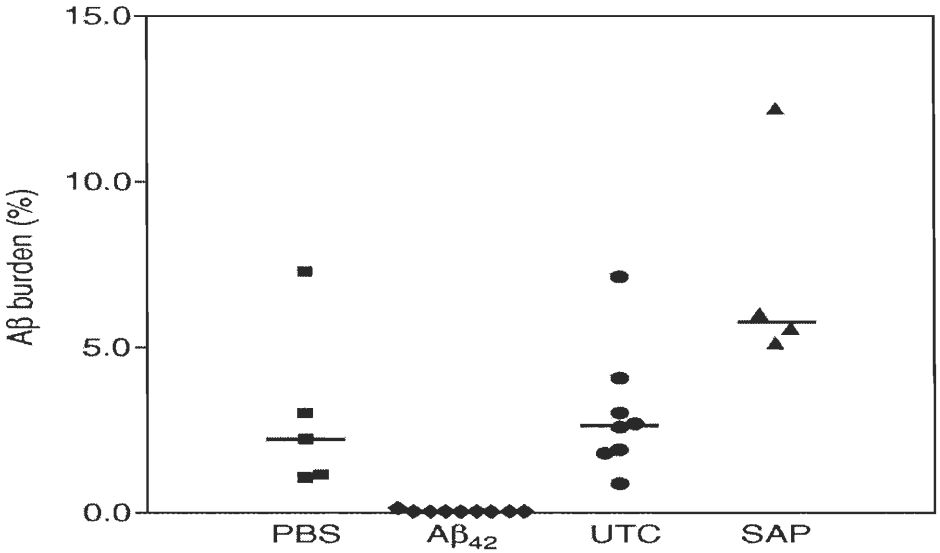


Figure 5. Reduction of Aβ burden in the hippocampus at 13 months of age in PDAPP mice immunized with $A\beta_{42}$. PDAPP mice were immunized beginning at 6 weeks of age. The percentage of the area of the hippocampal region occupied by Aβ deposits was determined by quantitative image analysis. Values for individual mice are shown sorted by treatment group. Horizontal lines represent the median values. The $A\beta_{42}$-immunized group had significantly fewer Aβ deposits than any of the other three groups ($P = 0.001$), which are not significantly different from each other (P 0.05). UTC, untreated controls; SAP, mice immunized with serum amyloid P. (Schenk *et al.*, 1999)

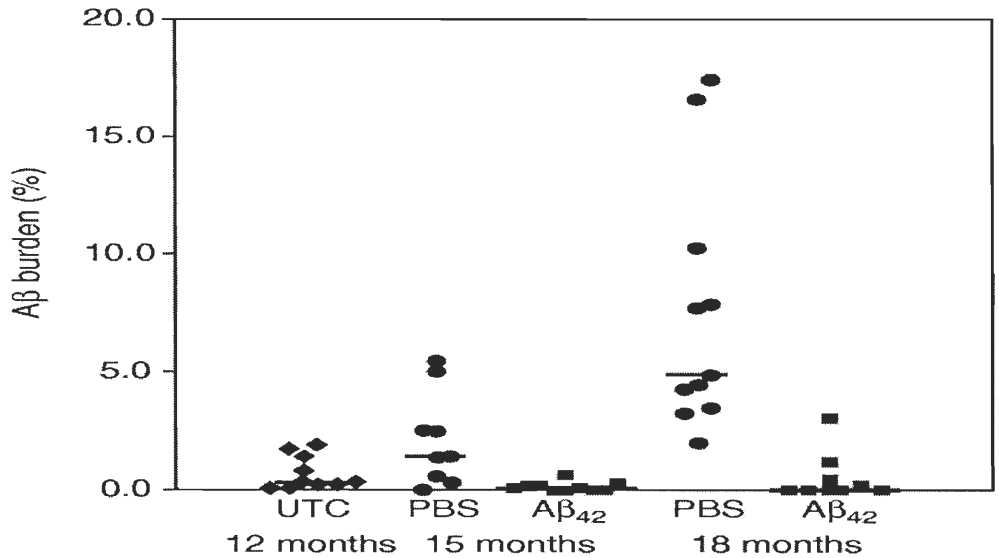


Figure 6. Quantitative image analysis of the cortical Aβ burden in older PBS- and Aβ-treated mice. Immunization of PDAPP mice was begun at 11 months of age. Amyloid burden was significantly reduced in the $A\beta_{42}$ group compared with the PBS controls at both 15 ($P = 0.003$) and 18 ($P = 0.0002$) months of age. The median value of the amyloid burden for each group is shown by the horizontal lines. (Schenk *et al.*, 1999)

The recent advances in genome sciences and the development of transgenic technology have provided a unique opportunity to study how genes associated with human cognitive dysfunction alter synaptic transmission between neurons in the mammalian brain. However, one of the difficult issues surrounding knockout and transgenic studies is the relevance of the animal phenotype to the disease state. In the case of models of Alzheimer's disease, mice have a short life span and therefore one can realistically question the relevance of changes seen over the space of a few years to a disease which, in humans, takes several decades to occur. It is similarly difficult to eliminate the influence that the disruption of the genotype of an animal may have upon developmental processes. However, the creation of inducible transgenics and knockout techniques will, to some extent, help to address these concerns. Clearly, both issues will remain a significant philosophical challenge for the interpretation of transgenic studies and must be considered carefully when extrapolating to human disease states. Nonetheless it is clear that transgenic studies have already provided, and will continue to provide valuable information regarding the significant role that APP and PS-1 mutations have in early-onset familial AD. (Seabrook and Rosahl, 1999)

The SCID Mouse

Severe combined immunodeficient (SCID) mice were created by Bosma *et al.* (1983). The SCID mouse has helped to create many scientific breakthroughs and is one of the most widely used transgenic animals in research. This is due to its almost nonexistent immune system which allows cells from other animals and humans to be engrafted onto the mouse for real life study. Although the SCID mouse does not always seem to be affected by these foreign cells, research involving the SCID mouse has given rise to many ethical concerns. For example, the long-term engraftment, proliferation and differentiation of human hematopoietic cells has been observed in SCID mice by exogenous compensation of human specific cytokines (Lapidot *et al.*, 1992) and by transplantation of human fetal organs (McCune *et al.*, 1988; Kyoizumi *et al.*, 1992; Chen *et al.*, 1994) and human adult bone (Heike *et al.*, 1995; Sandhu *et al.*, 1996). These methods are clearly not always appropriate with respect to cost or ethics. Recently, however, alternative attempts to maintain long-term circulation of human cells in mouse blood by the stable

supply of human specific cytokines have been reported (Nolta *et al.*, 1997; Goan *et al.*, 1995; Bock *et al.*, 1995). One was the transplantation of both human stem/progenitor cells and human IL-3 expressing stroma cells into SCID mice. Another was the use of transgenic SCID (Tg-SCID) mice integrated with human cytokine genes, human IL-3, GM-CSF and stem cell factor.

Of all the general transgenic mouse models, the SCID mouse is probably the most widely used and it certainly has the widest number of applications. It is used to monitor patient immune response to tumors (Bankert *et al.*, 2000). It is used as a model for human skin and the nature of an acute inflammatory response, which has recently been used to study the feasibility of an adenoviral vector as a means of therapeutic protein delivery for the treatment of impaired wound healing (Sylvester *et al.*, 2000). It is even used as a model for human lung cancer (Miyoshi *et al.*, 2000), and as a model for human head and neck tumors. The latter model was recently used to develop a novel method for suppressing human head and neck squamous cell carcinoma (HNSCC) by human peripheral blood lymphocytes (HuPBL) following local, sustained delivery of interleukin-12 (IL-12) by biodegradable microspheres to tumors (Kuriakose *et al.*, 2000).

Cancer Mouse Models

Transgenic animal technology has resulted in a plethora of murine models for cancer research providing insight into the complex oncogenic events contributing to the loss of cell cycle control and tumourigenesis. Besides studies designed to understand the disease process, these mouse strains provide controlled experimental systems to study gene-environment interactions and enable us also to study chemoprevention strategies. Cancer chemopreventive agents are chemical or dietary compounds, which reduce the risk of developing cancer in individuals carrying a higher risk of developing the disease. Non-transgenic experimental systems commonly used are based on treatments with a carcinogen and using tumour development or various preneoplastic biomarkers, e.g. aberrant crypt foci (ACF) in the colon, as end points. According to these protocols chemopreventive agents have been classified as blocking agents working in the initiation stage or suppressive or regressive agents working at later stages in the carcinogenic process. The SCID mouse, described earlier, has been one of the most widely used transgenic mouse models in human cancer research. Up to now only few mouse strains with genetically altered cancer related genes have been used in the study of chemopreventive strategies, including

the tumor-prone 'oncomouse,' the first patented transgenic mouse. In most of the models the treatment with a strong carcinogenic agent is avoided. Instead the animals develop tumours spontaneously because they carry highly penetrable disease genes analogous to those found in humans.

The p53 knockout mouse is a model analogous to the human Li-Fraumeni syndrome where the tumour suppressor gene p53 has been mutated. The mouse develops tumours at several sites with lymphomas, hemangiosarcomas, osteosarcoma and soft tissue sarcomas being the most important. The homozygous knockout, p53-/- develops such tumours early, at 4.5 months, in life whereas the p53+/- has a delayed onset, at 18 months. The latter genotype, created by Harvey *et al.* (1993), is highly susceptible to genotoxic carcinogens and is a candidate-screening assay for carcinogens (Harvey *et al.*, 1993). Studies in this model have confirmed the strong effect of calorie restriction on cancer development. Spontaneous tumour development in p53 null mouse was delayed by dehydroepisandrosterone (DHEA) and its analogue 16-αfluoro-5-androsten-17-one (Hursting *et al.*, 1997; Perkins *et al.*, 1997). These compounds cause a reduced food intake, but the effect is still present when the animals are pair fed and is more likely to be due to inhibition of glucose-6-phosphate dehydrogenase and reduced DNA synthesis, or steroid induced thymic atrophy and suppression of T cell lymphoma (Perkins *et al.*, 1997). Quercetin, d-limonene and all-*trans*-retinoic acid has no effect in this model (Hursting, *et al.*, 1995). (Alexander, 2000)

The Tg.Ac mouse model has four copies of the v-Ha-*ras* oncogene are located in tandem on chromosome 11 of strain FVB/N mice. There are hemizygous and homozygous versions, but the latter appears to respond more consistently. This model is good at detecting mutagenic and non-mutagenic carcinogens, including tumour promoters, but fails to detect ethyl acrylate and *N*-methyl-*o*-acrylamide. Ethyl acrylate requires cell proliferation to function as a stomach carcinogen. With dermal dosing as the current routine in this model, it might, therefore, fail to detect certain tissue-specific carcinogens of this type. There is also concern that the model may be oversensitive in other ways, in that it gives positive results with two out of five non-mutagenic non-carcinogens (resorcinol and rotenone). (Kirkland, 1998)

The Eμ-PIM-1 transgenic mouse model overexpressed the *pim*-1 oncogene in the T- and B-cells of the mouse. Less than 10% of the mice develop lymphomas, but they are strongly susceptible to *N*-ethyl-*N*-nitrosurea (ENU). T-cell lymphomas develop in more than 90% of mice treated with 200 mg ENU (Breuer *et al.*, 1991). The mouse is also highly susceptible to benzo(a)pyrene (Kroese *et al.*, 1997) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP; Sorensen *et al.*, 1996). This transgenic mouse model is thus excellent in studying the effectiveness and safety of different chemopreventive agents against T- and B-cell lymphomas.

The TgH*ras*2 mouse model carries five or six copies per cell of the human c-Ha-*ras* oncogene with its own promoter. This model is good at detecting mutagenic carcinogens, and also trans-species non-mutagenic carcinogens. However, it does not detect the mouse-only non-mutagenic carcinogen 1,1,2-trichloroethane, nor three out of three non-mutagenic non-carcinogens. (Kirkland, 1998)

The murine FAP models are by far the most frequently employed transgenic models in chemoprevention studies. These mice models all have germline mutations in the murine *Apc* gene analogous to the human *APC* gene, which is mutated in the inherited human syndrome of multiple adenomas in the large intestine. Unlike the human FAP syndrome, most of the tumours develop in the small intestine of the murine models. The Min mouse model is the most widely used and has an induced germline mutation in codon 850 of the *Apc*-gene giving rise to a truncated protein. Tumour formation starts at a very early stage in life, and thus this transgenic model is the most frequently studied regarding chemoprevention. The FAP transgenic mouse models have already given useful insights into colon cancer. First, *APC*-driven tumourigenesis in both the FAP syndrome as well as in sporadic colon cancer appears to be dependent on the inactivation of both *APC* alleles by mutations or loss of heterozygosity (Jen *et al.*, 1994; Levy *et al.*, 1994; Luongo *et al.*, 1994). This loss of APC function leads to loss of control of β-catenin, which acts as an oncogene when overexpressed (Polakis, P., 1999). Second, the effect of different NSAIDs (sulindac, piroxicam, aspirin, nimesulfide, etc.), which inhibit COX-2, causes the tumour number to be reduced by 80-95% (Beazer-Barclay *et al.*, 1996; Jacoby *et al.*, 1996; Mahmoud *et al.*, 1998; Nakatsugi *et al.*, 1997). Third, the n-3 polyunsaturated fatty acids, DHA and EPA, effectively reduce the tumour number and tumour growth particularly in the small intestine, but also in the colon. A plausible mechanisms could be that DHA inhibits COX-2 and EPA competes with arachidonic acid giving rise to accumulation of arachidonic acid, which may stimulate apoptosis (Chan *et al.*, 1998) and less active leukotriens and prostaglandins (Paulsen *et al.*, 1998). Fourth, soy isoflavones and vegetable fruit mixtures do not influence the tumour number (Sorensen *et al.*, 1998; van Kranen *et al.*, 1998), but a diet low in fat and high in fiber helps to suppress tumor development. (Alexander, 2000)

Chapter 4:

Transgenic Animals used for Research

The NGF Transgenic Mouse

A transgenic model overexpressing nerve growth factor (NGF) under control of the GFAP promoter in the brains of post-natal and adult mice was created by Kawaja and Crutcher (1997). These transgenic mice display elevated levels of NGF mRNA expression and markedly increased levels of NGF protein production in the cerebellum, in comparison with wild-type mice. Although the levels of NGF protein are highest during post-natal development and decrease with maturation, these levels remain relatively higher than those in the wild-type animals at the same ages (i.e. 100-fold and 50-fold higher NGF levels at post-natal day 14 and adulthood, respectively) (Kawaja and Crutcher, 1997). In response to these elevated levels of NGF, sympathetic axons arising from the superior cervical ganglia are seen invading the transgenic cerebellum. Sensory axons, like sympathetic axons, have also been found to aberrantly sprout into the transgenic cerebellum in response to local increases in NGF production.

Studies on this transgenic model by Kawaja *et al.* (1997) has given other exciting results. First, the temporal onset of peripheral axons entering the transgenic cerebellum reveals that the initial appearance of sensory axons precedes that for the sympathetic axons by at least 1 week. Furthermore, it has been revealed that sensory and sympathetic neurons require target-derived NGF for survival during embryonic and neonatal development. Second, sensory axons have been found to extend along blood vessels that pass through the gray matter layers, as well as extending a small number of new fibers within the granular cell layer. Sympathetic axons, on the other hand, are rarely seen in any of the three gray matter layers of the transgenic cerebellum. One possible reason for this differential distribution may be that sensory axons sprout from the blood vessels of the dura surrounding the cerebellum, along the blood vessels that pass through the gray matter and continue into the white matter. The route taken by sympathetic axons invading the transgenic cerebellum, on the other hand, remains uncertain. Third, it has been observed that NGF-immunoreactivities are co-localized to sympathetic axons which sprout into the transgenic

cerebellum. But the functional significance as to why sympathetic, and not sensory, axons preferentially sequester NGF at aberrant axonal branches still remains unclear. Fourth, it has been found that NGF-induced collateral sprouting of peripheral axons can occur under two conditions: (1) following transection of a spinal nerve, adjacent intact sympathetic and sensory axons extend new branches and provide functionally-relevant patterns of reinnervation to the denervated skin (Diamond *et al.*, 1987; Gloster and Diamond, 1995), which possesses elevated levels of endogenous NGF (Mearow *et al.*, 1993); and (2) increased production of NGF in peripheral tissues following damage or disease causes sympathetic and sensory axons to extend new branches and to inappropriately hyperinnervate the target tissue (Byers *et al.*, 1992; Constantinou *et al.*, 1994; Woolf *et al.*, 1994; Spitsbergen *et al.*, 1995). Studies on the NGF transgenic mice by Kawaja *et al.* (1997) has revealed that NGF-induced growth of aberrant sensory and sympathetic axons can occur in the absence of glial support (i.e., Schwann cells do not migrate into the cerebellum with these two populations of NGF-sensitive peripheral axons), and only the sympathetic axons sequester NGF at terminal branches.

The αMUPA Mouse

αMUPA is a line of transgenic mice created by Miskin and Masos (1997) and are used as a model for increased life span since, compared with their wild type (WT) counterparts, αMUPA transgenic mice spontaneously eat less (~20%) and live longer (average ~20%). αMUPA mice carry the entire cDNA of the murine urokinase-type plasminogen activator (uPA), including the coding sequence and ~1 kb 3'UTR, linked downstream from the promoter of the lens-specific αA-crystallin gene. αMUPA mice produce uPA mRNA in neuronal cells in multiple brain regions, most of which are devoid of endogenous uPA mRNA, including the hypothalamus, which plays a central role in the control of feeding and energy homeostasis. uPA is an ~48 kDa, secreted serine protease that specifically converts the abundant inactive zymogen plasminogen into plasmin, the ultimate blood clot-dissolving enzyme (Blasi *et al.*, 1987). Plasmin is a nonspecific, trypsin-like protease that can also directly degrade diverse extracellular components and can activate proenzymes of matrix degrading metalloproteases, thereby mediating extracellular proteolysis and cell adhesion and migration. Although plasminogen is the primary

physiological substrate for uPA, the enzyme can also directly cleave fibronectin (Quigley *et al.*, 1987) or activate growth factors from their inactive precursors through extracellular proteolytic cleavage (Mars *et al.*, 1993; Plouët *et al.*, 1997).

Table 7. Plasma corticosterone and food intake in young and old αMUPA and WT mice (Miskina *et al.*, 1999).

Parameter	Age (months)	αMUPA[a]	WT[a]
Corticosterone (ng/mL)[b]	3	168.5 ± 24.2*	114.3 ± 24.2**
	15	65.5 ± 9.9**	100.2 ± 20.9**
Food intake[c] (g/mouse/day)	3	2.03 ± 0.04***	2.74 ± 0.07**
	15	2.20 ± 0.05***	3.36 ± 0.15*

[a] Mean ± SE. Within parameters, mean values with different superscripts are significantly different ($p \leq 0.01$, $n = 10$).
[b] At 0800 h.
[c] Last 13 days.

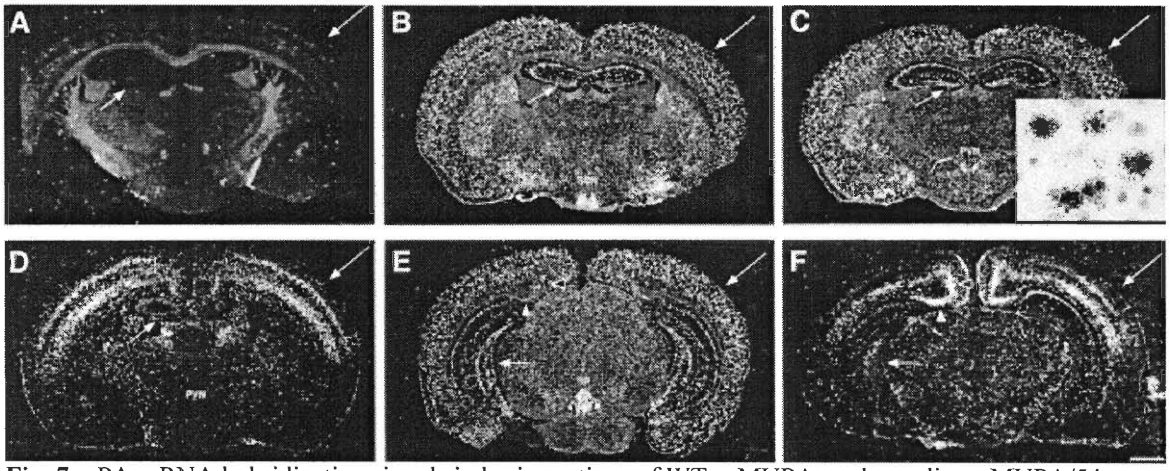


Fig. 7. uPA mRNA hybridization signals in brain sections of WT, αMUPA, and new line αMUPA/54. Coronal brain sections (12 μm) through the anterior ($a_{\¯}d$) or posterior (e,f) hippocampus were prepared from WT (a), αMUPA (b, c, and e) or a new transgenic line αMUPA/54 (d and f). $a_{\¯}b$, $c_{\¯}d$, and $e_{\¯}f$ are parallel or close sections. Microphotography was in dark field illumination. Symbols: short arrows, hippocampus; long arrows, neocortex; full arrowheads, presubiculum; empty arrowheads, retrosplenial cortex. SCN, suprachiasmatic nucleus; PVN, paraventricular nucleus; IP, interpeduncular nucleus. Bar = 0.9 mm. (Miskina *et al.*, 1999)

So far studies involving the differences between αMUPA transgenic mice and wild type (WT) mice have given interesting results. First, in αMUPA transgenic mice, the spontaneously

reduced food consumption coincides with a significant reduction of body temperature compared with WT mice. This temperature reduction seems to contribute to the anti-aging effect; possibilities, such as decreased DNA damage and protooncogene expression, have been discussed (Lane *et al.*, 1996; and Nakamura *et al.*, 1989). Second, plasma corticosterone levels differ between αMUPA and WT in two respects: first, at the young age it is higher in αMUPA than in WT; second, within αMUPA the level declines in aged mice, whereas it remains unchanged within WT. These studies have shown that corticosterone reduction with age could be beneficial since normal aging seems to increases the susceptability to corticosterone-mediated neurotoxicity, which may be caused by dysregulation of neuronal calcium homeostasis (Landfield and Eldridge, 1994). Third, the increased αMUPA life span is not supported by maintaining the youthful state of the thymus; thus maintaining the youthful state of the thymus through old age does not significantly increase life span as previously thought (Weindruch and Suffin, 1980). (Miskina *et al.*, 1999)

Transgenic Mouse Models in Learning

Animals can learn because of changes in the brain which allow new information to be acquired, stored and later recalled. At the cellular level, these changes probably occur at synapses — the junctions between nerve cells. This idea is formalized in the Hebb rule, which states that a synapse between cell A and cell B will be strengthened if the two cells are active at the same time (Hebb, 1949). Neuroscientists have explored the properties and behavioural implications of the Hebb synapse by studying an experimental model of synaptic plasticity known as long-term potentiation (LTP) (Bliss and Collingridge, 1993). Synapses that show LTP are found in several parts of the brain, notably in the hippocampus (a cortical structure which, in humans, is required for the formation of autobiographical memories). If drugs are used to block the induction of LTP, rats have trouble finding their way around a maze, suggesting that LTP is necessary for spatial learning (Morris *et al.*, 1986). Thus by using transgenic mice, researchers are able to explore what happens to LTP and learning when specific proteins are overexpressed or deleted. (Bliss, 1999)

Doogie, a new 'smart mouse' strain.
(Genetic engineering boosts intelligenece. BBC News, September 1, 1999.)

In 1999, Tang *et al.* (Tang *et al.*, 1999) created 'smart mouse' and showed that LTP is considerably enhanced in transgenic mice with improved learning performance. They actually made two different transgenic mouse lines that overexpress the NR2B subunit of the NMDA receptor postnatally in the forebrain. The NMDA (N-methyl-D-aspartate) receptor binds to the excitatory neurotransmitter glutamate and controls the initiation of LTP in most hippocampal pathways. The receptor forms a channel that does not open unless two conditions are satisfied simultaneously — glutamate must bind to the receptor, and the membrane in which the receptor is embedded must be strongly depolarized. Once the channel opens a flux of calcium ions enters the cell, triggering the induction of LTP. The NMDA receptor is made up of an NR1 subunit, which is obligatory for channel function, and a selection of developmentally and regionally regulated NR2 subunits (A to D). The functional properties of the receptor depend on its subunit composition. For example, the glutamate-evoked current (which is important in determining the amount of Ca2+ that enters the cell) has a longer duration in receptors containing NR2B subunits than in those containing NR2A subunits. It has been found that the proportion of NR2B subunits is higher in young animals than in adults (Monyer *et al.*, 1994), and this may account for the greater degree of LTP seen in young animals (Harris and Teyler, 1984). (Bliss, 1999)

Both mouse lines turned out to have similar physiological and behavioural effects. As adults, these transgenic mice show an increase in NMDA-receptor-mediated current, and an enhanced level of LTP normally seen only in young animals. And this seems to make a smarter mouse. Glutamate-evoked NMDA-receptor-mediated currents are larger and of a longer duration in cultured neurons from these transgenic animals than from normal animals. The transgenic mice showed improved learning scores compared with normal mice in three different tests of their ability to acquire and retain information. In one of these tests, fear conditioning, a tone was paired with a mild foot-shock in a particular box. The mouse would freeze, indicating memory of the

shock, when it was replaced in the box some time later. This 'context specificity' is thought to depend on the hippocampus. They found that associative learning was enhanced in the transgenic mice. Furthermore, when the animals were repeatedly given the tone alone or repeatedly returned to the box in which they had been trained, without being given further shocks, the conditioned response was extinguished more rapidly in the transgenic animals than in normal mice (Fig. 4). Tang *et al.* regard this as further evidence for improved learning — an interpretation that requires extinction of the response to be seen as active relearning, rather than a passive forgetting of the previously learned association. In two other tasks (object recognition, shown in Fig. 9, and the water maze, in which animals have to locate a submerged platform in a pool of opaque water), the transgenic animals again scored higher than normal mice.

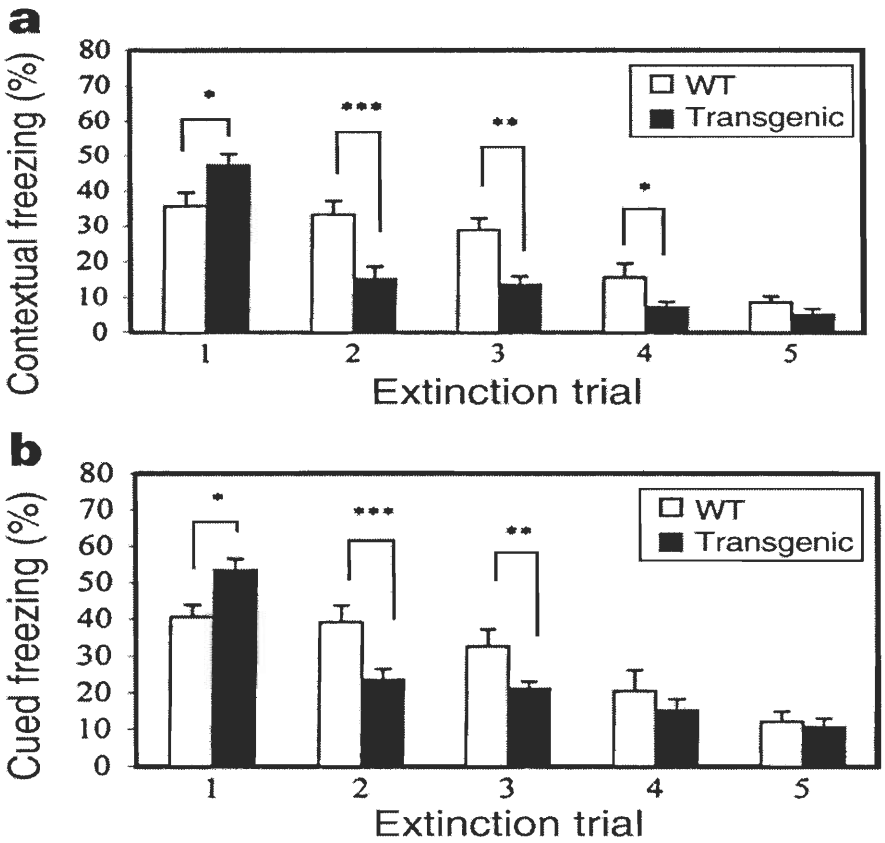


Figure 8. Transgenic mice exhibit faster learning in fear extinction. **a**, Faster fear extinction to contextual environment in transgenic mice. Either wild-type ($n = 8$) or transgenic (Tg-1, $n = 7$; Tg-2, $n = 8$; data plotted together) mice were subjected to five extinction trials 24 h after enhancement of both contextual and cued fear memory. **b**, Faster fear extinction to the tone in transgenic mice. The value in each column represents percentage of freezing rate; data are expressed as mean □ s.e.m. Asterisk, $P < 0.05$; double asterisk, $P < 0.01$; triple asterisk, $P < 0.001$, *post hoc* analysis. (Tang *et al.*, 1999)

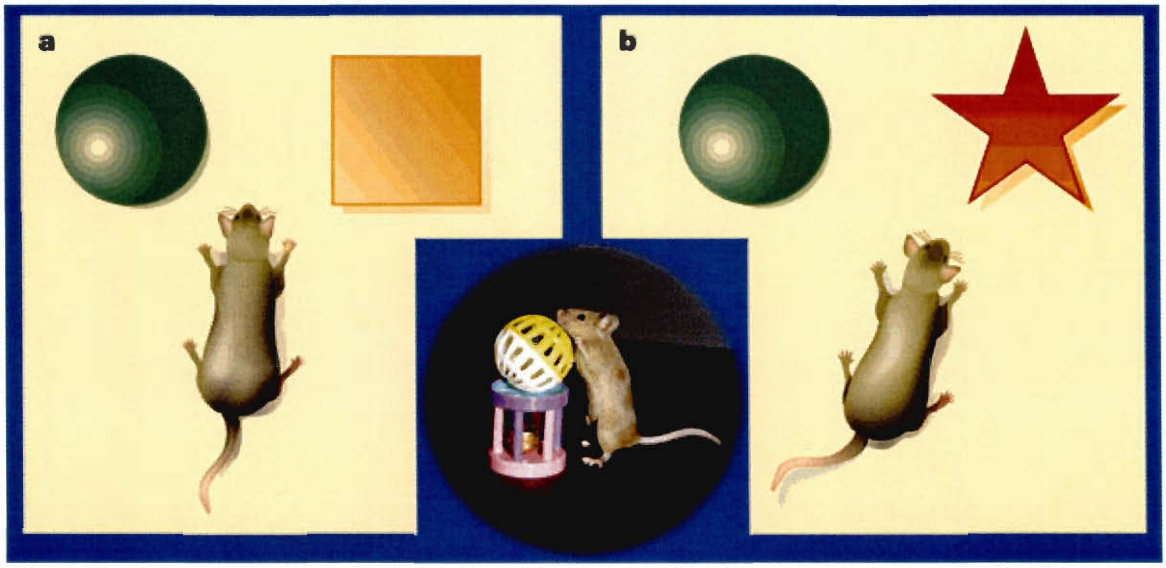


Figure 9. Object-recognition task. a, In an initial training session the mouse explores two objects in a box, devoting roughly equal time to each. b, When the mouse is then re-exposed to one of these objects, together with a new object, it spends more time exploring the new object. Tang *et al.* found that this bias is enhanced in transgenic mice overexpressing the NR2B subunit of the NMDA receptor, indicating improved recognition memory. (Bliss, 1999)

An improvement in LTP has also been reported in other transgenic mice. When the genes encoding postsynaptic density-95 (PSD-95; a cytoplasmic protein that binds the NMDA receptor) (Jia *et al.*, 1996) and GluR2 (a subunit of the AMPA subtype of glutamate receptor) (Jiaet al., 1996) were deleted, both showed enhanced LTP. However, their performance in the water maze was impaired. The PSD-95 knockout mice were also found to be defective in a form of activity-dependent synaptic plasticity, long-term depression (LTD). By contrast, LTD appeared normal in Tang and colleagues' mice. This may explain the different responses in the water maze — bidirectional changes in synaptic efficacy are thought to be important for efficient storage in neural nets (Willshaw and Dayan, 1990), another hypothesis that could be tested with the help of future transgenic animal models. (Bliss, 1999)

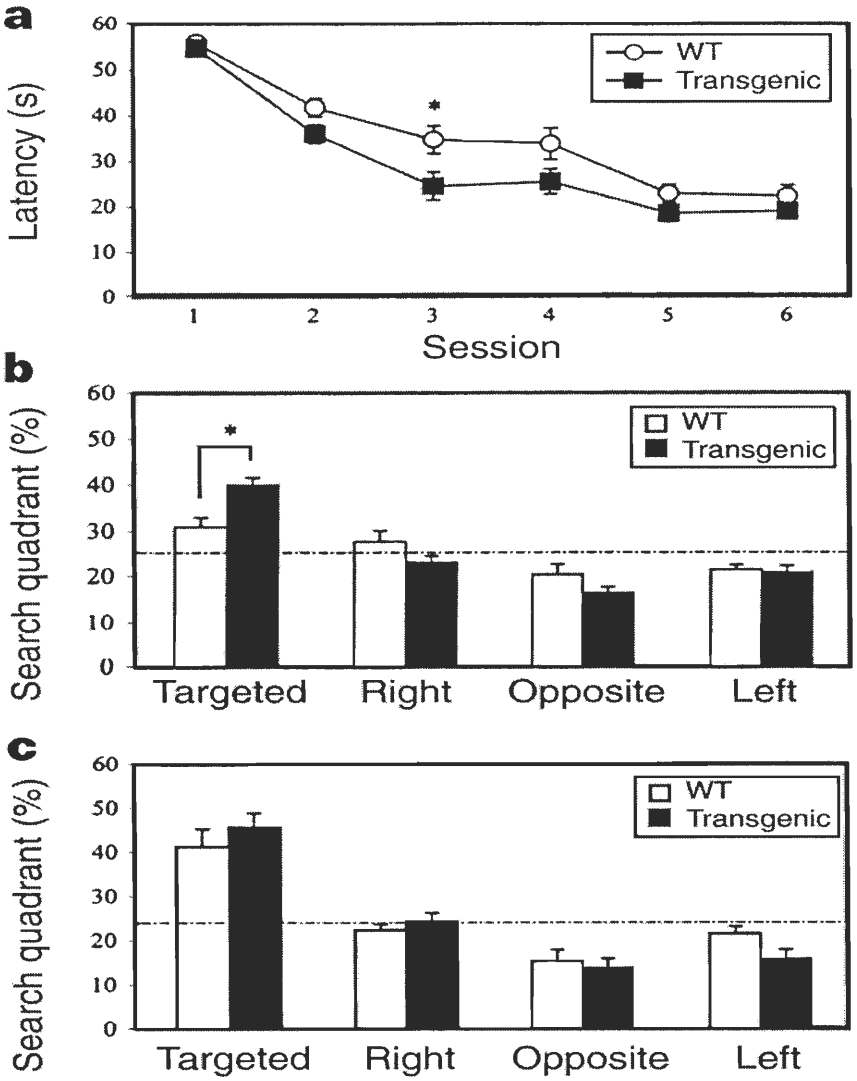


Figure 10. Enhanced performance in the hidden-platform water maze task by transgenic mice. **a**, Escape latency (mean ☐ s.e.m.) in water maze training (Tg-1, $n = 13$; wild-type, $n = 15$). **b**, Place preference in the first transfer test conducted at the end of the third training session. Transgenic mice spent more time in the target quadrant than other quadrants, whereas control mice did not show any preference for the target quadrant at this stage. **c**, Place preference in the second transfer test carried out at the end of the sixth training session. Both transgenic and wild-type mice exhibited strong preference for the target quadrant where the hidden platform was previously located. Asterisk, $P < 0.05$, *post hoc* analysis in **a**, and Student's *t*-test in **b**, between transgenic mice and controls. (Tang *et al.*, 1999)

Chapter 5:

Transgenic Farm Animals

Transgenic Livestock

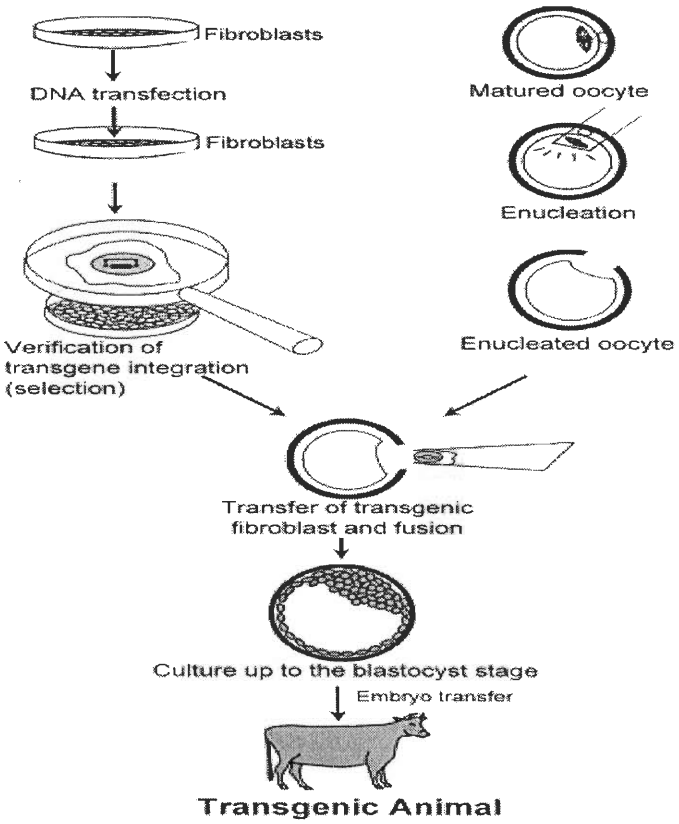
Microinjection of foreign DNA into pronuclei of fertilized oocytes has been the only successful method for the generation of transgenic livestock (Table 8). Although this procedure works reliably, it is inefficient (1-4% transgenic offspring/transferred microinjected zygotes), results in random integration into the host genome and variable expression due to position effects (Pursel and Rexroad, 1993; Wall, 1996). In addition, it is time consuming and requires substantial intellectual, financial and material resources. Recent reports on the generation of transgenic sheep and cattle (Schnieke *et al.*, 1997; Cibelli *et al.*, 1998) via somatic nuclear transfer inspired great expectations about this elegant approach to improve the generation of transgenic livestock. Fetal fibroblasts were transfected in vitro, screened for transgene integration and then transferred into enucleated oocytes. After fusion of both components and activation of the reconstituted nuclear transfer complexes, blastocysts were transfered to synchronized recipients and gave rise to transgenic offspring (Figure below). Compared with the microinjection procedure in which screening for transgenesis and optimal expression of the transgene takes place at the level of the offspring, cloning by nuclear transfer can accelerate the time-consuming transgenic production by prescreening of donor cells for the optimal expression of the desired trait in vitro and 100% transgenic offspring. As of today a variety of cell types has been successfully employed as donors in nuclear transfer (Fulka *et al.*, 1998). However, the overall efficiency of nuclear transfer is low. Factors affecting the success of nuclear transfer are poorly defined and the percentage of live offspring does not exceed 1-3% of the transferred reconstituted embryos (Cibelli *et al.*, 1998; Wilmut *et al.*, 1997; Wakayama *et al.*, 1998). (Niemann, 2000)

Methodology	Integration	Expression	Mouse	Livestock
Gain-of-function				
Microinjection				
minigene constructs	random	variable	+	+
with regulatable promoter	random	inducible (Mt/TET)	+/+	+/–
artificial chromosomes	random	integration site independent (YAC, BAC)	+	...
Retroviral infection	random/site specific	variable?	+	+?
Sperm (atogonia) mediated	random	?	+	+?
Targeted chromosomal integration (Flp, Cre/lox)	defined	controlled	+	–
Nuclear transfer with transgenic donor cells	random/defined[a]	variable/?	–	+/+
Loss-of-function				
ES cells+gene knockout	defined	abolished in all cells	+	–
Cell-type-specific knockout	defined	abolished in specific cells	+	...
Inducible knockout	defined	abolished upon induction	+	–
Somatic nuclear transfer with knockout cells	defined	abolished	–?	–?

[a]Pharmaceutical Proteins Limited (PPL) has recently announced the birth of transgenic lambs produced by the gene targeting method and nuclear transfer.

Table 8. Methodological repertoire for the production of genetically modified mice and large farm animals. +=shown; -=not shown; ?=questionable; approaches were validated according to germline transmission and expression data. (Niemann, 2000)

Figure 11.



Despite all the setbacks in creating transgenic farm animals, some successful transgenic lines have been developed. An Australian group has generated transgenic pigs bearing a modified

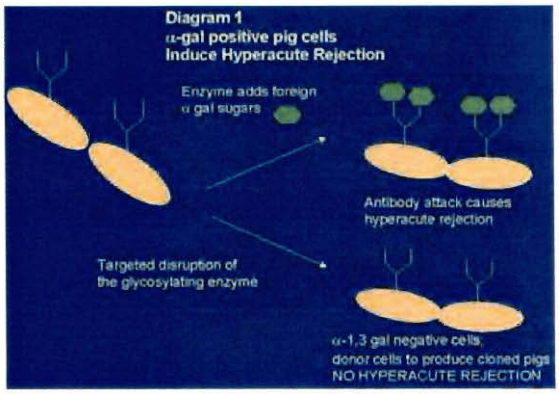
porcine growth hormone (hMt-pGH) construct that can tightly be regulated by zinc feeding. The transgenic animals show significant improvements in economically important traits such as growth rate, feed conversion and body fat muscle ratio. These animals are close to being released to the market (Nottle *et al.*, 1999). Transgenic sheep carrying a keratin-IGF-I construct show expression in the skin and the clear fleece is about 6.2% greater in transgenic vs. nontransgenic animals. These animals are also being prepared for commercial application (Damak *et al.*, 1996a; 1996b). In both projects, no adverse effects of the transgene on health or reproduction are observed.

In the area of biomedicine, numerous transgenic farm animal lines have been developed in a matter of years, some of which are already in advanced clinical trials. Several recombinant proteins have been produced in large amounts in the mammary gland of transgenic sheep and goats, purified from milk and biologically characterized (Houdebine, 1994; Meade *et al.*, 1999). Several products such as human antithrombin III (ATIII), α_1-antitrypsin, tissue plasminogen activator (tPA), α-glucosidase and lactoferrin are currently in advanced clinical trials and the first products are expected to be soon on the market (Ziomek, 1998; Meade *et al.*, 1999).

Xenotransplant Providers

Xenotransplantation is also a promising area in which transgenic pigs are close to clinical application. To overcome the growing shortage of human organs, transgenic pigs have been generated that express human complement regulatory genes. This approach enables overcoming the hyperacute rejection response of patients. Recenty all the four known causes of xenograft rejection (diagrams 1-4) have been devised and shown to work in cell-based experiments. On March 5, 2000, PPL Therapeutics announced that it had overcome the major cause of xenograft rejection (diagram 1) by producing five pigs (picture below) which should become the industry standard for xenotransplantation – a pig lacking the alpha 1-3 gal transferase gene. Another promising area of application for transgenic animals will be tissue engineering. Recently, neuronal cells were collected from bovine transgenic fetuses, transplanted into the brain of a rat model for Parkinson disease and resulted in significant improvements of the neurological

symptoms (Zawada *et al.*, 1998). This indicates that genetically modified livestock cells may serve as a suitable source for xenogenous tissue in certain diseases.



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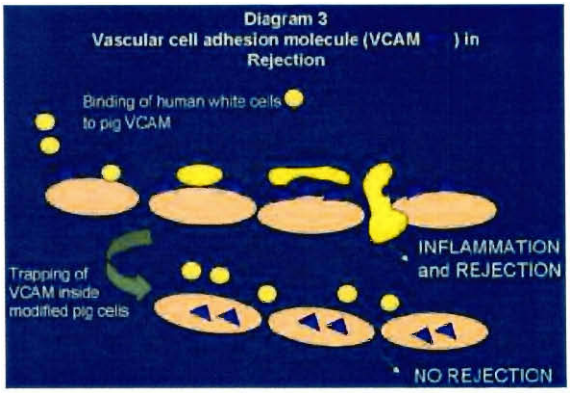

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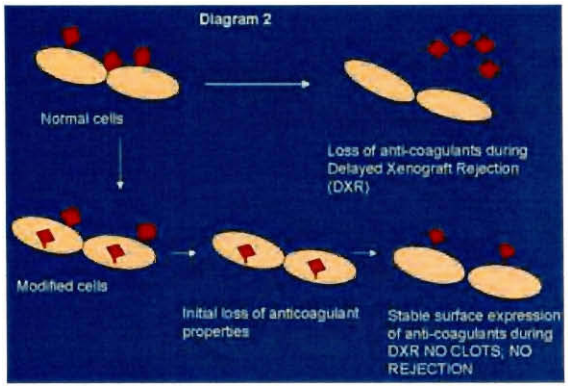

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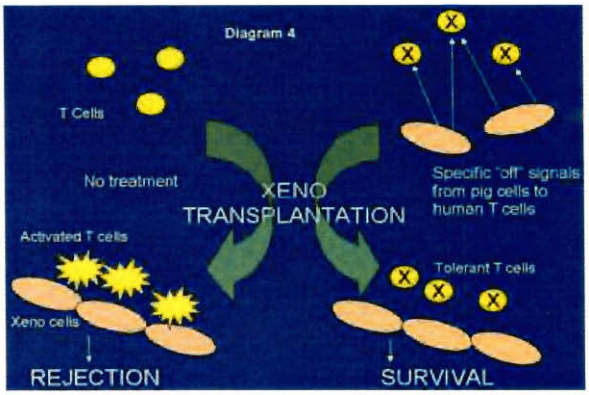

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PPL Therapeutics' five healthy female piglets, Millie, Christa, Alexis, Carrel and Dotcom, born on 5 March 2000, as a result of nuclear transfer (cloning) using adult cells.

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Chapter 6:

Ethical & Personal Views on Transgenic Animals

As a starting point for a dialogue about the acceptability of a particular breeding goal or biotechnology, one may consider the implications for all the parties involved – i.e. for the animals, humans and the environment. Next, those implications – potential risks and benefits – must be weighed against each other. In moral decision making, one seeks a balance between intuitions, principles and relevant facts, notwithstanding the fact that our intuitions may change with new information (Boer *et al.*, 1995). To enable the detection and identification of the issues, and the weighing of the concerns, different models have been developed. The general view in our society is that it is acceptable to use animals in, e.g. farming and research if this is done humanely. This view is reflected in principles of humane use of animals, such as animal protection laws, which state, e.g. that no harm must be done unless necessary, that the harm must be outweighed by benefits, and that some types of harm should be prohibited (MAFF, 1995). (Christiansen and Sandøe, 2000)

I personally agree with the above utilitarian point of view. Harm has been done to animals during transgenic research and development either through ignorance or by mistake. For example, a major case exposing welfare problems associated with the use of biotechnology is the case of the "Beltsville pigs". The "Beltsville pigs" contained human growth hormone genes to accelerate growth, but suffered from health problems such as lameness, ulcers, cardiac diseases and reproductive problems (King, 1996; Rollin, 1997). Some transgenic calves have also been found to be behaviourally retarded and to have difficulties surviving (Mepham, 1995; Rollin, 1997). Attempts to create transgenic sheep with increased growth have resulted in unhealthy animals (Rollin, 1996); and equally, an attempt to produce transgenic cattle with double-muscling resulted in a calf, which within one month was unable to stand up on its own (Rollin, 1996). Furthermore, our current understanding of physiology is not always complete – e.g. the relation between growth hormone genes and diabetes, kidney diseases and bone malformations is unclear

(Mepham, 1994) – and this makes it hard to foresee what the consequences for animal welfare would be of the selected traits. Finally, there is a risk of unrelated, harmful mutations. When creating transgenic animals, foreign DNA is inserted into the hosts' DNA. The foreign DNA may, however, be integrated in the genome in a way, which causes mutations. Such unpredictable responses from totally unrelated genes have been reported in mice showing lethal or deforming mutations (van Reenen and Blokhuis, 1997).

Table 9. Examples of unexpected effects in transgenic animals. (Prieto *et al.*, 1999)

Transgene/TA	Effect	Reference (year)
MTh-bovine GH/pig	Gastric ulcer, cardiomegaly, arthritis	Pursel et al. (1989)67
mWAP/pig	Failure to lactate (agalactia; characteristic mammary gland phenotype)	Shamay et al. (1992)112
baLac-bbcasein/mice	Short lactation	Bleck et al. (1995)110
rWAP-EPO/rabbit	Infertility, agalactia, premature death	Massoud et al. (1996)111
MTh-β-Gal-transferase/mice	Impaired mammary gland development	Hathaway and Shur (1996)109
mWAP-α1-2FUT I/rabbit	Lactose free milk (changes in milk protein quality and content)	Prieto, unpublished results

MTh–mouse metallothionein promoter. mWAP–mouse whey acidic protein promoter. rWAP–rabbit whey acidic protein promoter. EPO–human erythropoietin. baLac-bbcasein–bovine αlactalbumin promoter-bovine β-casein. α1-2FUT–human α1-2 fucosyltransferase "H."

Transgenic technology has also had many benefits for animals. The administration of natural or recombinant somatotropin (ST), or growth hormone-releasing hormone (GHRH) and its analogs, accelerates muscle growth and reduces fat deposition in most farm animals, and these exogenous agents are in widespread commercial use in a number of countries. ST is very effective in pigs (Etherton *et al.*, 1986) and the administration of recombinant bovine ST (bST) increases milk production in dairy cows (Bauman and Vernon, 1993). The long-term delivery of these exogenous agents is accomplished through injectable slow-release formulations and can cause many discomforts in the animals. Administration of high doses of ST to growing pigs or steers may have adverse consequences on some aspects of health, including increased incidence of osteochondrosis, cartilage soundness and stomach ulcers (Sejrsen et al., 1996). In dairy cows, fecundity and fertility are negatively affected when bST is administered before breeding (Burton et al., 1994), in relation with the negative energy balance of the animal. Administration of exogenous pST to lactating sows results in severe energy deficience and difficulties in adjusting internal temperature, which usually results in high mortality rates (Cromwell et al., 1989). However, transgenic technology has allowed the creation of transgenic animals with GH or IGF-I genes coupled to promoters that enable lower production of GH through time control or tissue specificity of gene expression. This has, for example, caused no apparent physiological trouble in transgenic pigs (Nottle *et al.*, 1997). Transfer of the salmon GH gene in salmon or trout, as opposed to exogenous administration of hormones, results in a few symptoms of acromegaly in

the fish (Devlin et al., 1997). The transfer of disease-resistance genes into animals can also treat diseases that might be otherwise untreatable, provide a more natural way for animals to combat disease, and replace chemical or antibiotic therapies that may be costly, harmful to the consumer of animal products or induce antibiotic resistance in germs threatening human health. (Bonneau and Laarveld, 1999)

There are a number of religious groups opposing the creation of transgenic animals, partially because they feel that it is an effort of trying to play God. Others believe that human beings should leave animal genomes intact since animal integrity, or the intrinsic value of animals that is naturally evolved, should be respected (Vorstenbosch, 1993; Thompson, 1997). However, man has already engaged in genome manipulation for thousands of years through selective breeding of animals. For example, the dairy cow now produces 10 times more milk than her calf would traditionally suckle from her (D'Silva, 1998). Current transgenic technology can also be used to redress welfare problems created through selective breeding (Irrgang, 1992). It is also hard to see why transgenic technology is dismissed on the basis that it is unnatural if it is acceptable to, e.g. dam rivers and build cities (Rollin, 1996).

One of the major concerns relating to humans is the "slippery slope" argument, i.e. the fear that what can be done with animals will also be done with humans (Schroten, 1997). Thus, the "slippery slope" argument is concerned not only with a potential technological development, but also a potential change in attitudes regarding what is considered acceptable. However, if at some stage it does become possible to genetically engineer humans, it still does not follow that we have to do so (Sandøe and Holtug, 1993). Transgenic technology will probably be used to treat serious illnesses and hereditary diseases in humans, though, but this is just a change from an "unnatural state" to a natural or normal state.

There is some concern about the risk of loosing genetic diversity through biotechnology. Although this would allow a standardisation of, e.g. dairy products, the loss of genetic diversity makes the animals more vulnerable to diseases and other challenges (Boer *et al.*, 1995). However, some see a potential increase in genetic diversity, as genes are more often added to a species than removed. This gives rise to another concern, however, since distinctions between species may become less distinct, or blurred (Sandøe and Holtug, 1993). The loss of genetic diversity may be considered irreversible (Boer et al., 1995), although the potential exists to preserve genetic material (MAFF, 1995), which could prove useful in the preservation of endangered species. Some also argue that an extensive gene pool may still be available from hobby breeders (Rollin, 1997). (Christiansen and Sandøe, 2000)

There is also concern that if transgenic animals should escape or be released in the wild, the consequences could upset the ecological balance. There is a potential for these animals to replace existing animals in nature, e.g. if they manage better in that habitat or pass on infections to other species. Such infections may develop due to an introduced disease resistance or unpredictable pathogens (Sandøe and Holtug, 1993; Rollin, 1997). Precautions against escape and genetic disadvantages of the transgenic animals, however, make this scenario unlikely (Sandøe and Holtug, 1993), although some aquaculture animals have been known to escape into natural aquatic ecosystems (Kohler et al., 1992). (Christiansen and Sandøe, 2000)

So far there are a countless number of successful transgenic animals that have been made to date. Economics puts a lot of pressure for success in creating transgenic animals due to the high costs, amounts of time, and intellectual capacity that goes into creating just one transgenic animal. And due to the economic worth of transgenic animals, they are the best treated animals in any laboratory or farm. Every mistake that does occur during creation of transgenic animals generally does not go in vain; scientists generally communicate with each other very well about these problems so as to be able to solve them, and thus the success rates of generating perfectly healthy animals is steadily rising. After all, we did not fly to the moon on our first try.

©NASA. Picture taken by the Apollo 17 astronauts on Dec. 7, 1972.

Chapter 7:

Conclusions & Recommendations

Transgenic animals are poised to dramatically increase the well-being of countries. Farm animals producing complex biopharmaceuticals means that former and more expensive methods of harming and sacrificing organisms can be abandoned. Farm animal production can be made more efficient and has the potential to produce the same amount of food using fewer animals. This could reduce problems of pollution and would be of great benefit to the environment (Mepham, 1994). Disease models such as the PDAPP transgenic mouse model help to create cures for diseases such as Alzheimer's which would end the suffering of millions of people and billions of dollars in temporary treatments that could be used more beneficially elsewhere, including environmental protection. In other words, a fraction of the money saved due to permanent treatments and products developed with the help of transgenic animals could be put back into the environment. In this way even plants and animals in the environment will benefit from transgenic animals as long as the governments of countries will uphold these rules or benefits. The ever smaller number of problems that occurs making transgenic animals is thus a small price to pay in the grander scale. There, however, needs to be regulatory agencies to monitor that there is no needless suffering on the part of the animals. If there are other feasible methods in the research and production of medicine and food, these should be used instead. Committees and agencies that deal with the ethical issues and any possible problems that may result from transgenic technology need to be set up and maintained. The benefits of transgenic animals to society, however, are so overwhelming that it would surely be unwise to abandon them.

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