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IQP-43-DSA-8862
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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

submitted to the faculty

of the

WORCESTER POLYTECHNIC INSTITUTE

in partial fulfillment of the requirements for the

Degree of Bachelor of Science

By

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December 19, 2000

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ABSTRACT

Transgenic animals have foreign DNA incorporated into their genomes so that all of their cells have been genetically altered. Such animals have been used successfully for various important functions such as disease models, transpharmers, xenotransplanters, enhancement engineering, and in basic biological investigations. In the project, the legal and ethical issues surrounding these animals were reviewed which brought us to the conclusion that is created and utilized in a controlled manner, transgenic animals could bring powerful advantages to society.

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EXECUTIVE SUMMARY

A transgenic animal carries a foreign gene (transgene) in its genome so that all of the cells including the germ line are genetically modified. These animals that have the foreign gene incorporated into their genome, pass it to their offspring, and express that gene along with their own genomes. This is the basic idea of transgenesis, and it has been becoming increasingly popular over the past couple of decades.

Transgenic animals are used for a variety of useful purposes. They are used as disease models, to help study the diverse aspects of a specific human disease, with the goal of finding a cure or producing a therapeutic. Secondly, they are used as pharmaceutical producers, in which they produce drugs of medical purpose. Transgenic animals can also be used as xenotransplanters, engineered to minimize organ rejection during animal-to-human transplants. In enhancement engineering, animals are manipulated to possess superior traits such as less fat in beef, or resistance to certain pathogens. The last use of transgenic animals is in basic biological studies in which they are used to improve our knowledge of fundamental biological processes.

The purpose of this interactive qualifying project was to describe this new biological technology, transgenics, and to determine the impact of this influential technology on society. This was accomplished by analyzing the ethical and legal issues to decide if the pros outweighed the cons and if transgenic animals would overall be an asset to society.

In conclusion, we agreed that in most cases transgenic animals are valuable to the community without causing needless suffering to the animals. We especially found

useful transgenic animals as transpharmers, which produce valuable pharmaceuticals in their milk, and disease models, which are essential in finding cures to terminal disease. We also concluded that xenotransplanters may be even more beneficial in the future. We decided that all of these medically important reasons were far too important to overlook, especially if all precautions are taken to minimize potential suffering by the transgenic animal. If transgenic animals are used in the proper way and are not hurt they should be used to bring about great benefits to mankind.

PROJECT OBJECTIVE

The purpose of this interactive qualifying project was to describe a new biological technology, transgenics, and to determine the impact of this powerful technology on society. We began by describing the different techniques used to make transgenic animals, and then gave examples of transgenic animals that have been made and their purposes. The legal and ethical issues were also analyzed, allowing us to decide where we stand on the issue.

We accomplished this goal by dividing the paper into six chapters: an introduction, examples of current transgenic animals, methods, legal issues, ethical issues, and conclusions and recommendations. In view of the research performed in this project, both authors came to a common positive conclusion about the controlled use and optimistic future of transgenic animals.

CHAPTER 1: INTRODUCTION

A transgenic animal is one in which foreign DNA has been introduced into the genome of the animal so that all the cells including the germ line (sperm and ova) are genetically altered. These animals having the foreign gene incorporated into their genome, pass it onto their offspring, and express that gene along with their own genomes. Transgenic animals are currently one of the most popular research tools available in the field of biology. These animals are tools of the trade for scientists who use these model systems to probe basic mechanisms of developmental biology, physiology, and pathogenesis. Once the transgenic model is made, researchers can use them for different purposes. Almost every cell of a living organism has a nucleus, which has chromosomes in it. A gene is a part of the chromosome, which is passed on for generations and contains the hereditary information. It is the manipulation of this genome that creates transgenic models (Dziadek, 1996).

FIGURE 1.1 (Lewis, 2000)
Shows a figure of a transgenic mouse.

Altering the genome of an animal helps in understanding gene regulation and gene functioning of the whole animal. The knowledge gained from these studies helps researchers develop animal models of human disease, such as the Alzheimer's mouse. Researchers have been genetically modifying farm animals for agricultural and biotechnological research for thousands of years by traditional selective breeding, but are now doing it faster and between species by inserting transgenes. Transgenic animals are also proving to be valuable tools in the drug development process itself, providing a more accurate way to test the effectiveness of drugs and develop new ones, such as humulin. In another exciting development, transgenic animals are providing organs for transplantation (xenotransplantation) that are less likely to be rejected than the organs currently being used.

When preparing transgenic animals, the genome is manipulated by various recombinant DNA methods so that expression of the foreign material occurs. First, choices must be made about which transgene is worth inserting in another animal. Great care must be given to ensuring a minimum of animal suffering, while maximizing the medical benefit. Next, an egg is extracted from the host animal usually by saline flush. In vitro fertilization of the sperm and egg takes place next, in a test tube. The next step is introduction of the recombinant DNA into the egg, which can be done by retroviral infection of the egg or pronuclear injection and injection of blastocytes. After the injection, the embryos are cultured and implanted. Later the presence of the transgene in the animals is tested by PCR amplification.

Many legal, ethical and moral debates are raised by this topic. Some people agree with animal patenting arguing that because organisms are alive, they can be made useful

by patenting for economic reasons. Others debate that animal patenting should be illegal. One of the most famous transgenic court cases is the oncomouse/Dupont case. Oncomouse was the world's first patented animal.

There are of course many moral issues that go along with creating transgenic animals. Members of diverse religions argue that animals are sacred and altering or creating new life forms is an attempt at playing god. Researchers, scientists and people in the biotechnology field attempt to create them because of their potential to aid the human condition with medical advances. Others, such as animal rights activists, environmentalists, and some of the general public find it to be inhumane and immoral. The outcome of our research into this subject has convinced us that although in some cases transgenic animals undergo suffering, much can be done to minimize the suffering while still contracting great medical benefits.

CHAPTER TWO: EXAMPLES OF TRANSGENIC ANIMALS

TYPES OF TRANSGENIC ANIMALS

Transgenic animals have been used in a wide variety of applications. For this paper we have categorized them into the following five categories:

1.) Pharmaceutical producers and transpharmers- these animals are used to produce drugs of medical importance. If these drugs are secreted into the milk of the transgenic animal, the animals are known as transpharmers.

2.) Disease models- these animals are used to study various aspects of specific human disease, with the goal of helping find a cure, or produce a therapeutic.

3.) Xenotransplanters- these animals are engineered to minimize organ rejection during animal-to-human transplants.

4.) Enhancement Engineering- these animals are engineered to possess a "superior" traits like production of greater quantities of leaner meat, increase disease resistance, or increased reproduction.

5.) Basic biological investigations- these animals are used to improve our knowledge of fundamental biological processes.

PHARMACEUTICAL PRODUCERS AND TRANSPHARMERS

Animals larger than mice have been used with the aim of creating large quantities of pharmaceuticals from animals. One such case is transgenic pigs that can make human hemoglobin in their blood. In 1992, DNX scientists produced human hemoglobin in pigs. This hemoglobin was extracted from the pig's red blood cells, purified and used in human transfusions. The transgenic hemoglobin would not carry HIV, nor would it have to be typed before transfusion (Lee, 1993). This is just one of the many ways transgenic animals have been made to create pharmaceuticals.

Transpharmers are transgenic animals that produce pharmaceuticals in their milk. The primary advantage of having an animal secrete a pharmaceutical in its milk is the animal does not need to be sacrificed to obtain the drug. The milk can be directly given to the patient, if the drug is ingestible. If the drug is not ingestible, then it is purified from the milk. A second advantage of producing the drug in the milk is that it is secreted into the mammary gland, which prevents it from circulating free in the animal's body. This approach is remarkably well suited for hormones that are biologically active in small contents in the bloodstream. Using milk as a source of recombinant proteins was first demonstrated by Simons et al. in 1987, who generated transgenic mice that secreted sheep beta-lactoglobulin in their milk. Later it was shown that larger farm animals are actually more valuable for this process, while the smaller animals (such as mice) produce only small amounts of protein (Houdebine, 1997). Using transgenic animals to produce recombinant proteins in their milk has a number of uses, such as research purposes and therapeutics. The mammary gland is the best studied transgenic system for protein production.

DISEASE MODEL INTRODUCTION

Transgenic animal studies help us understand the cause of many diseases and may point the way to their treatment. Disease models are animal models of human disease that have been created to duplicate portions of the disease, so that portion can be studied. Such models are created to test certain chemicals that might have an effect on the onset of the disease, slow its course, or alleviate symptoms. Since they are models of the disease they render a way to study of the disease without using humans (Eynon and Flavell, 1999). Alzheimer's mouse is one of the many examples of disease models important in transgenic technology today. It was created in part here at WPI in Prof. Adams' lab, and mimics the onset of this costly neurodegenerative disease (Games et al., 1995). Such mice show memory loss, behavioral deficiencies and the classical symptoms of the disease such as the accumulation of beta-amyloid plaques and neuronal cell death (Guenette, 1999).

XENOTRANSPLANTERS

Xenotransplantation is the process of using animal organs to replace human organs. When a major organ fails, the main goal is to get a human transplant for that organ. One main problem is that there is a shortage of human donors. So one solution was xenotransplantation. It has been done so far with moderate success using primates as donors. One of the problems with this process is organ rejection. Rejection can happen very quickly, in a matter of minutes to hours. Hyperacute rejection causes excessive blood loss, thrombosis and edema, ultimately leading to destruction of the new organ. Even if this type of rejection were avoided by getting rid of natural antibodies, then a

person would still have to worry about acute vascular xenograft rejection, which can take months to occur. This rejection involves xeno-reactive antibodies. Transgenic animals are currently being constructed that have genetic modifications to prevent this type of rejection from happening, thus allowing themselves to be suitable organ donors to a human (Houdebine, 1997).

ANIMAL ENHANCEMENT ENGINEERING INTRODUCTION

In animal enhancement engineering, genes are implanted into livestock to amplify certain portions of their body. In one such case, bovine growth hormone was inserted into the genome of a pig (Lee, 1993). Not only does this save money by creating larger animals but it creates leaner meat. Transgenic researchers have intentionally terminated experiments with these animals due to the fact that these animals developed arthritis.

USE OF TRANSGENIC ANIMALS IN BASIC BIOLOGICAL INVESTIGATIONS

In basic biological investigations, transgenic mice are sometimes used to analyze sex determination. A gene called Sry from the murine Y chromosome was inserted into the female mouse embryo (a mouse with two X chromosomes). The result was two male mice, which proved that Sry was indeed the male-determining gene. This was a significant finding because it gave the researchers hope that this was probably true in humans (Lee, 1993).

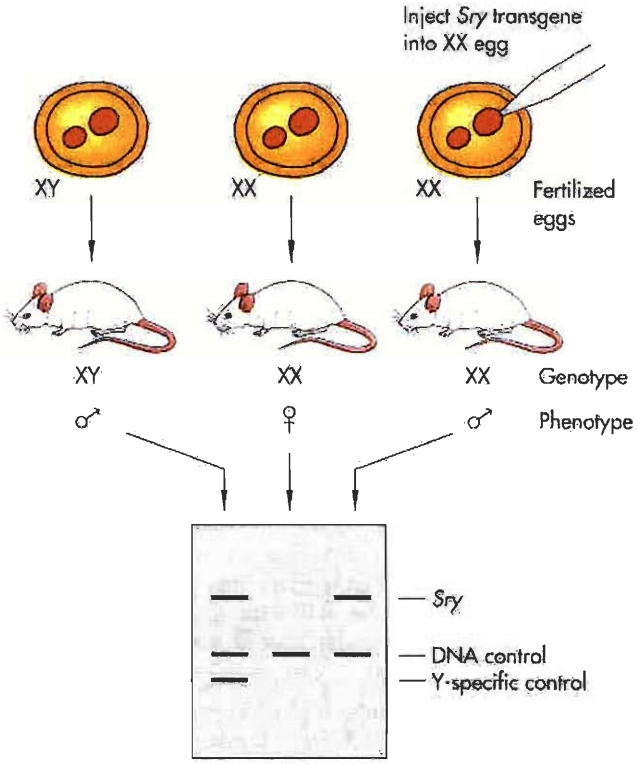

FIGURE 2.2 (Watson et al., 1992)
Shows a diagram of Sry transgene injected into XX egg.

Transgenic methods can also be applied to study the molecular basis of milk biogenesis. This is done through studies conducted with transgenic mice. Alpha-lactalbumin is necessary for normal milk formation. It has two roles: It is a significant nutritional constituent of milk, and it is a regulatory molecule responsible for increasing synthesis of milk (in most species). Transgenic mice were used to show whether or not milk formation is sensitive to alpha-lactalbumin synthesis. Studies were performed on mice that had no murine alpha-lactalbumin genes, and in mice in which the genes were replaced with human alpha-lactalbumin genes. The end results showed that human alpha-lactalbumin can functionally replace the murine protein and alpha-lactalbumin concentrations in the cell are not limiting for lactose synthesis (Colman, 1996). Thus,

showing us one way how production of proteins in the milk of transgenic animals can help scientists gain knowledge about milk biogenesis.

CHOICE OF ANIMAL SYSTEM

Transgenic technology was basically invented on mice, and later applied to rabbits, pigs, sheep, cows, goats, etc. Table 2.1 shows the generation times of the mouse compared with that of the rabbit, pig, cow and sheep. It is costly for the researcher to spend extra time and money on livestock before they know what to expect from an experiment. Initiating an experiment with mice provides information on the sufficiency of the arrangement of the DNA construct and the ability of the mammary gland to yield proteins of the preferred quality (Colman, 1996).

SPECIES	Go BIRTH	Go ADULT	G1 BIRTH (FIRST MILK)	G1 ADULT	G2 BIRTH	G3 BIRTH (G2 HERD)
			mo			
Sheep	5	13	18 [9]	26	31	44
Goat	5	13	18 [9]	26	31	44
Cow	9	23	32 [12]	46	55	78
Pig	4	11	15	22	26	37
Rabbit	1	6	7	----	----	----
Mouse	0.75	2.5	3.25	----	----	----

TABLE 2.1 (Colman, 1996).
Shows the livestock generation times.

There are many qualities that make one animal species a better candidate for transgenic study than another. They include the length of time to milk production, the

volume of milk produced, the litter size, and the disease status as seen in table 2.2 (Colman, 1996).

SPECIES	VOLUME OF MILK	TIME TO MILK PRODUCTION	EASE OF GENERATING FOUNDERS	HEALTH
Sheep	+++	+++	++	++
Goat	+++	+++	++	++
Cow	++++	++	+	++
Pig	+	+++	++++	+++
Rabbit	+	++++	++++	+++

TABLE 2.2 (Colman, 1996)
Shows the relative trangenic merits of various species.

TRANSGENIC MICE

Mice, by far, have been and are currently the most popular species for transgenesis. The mouse may be a small animal, but it is ideal to work on before getting into the more expensive and problematic larger species. Mice are often used just for pilot studies. Mouse models are not always the most dependable because protein quality and yields differ greatly from larger animals.

FIGURE 2.3 (Nature, 2000)
Shows a picture of a transgenic nude mouse.

FIRST EXPERIMENTS

The first transgenic mouse experiments were performed in the early 1980's. A monkey virus, SV40, was injected into mouse blastocysts at an early stage in development. These blastocysts were then implanted into the oviducts of pseudopregnant female mice. Southern blotting indicated that some of the offspring incorporated SV40 DNA in their genome (Gordon et al., 1980).

The first transgenic animal using a human transgene was made in 1982 by Richard Palmiter. He was the first to show that injecting a foreign gene into the germ line can change the appearance of an animal (Fernandez and Hoeffler, 1999). He created **"supermouse"** by fusing the promoter region of the mouse gene for metallothionein-I (MT-I) to the structural gene coding for human growth hormone. He made this abnormally large mouse by microinjecting fertilized eggs. The results proved that 70% of the mice that had incorporated the transgene showed high levels of human growth hormone in their serum, and their phenotypes were different from the control mice in that they grow significantly larger (Palmiter et al., 1983).

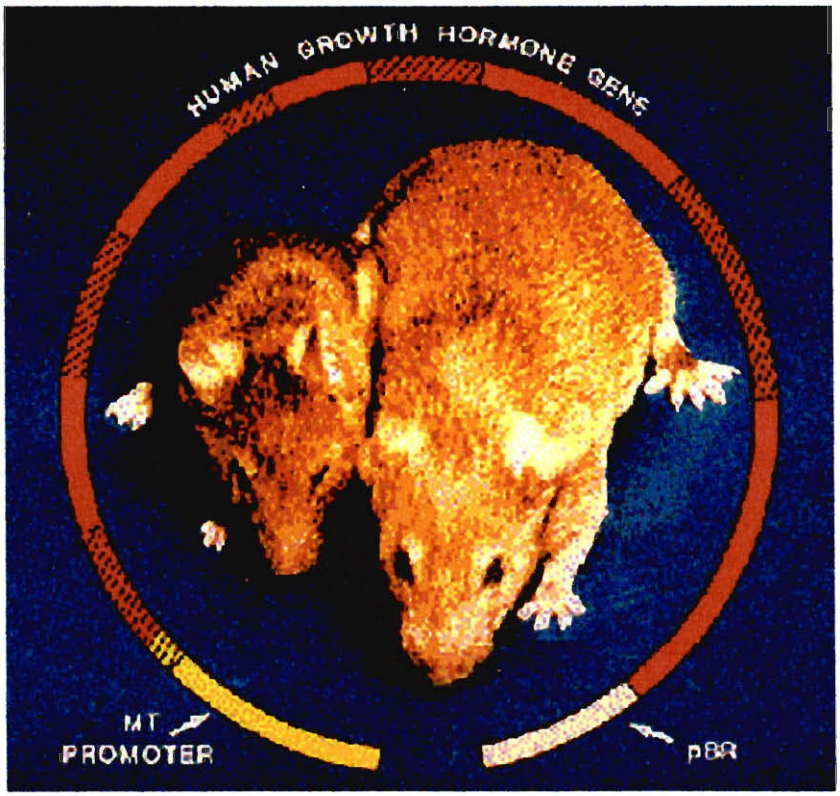

FIGURE 2.4 (Palmiter et al., 1983)
Shows a photo of the first transgenic animal, repressing a human gene. The **"supermouse"** (right) compared with a non-transgenic littermate (left).

MOUSE DISEASE MODELS

HIV MOUSE

One of the most crucial animal disease models is the HIV mouse, which was developed by Vogel et al., in 1988. The human tat gene was put under the control of HIV-1 LTR and it was introduced into mice as a transgene (Taverne, 1993). When this was done, skin lesions appeared that resembled what is known as Kaposi's sarcoma. Transgenic mice containing this tat HIV transgene develop a spontaneous and deadly disease resembling human AIDS (Merlino, 1991). The mice models have allowed

conclusions to be reached that were otherwise impossible to show. This mouse proved that HIV-nephropathy is a direct result of viral infection and not a complication of immune dysfunction or drug abuse (Taverne, 1993). We have come a long way in the search for answers about the devastating disease AIDS, and transgenic mice have helped us in that battle.

NOD MOUSE

A second important mouse disease model is the NOD (non-obese diabetic) mouse. Diabetes is a very attractive autoimmune disease to be studied because it affects so much of the human population, and recently great animal models for the disease have been constructed, like NOD mice. These mice spontaneously develop an insulin-dependent diabetes mellitis (IDDM), which has several features in common with the human disease. CD8+ cells are thought to be the main mediators of cell death in the NOD mice, so much work focused on those cells in the model. Other areas of investigation include the antigen presenting capacity of dendric cells found in the NOD mice by using a TNF-alpha transgenic mouse model. TNF-alpha mice express TNF-alpha only in their islet cells, and as they grow they develop diabetes (Eynon and Flavell, 1999). TNF-alpha is used because of the linkage between this and diabetes: Yang et al., showed that NOD mice injected with neutralizing anti-TNF-alpha antibodies protected against diabetes, and injection of recombinant TNF-alpha provoked pathology (Green and Flavell, 1999). The prospects of the studies of TNF-alpha NOD mice are to design treatments that prevent beta-cell destruction associated with IDDM (Eynon and Flavell, 1999). Recently, diabetes n these NOD mice was cured using gene therapy with an adeno-associated virus expressing human insulin (Lee et al., 2000).

MS MOUSE

Multiple sclerosis (MS) is yet another disease in which mice make excellent disease models. The pathophysiology of experimental allergic encephalomyelitis (EAE) in some animals simulates that of the human disease, MS. It is a common autoimmune disease of the nervous system, where inflammation is observed in the CNS associated with the presence of lymphocytes and macrophages. Since T cells play a major role in EAE, T cell receptor transgenic models of peripheral tolerance have been made. The symptoms in both MS and models are similar, ranging from changes in vision to weakness and paralysis. The TCR transgenic mice have been used to gain perceptiveness into the cellular regulation of EAE tolerance, pathogenesis and functions (Wong et al., 1999).

ALZHEIMER'S MOUSE

The first Alzheimer's mouse model was created in 1995 by Games et al. Alzheimer's patients display memory loss and disorientation due to a degeneration of the brain. Analysis of Alzheimer brains at autopsy show a "spongiform" pathology with holes in the brain. Neurodegeneration occurs close to the sites of "senile plaque" formation. The dominant protein of these plaques is **amyloid**, which is neurotoxic. Thus current strategies for therapies often focus on reducing plaque formation. However, such studies are difficult and slow to perform in humans, thus an animal model was desperately needed. A mouse model was created, because only costly orangutans occasionally get plaques. Transgenic mice were created, in part here at WPI, those express large levels of human mutant APP (amyloid precursor protein). The mutant gene mimicked an aggressive early-onset version of the disease in humans. These mice

experienced neurotic plaques, synaptic loss, microgliosis, and astrocytosis, all symptomatic of Alzheimer's disease. This model established that amyloid deposition in the brain is necessary and adequate for initiating the disease (Games et al., 1995).

Recently, this mouse model was used to produce the first Alzheimer's vaccine. The transgenic mice were immunized with Abeta42, which is the amino-acid form of the peptide found in the amyloid plaques in the disease. They injected the peptide either before or after the onset of the disease. In both cases, their results proved that prevention of plaque formation, or the removal of existing plaques via the vaccine, reverses brain neurodegeneration (Schenk et al., 1999). The results of a phase-I human clinical safety trial with this vaccine indicated it has no major side effects in humans (Helmuth, 2000).

TRANSGENIC RABBITS

Rabbits are another good example of animals favored by researchers in transgenesis. Although they are larger than mice, they are small compared to other livestock, which is beneficial. Also on the up side, rabbits produce on average eight offspring, and up to forty embryos can be gathered. Rabbits have a few advantages over mice because they are larger and it is easier to do certain applications on them. Like mice, transgenic rabbits can also produce recombinant proteins.

RABBIT TRANSPHARMERS

In 1991 foreign antigens were produced in rabbit blood. In 1996 foreign antigens were produced in rabbit milk. To this date many important proteins have been secreted in

the milk of the rabbit including: Human interleukin 2, tissue plasminogen activator, chymosin, human erythropoietin and human IGF-1 (Houdebine, 1997).

One disadvantage in using transgenic rabbits over mice is that they are more costly because they need more space due to their larger size. They make up for it because they can produce tens to hundreds of grams of recombinant proteins in their milk, which is actually cost effective compared with mice (Fan et al., 1999). In addition, transgenic rabbits are great models for the study of lipid metabolism (mice are not) because of the similarities of the lipid systems of rabbits and humans (Houdebine, 1997).

RABBIT DISEASE MODELS

One example of a current transgenic rabbit is the model made for human hypertrophic cardiomyopathy. This is a disease in which the walls of the heart thicken because of defective cells in the heart muscle (Kunz and Finkel, 1987). It is caused by certain mutations in the genes for sarcomere proteins. Transgenic rabbits have been created containing this mutant gene as a model to study this disease. Cardiac expression of beta-Myhc-Q (403), the mutated gene, resulted in interstial fibrosis, myocyte and myofibrillar disorder, hypertrophy and death. These were many of the same symptoms seen in humans with the disease (Marian et al., 1999).

TRANSGENIC PIGS

Transgenic pigs have been produced for four reasons: 1) to improve their productivity, 2) to enhance their resistance to disease, 3) for biomedical products and 4)

as models for human disease. Pigs are great farm animals to work with because they are smaller than most other livestock, which is more cost effective. Unlike the larger farm animals, pigs produce a litter. Transgenic pigs improve farmer's productivity because it saves them money by better efficiency in food utilization. Which means there are 10-15% more pigs because it costs less. The farmer ends up producing pork with lower fat content at a lower cost (leaner meat). There is also 15% less waste being produced (Houdebine, 1997).

The second reason transgenic pigs have been produced is because of possibilities for reducing disease susceptibility. This comprises genes known to modulate non-specific host defense mechanisms, genes for specific disease resistance, genes encoding an immunoglobin specific for a common pathogen, genes for a viral protein that can interfere with viral replication, and genes for antisense RNA as antipathogenic agents (Houdebin, 1997).

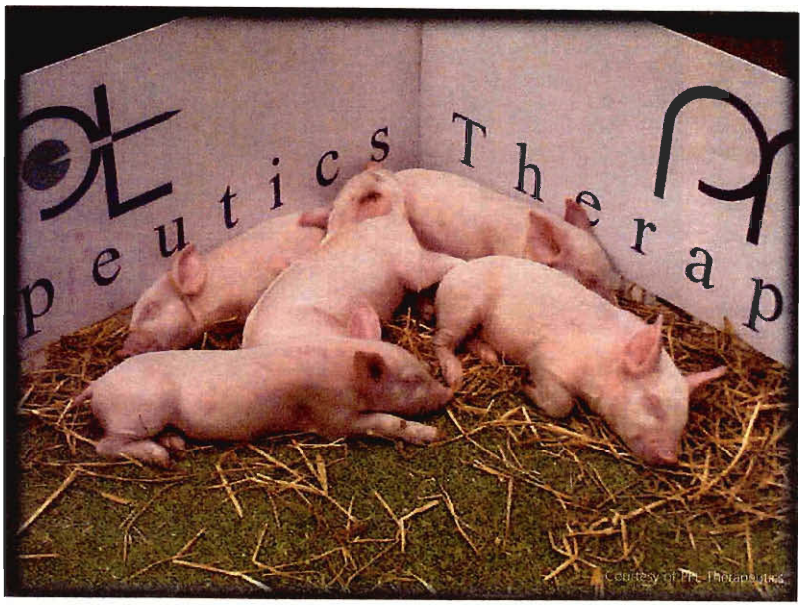

FIGURE 2.5 (Lewis, 2000)
Shows a figure of transgenic mice.

PORCINE DISEAES MODELS

One example of a transgenic pig model for disease was created by Petters in 1994. These pigs had a defective rhodopsin gene. This allowed further study of the disease retinitis pigmentosa. Pigs are better than mice for studies concerning the eyes, because humans and pigs have eye similarities such as the size, retinal physiology and anatomy, that mice do not share (Houdebine, 1997).

PORCINE TRANSPHARMERS

Transgenic pigs have been created that produce important products like human hemoglobin and human protein C that are used for medical applications (Houdebine, 1997). The porcine mammary gland can carry out many of the processes that are necessary for recombinant synthesis of complex human proteins and produce them at high levels. One of the advantages of using the porcine mammary gland as a bioreactor is the high cell density comparative to that of the cell culture (Morcol et al., 1994).

Human hemoglobin A (HbA) has also been expressed in transgenic swine (TgHbA). The TgHbA is interchangeable with the HbA in that they have the same isoelectric pH, mass, oxygen affinity, cooperativity, Bohr effect, and allostery. The transgenic swine system serves as a superior source for the production of human HbA for therapeutic applications (Manjula et al., 1998).

PORCINE XENOPLANTS

One future prospect for pigs is inserting certain genes so that one day they can supply organs for xenotransplantation into humans. The pig thus far is the most

appropriate donor for humans. Much work is being done to overcome the immunological obstacles of hyperacute rejection, delayed xenograft rejection (DXR) and acute vascular xenograft rejection to make the transplantation work. The focus of researchers has not been on just the recipient of the graft, but also on the donor.

Transgenic pigs have been created that have genetic modification, which will inhibit the activation of the complement cascade, which in turn prevents hyperacute rejection. DXR is controlled by transgenic inhibition of endothelial cell activation.

Pig to primate xenotransplantations are currently in the works, which provides us with a bi-animal system for investigating how these rejections can be prevented (French et al., 1998).

TRANSGENIC SHEEP

Transgenic sheep have been generated for various reasons. Like mice and rabbits, they can produce valuable proteins in their milk. The obvious advantage in using sheep over mice is that they are larger and can produce more milk, hence more protein. Sheep milk is very much like cow's milk. It is high in minerals and calcium. Lactose is also the major carbohydrate. These components found in the milk can be easily removed, and will make the process of separating proteins from each other easier (Houdebine, 1997).

Proteins from sheep milk have now been used in trials for cystic fibrosis and congenital emphysema. There are a few drawbacks to this technology, which involve an inability to control targeted insertion of the transgene into animal genome, extensive timelines to production of herds, and unpredictable expression levels.

One example of a current transgenic sheep is Polly. Polly is a cloned sheep containing the human gene-encoding factor IX. This protein is involved in the prevention of hemophilia (Colman, 1999).

Two techniques have been used to increase the trans-product productivity in sheep: 1) use of the ovine growth hormone gene, in which regulation has been changed by use of an ovine metallothionein promoter. This approach can boost the growth rates of sheep while keeping them in fine health. 2) The modification of intermediary metabolism by introducing the cysteine biosynthetic pathway in sheep (Ward and Brown, 1998).

The sheep mammary gland can serve as a bioreactor for producing pharmalogically active proteins. This is done by marking the expression of sequences that encode non-milk proteins to the mammary gland of transgenic sheep. The sheep have been constructed which have a gene with the human blood clotting factor VIII cDNA under the transcriptional control of a fragment of the mammary gland promoter of sheep beta lactoglobulin gene. These sheep have produced human blood clotting factor VIII in their milk (Niemann et al., 1999).

TRANSGENIC GOATS AND COWS

Transgenic goats and cows are good prospects for milk production. Using the goat's beta casein promoter researchers can produce human pharmaceuticals in their milk. Transgenic cows have been used for enhancement of productive efficiency. Bovine somatotropin was adopted for use in the dairy industry. Tests were done using bovine somatotropin group and a control group over a period of eight years. 340 herds

were involved with 80,000 cows and 200,000 lactations. The overall results showed that bovine somatotropin improved lactation yield and persistent consistency. There were no negative effects on cow stability or herd life (Bauman et al., 1999).

CHAPTER 3: TRANSGENIC METHODS

The construction of a transgenic animal is done in several steps as follows:

1. Preparation of DNA: transgenes are constructed, with prudent choosing of the promoter
2. Egg maturation and extraction
3. Fertilization of the sperm and egg in a test tube; in vitro fertilization
4. Embryo culture/embryo collection
5. The foreign DNA is introduced into the zygote by several methods:
    a) pronuclear microinjection
    b) transfection of DNA into embryonic stem (ES) cells
    c) retroviral infection of ES cells or embryos
6. Embryo implantation
7. Screening offspring by southern blotting or PCR

PREPARATION OF DNA

DNA is prepared by using recombinant DNA methods. The gene should contain three things, the structural gene desired, the vector DNA that allows the molecules to be incorporated into the host DNA molecules, and the promoter and enhancer sequences, which allow the gene to be expressed by host cells. The structural gene is just the DNA sequence that codes for a protein (Maclean, 1994). The structure of a gene is as follows: it is composed of various DNA segments exons, introns, promoters, silencers and

enhancers. Exons, which code for specific portions of the gene, are separated by the introns, which are the non-coding part of the gene essential for most advantageous expression of the gene. The gene also consists of a promoter, which is the major regulatory element and is situated in front of the first exon, controlling the initiation of transcription and the rate of transcription. It also plays an important role in controlling the rate of activity of other genes. Enhancers and silencers are the other regulatory elements, which also have an essential role in gene transcription. Additional regulatory steps determine how much functional protein is produced. This includes mRNA splicing, transport and stability of mRNA in the cytoplasm, efficiency of translation into the protein and stability of protein and post-translational modification, which is necessary for complete protein function.

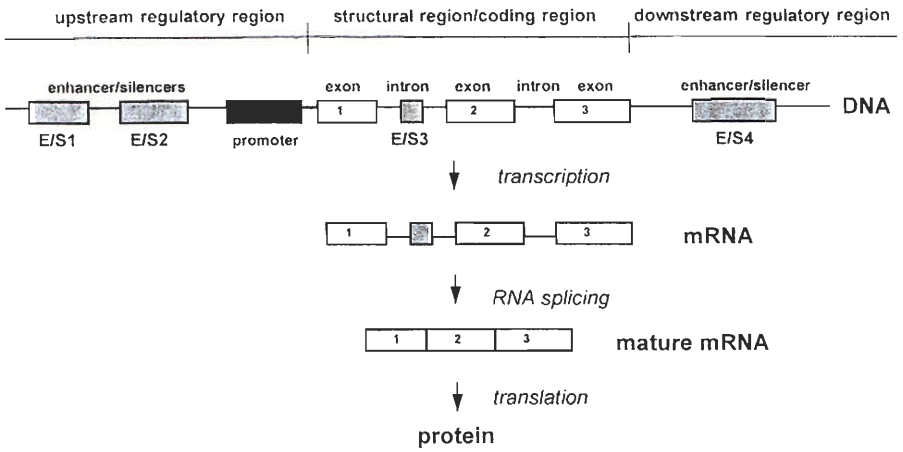

FIGURE 3.1 (Dziadek, 1996)
Shows the main elements of a gene. The open boxes 1, 2, and 3 are exons and are separated by introns. The black box is the promoter. Shaded boxes are Enhancers or Silencers.

The transgene is prepared in the laboratory by using restriction enzymes and ligase to recombine different functional regions of genes from different species. The restriction enzymes recognize a specific sequence in the DNA and cleave it in both strands at every point that target sequence occurs. The proper restriction enzymes as well as the correct ligases, which are enzymes that seal DNA fragments by noncovalent interactions, must be used in order to form the correct transgene expression cassette, which is done in vitro (Lewin, 1997). When DNA molecules are cut, the ends have an overhanging piece of single-stranded DNA. These "sticky ends" base pair with any DNA molecule containing the complementary sticky end. Both of the DNA preparations (the transgene and the vector it is inserted into) have complementary sticky ends and thus pair with each other when mixed. DNA ligase, enzyme covalently links the two into a molecule of recombinant DNA (www.godriva.com/~jkimball/BiologyPages/R/RecombinantDNA.html).

The procedure involves treating the DNA from transgene and vector with the same restriction endonuclease, for example, BamHI. BamHI is used to cut the same site on both molecules

5' GGATCC

3' CCTAGG

Once the transgene expression cassette is constructed polylinker sequences are added to modify the ends of the completed construct. The polylinker sequences contain the target site for the restriction enzyme and allow the construct to be inserted into different vectors for cloning and testing, by creation of "sticky ends" at which the foreign DNA can be inserted (Lewin, 1997).

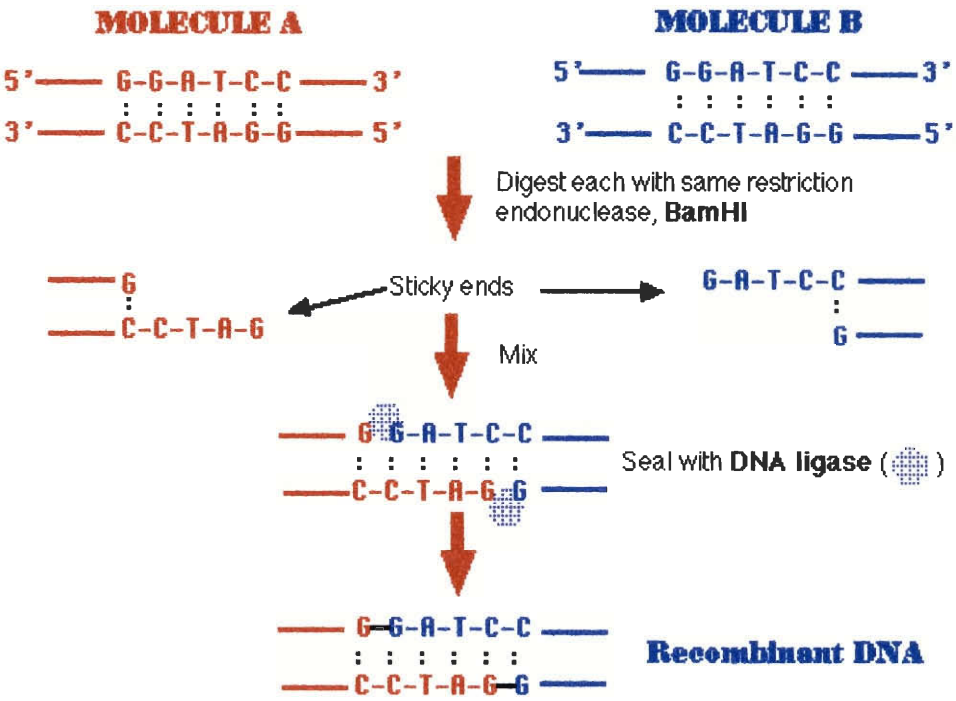

FIGURE 3.2 (www.godriva.com/~jkimball/BiologyPages/R/RecombinantDNA.html)
Preparation of Recombinant DNA.

EGG MATURATION AND EXTRACTION

After preparation of the DNA of choice, the next step is, to obtain fertilized eggs for one of the several methods for introduction into the zygote. There are many ways to obtain fertilized eggs; one basic approach is to set up natural matings without the use of gonadotrophins, in which environmental conditions regulate the timings of ovulation and fertilization, instead of hormones. In order to increase the number of fertilized egg production, which is used for the recovery of the preimplantation embryos in experiments such as microinjection of the eggs, gonadotrophins are often administered to females prior to mating. This increases the number of eggs that can undergo ovulation, which is

also known as superovulation. Newly ovulated eggs, surrounded by cumulus cells, are found in the upper part of the oviduct of the mouse and are very enlarged at this time. The embryos are collected by having the animal lie on its back, while the embryos are surgically isolated from the oviduct of donors. The oviduct and the ovary are retracted from the abdomen of the donor. The oviduct is collected by flushing with 50-ml phosphate-buffered Dulbecco's medium (PBS), also known as saline flushing. The isolated zygotes are cultured in fresh medium and later are classified morphologically with the aid of microscopes. The pronuclei of the zygotes are saved for later DNA microinjection (Hogan et al., 1986).

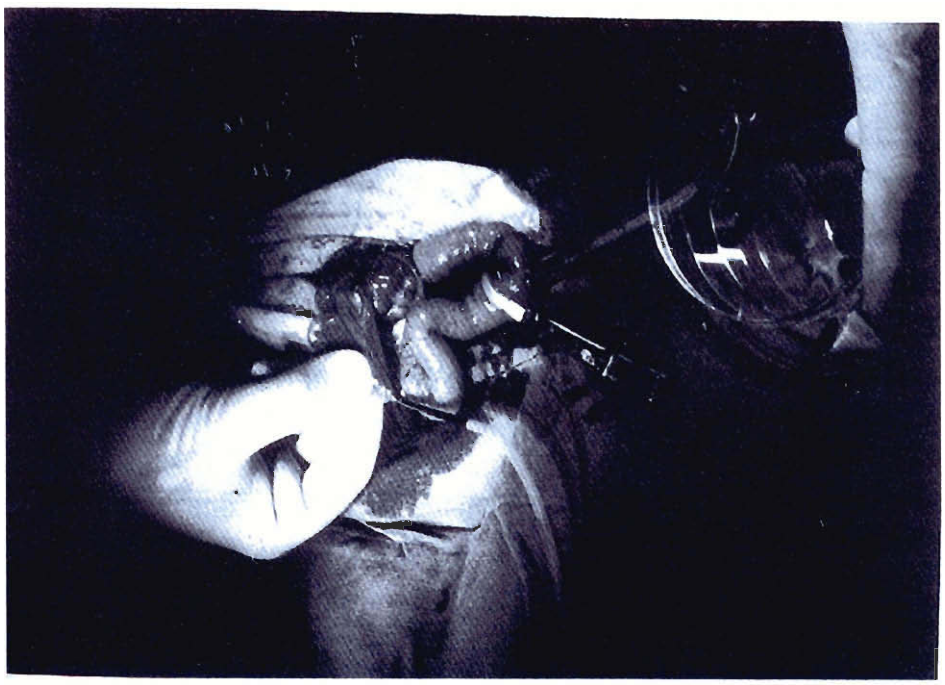

FIGURE 3.3 (Maclean, 1994)
Surgical embryo collection by flushing of an oviduct.

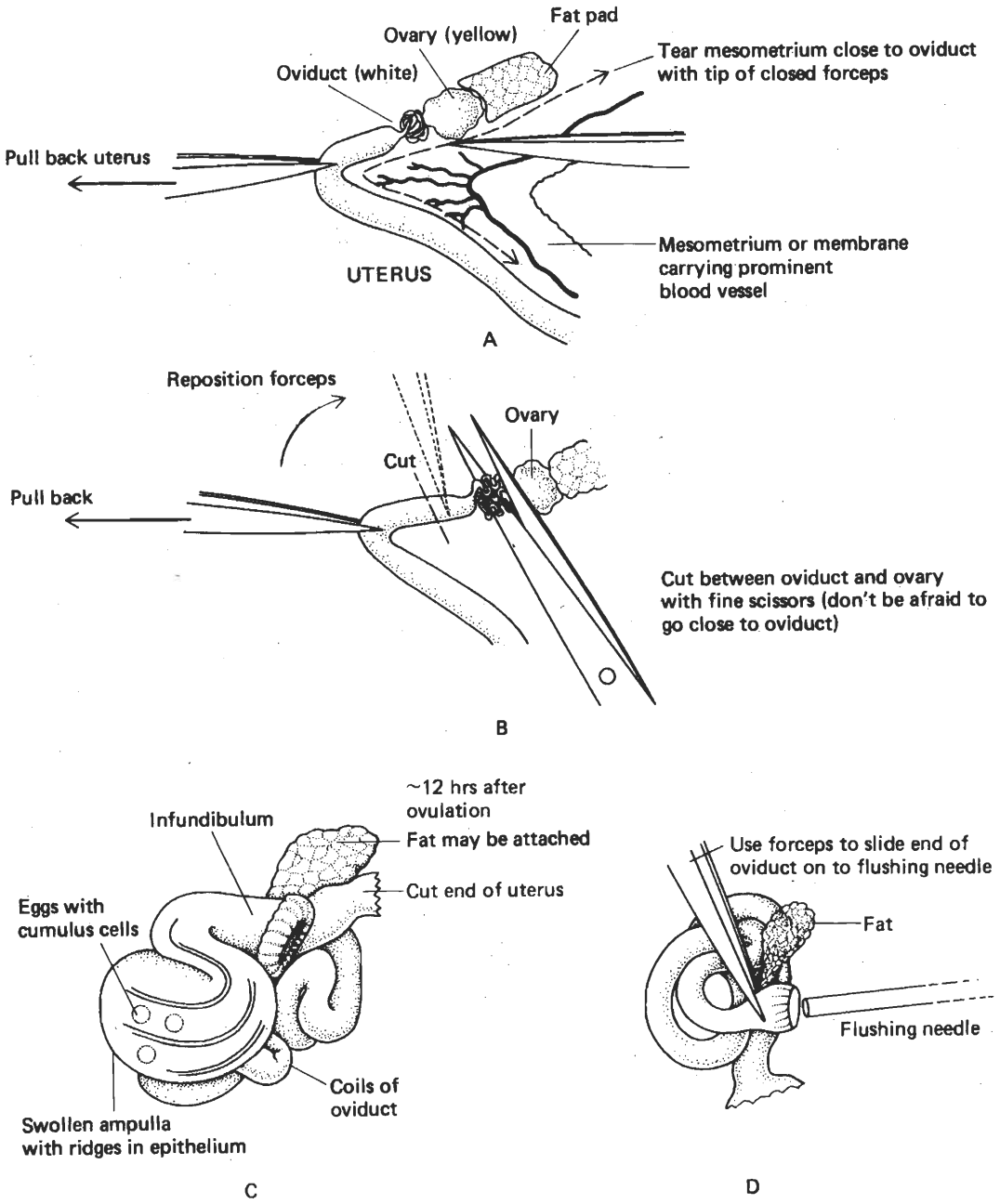

Figure 3.4 (Hogan et al., 1986)
Collecting embryos from the oviduct.
A) A cut made between the oviduct and the ovary.
B) Second cut between oviduct and the ovar y.
C) Eggs released by tearing the ampulla.
D) Embryos recovered by flushing the oviduct.

Blastocytes can be flushed from the uterus before the cloned DNA is introduced into the embryonic cells. They are isolated from the embryo and later transferred to the foster mouse to develop to term (Hogan et al., 1986).

IN VITRO FERTILIZATION

Embryos can also be collected through an alternative procedure, in vitro fertilization. There are two major advantages to this procedure. The biggest advantage to this technique is one can obtain large numbers of early-cleavage-stage embryos that can be developed more or less at the same time than those collected after natural mating. The second advantage is that there is no need to set up natural matings for fertilization and the females do not need to carry litters to term. The in vitro fertilized eggs are later transferred to a pseudopregnant foster mother (Hogan et al., 1986).

> **"The in vitro fertilization technique involves superovulating females and fertilizing the eggs so obtained with sperm taken from the epididymis of the male. This has been found to give more synchronous development than sperm from the vas deferens" (H.Pratt, unpubl.)**

The process of intro fertilization is as follows: females are injected with PMS and hCG as in superovulation. Males are killed, and the epididymis is dissected out, it is placed into a 500-microlitre drop of pregassed Whittingham's medium under oil. The sperm is squeezed using forceps, which is then incubated at 37°C to capacitate them. Later females are killed and their oviducts are dissected out. They are released into a 1000 microliter drop of pregassed Whittingham's medium. The oviducts are incubated at 37°C. 100 microliters of sperm is added to the drops containing eggs. Both sperm and

eggs are incubated for 4 hours at 37° C eggs are transferred into pregassed sterile M16, taking as few sperm as possible. The eggs are then fertilized (Hogan et al., 1986).

EMBRYO CULTURE/EMBRYO COLLECTION

In order to collect embryos to be treated, oviducts are removed from the pseudopregnant females and bunches of pronuclear embryos are gathered from the oviducts by one of two methods, flushing or by dissection into a microdrop of sterilized buffered medium. The embryos are bunched together with sticky follicular cumulus cells that must be eliminated by treatment in a series of microdrops. The embryos are made free of cumulus cells, debris and enzyme by transferring them from drop to drop. Lastly the embryos are transferred to a petri dish full of medium that will be placed under a microscope (www.criver.com/techdocs/transgen.html).

INJECTION OF DNA INTO THE ZYGOTE

There are several methods in which the DNA gets incorporated into the host animal's genome before it becomes a transgenic animal. One of the first and most widely known techniques is DNA microinjection into the pronucleus of a fertilized egg. The second method is also widely used, the embryonic stem cell method. Last is the retroviral infection of embryonic stem cells or embryos. All three methods have their pros and cons while some are more useful than others.

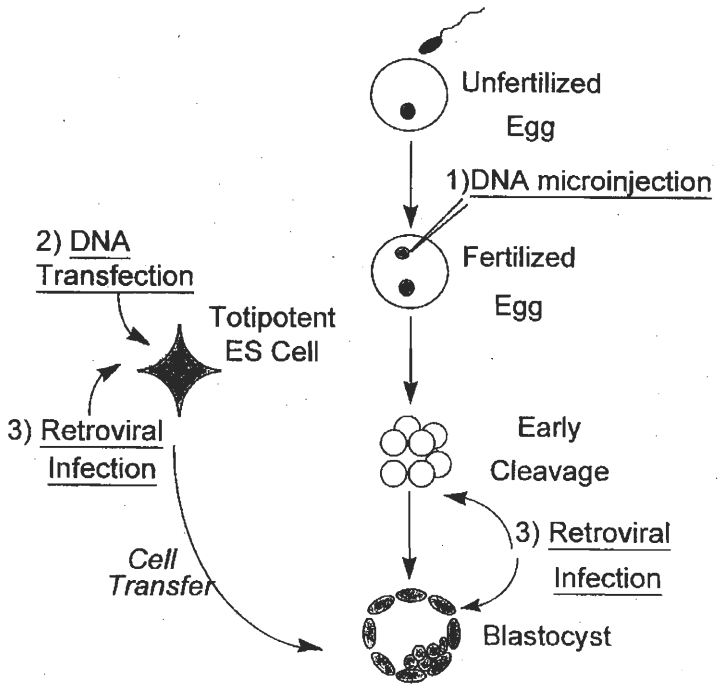

FIGURE 3.5 (Fernandez and Hoeffler, 1999)
Different strategies for the generation of transgenic mice.
1) DNA microinjection into the pronucleus of a fertilized egg.
2) Transfection of DNA into embryonic stem cells.
3) Retroviral infection of embryonic stem cells.

MICROINJECTION

Gordon et al., reported the first production of transgenic mice using the pronucleur microinjection technique in 1980. A vector-gene construct containing the herpes simplex virus thymidine kinase gene and a piece of simian virus 40 genome was microinjected into several hundred mice eggs (Glick and Pasternak, 1998). The transgene was reported to have incorporated itself into the mouse genome, but it did not express itself. In 1982, the first visible changes in transgenic mice were detected by Palmiter et al., for mice expressing the rat growth hormone. The pronuclear stage of the

zygote is easy to inject as they swell during the one-cell stage and they are in the exact optimal state for microinjection. So they reach the maximum size before the nuclear membrane disappears and the transgene DNA is introduced into the zygote immediately after fertilization. Glass pipette attached to micromanipulator is used to inject very small quantity of solution containing DNA into egg. In this way several hundred copies of cloned gene are injected into the male pronucleus of the fertilized egg.

In the pronuclear microinjection technique, hundreds of copies of the transgene get incorporated directly into the pronuclei of a newly fertilized egg. The fertilized egg contains two pronuclei, one from the sperm and one from the egg; these eventually form the nucleus of the one-celled embryo. After injection the two pronuclei fuse together to form a diploid zygote nucleus, the zygote will divide by mitosis to become a two-cell embryo (Watson et al., 1992). The microinjection method is also called the preferred method because it is applicable to a wide variety of species. It is achieved in three steps: In the first step, the donor females are stimulated to superovulate to increase the amount of available fertilized eggs. A normal female mouse produces 5-10 eggs, while a superovulated mouse produces about 35 eggs. This is achieved by giving the mouse two injections. The first is an injection of pregnant mare's serum, and 48 hours later they are injected with human chorionic gonadotropin. Next the superovulated mice are mated and then killed so the fertilized eggs can be flushed from their oviducts. Last the eggs are collected and microinjection occurs (Glick and Pasternak, 1998). The transgene is presented into the zygote at the pronuclear period, which follows fertilization (the earliest time possible) because it has to get incorporated into the host genome before the first cleavage and before the doubling of genetic material. If the transgene does not integrate

into the host at that time, a mosaic animal is formed in which some of the cells do not carry the new gene (www.criver.com/techdocs/transgen.html). If the DNA gets integrated in the first division, every cell will have the transgene. The embryos are then transferred to the oviduct of the pseudopregnant foster mother (Watson et al., 1992).

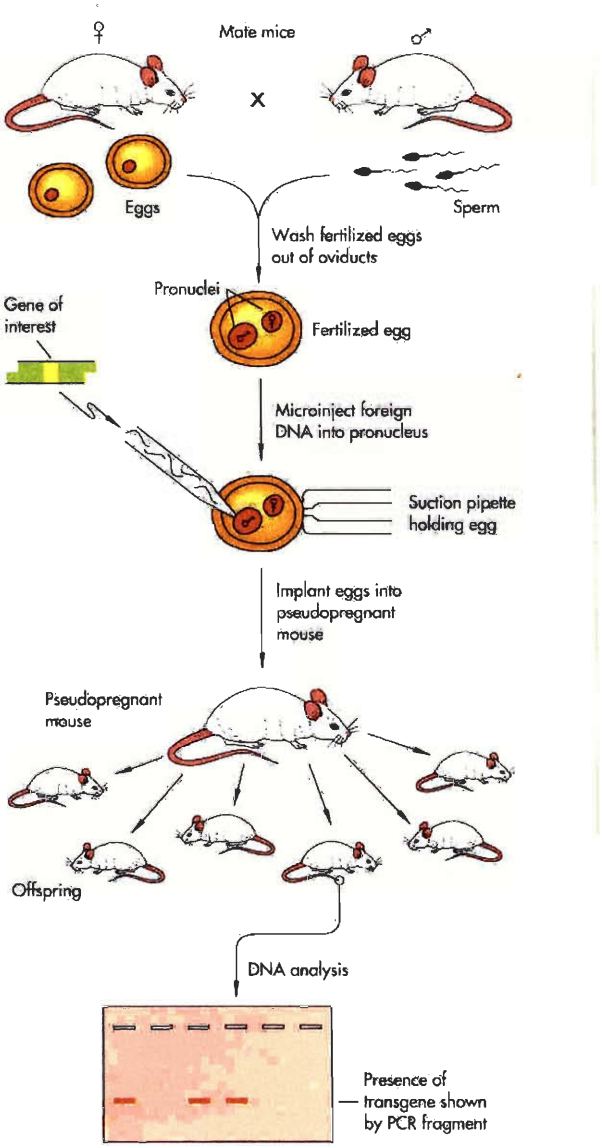

FIGURE 3.6 (Watson et al., 1992)
Shows the microinjection technique. Eggs are taken from the donor female and purified samples of the transgene are microinjected into the pronucleus.

Only about 5 % of the eggs will become transgenic animals, and there is a high probability that the injection itself will kill the zygote. After that, 25 % of the implanted eggs develop into offspring and 25 % of those become transgenic animals. Out of 1000 eggs, only 30–50 become transgenic animals.

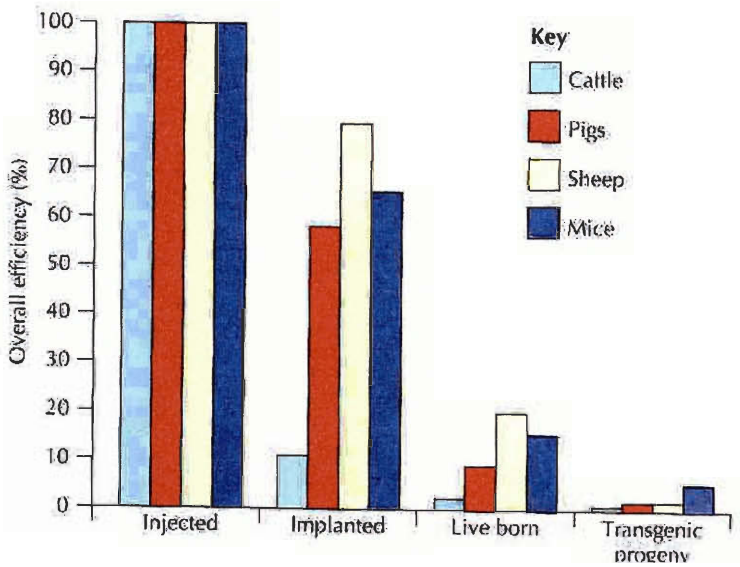

FIGURE 3.7 (Glick and Pasternak, 1998)
Shows the overall effiency of the transgenesis process after microinjection

There are four major problems that go along with this microinjection technique, 1) there is no control over how or where the transgene will be integrated, 2) there can be multiple copies of the transgene, 3) It can disrupt the normal function of the gene (knockout the function of the gene), because the integration is random the transgene can insert itself into functional genetic sequences which can either be insignificant or lethal and 4) It may not even get expressed (Glick and Pasternak, 1998).

EMBRYONIC STEM CELL METHOD

By using the method of transfection of DNA into embryonic stem cells one can manipulate and control DNA integration, which is done by introducing the foreign DNA into the embryonic stem cells (ES cells). ES cells are pluripotent, undifferentiated cells, which have the ability to differentiate into any type of cell such as somatic and germ cells (www.godriva.com/~jkimball/BiologyPages/T/TransgenicAnimals.html). In cell culture these cells can be easily genetically engineered without changing their pluripotency (Glick and Pasternak, 1998). The ES cells are captured by culturing the inner cell mass of mouse blastocytes. They are grown as any other cells are, except they must be prevented from differentiating. This is achieved by growing them on a feeder layer of fibroblasts or by adding Leukemia Inhibitory factor to the culture medium (Watson et al., 1992). After the cells are transfected with transgene, it is noted that some of the cells have DNA incorporated at nontarget sites, while others have the DNA incorporated at its target site. In more cells than not, the transgene will not be incorporated at all.

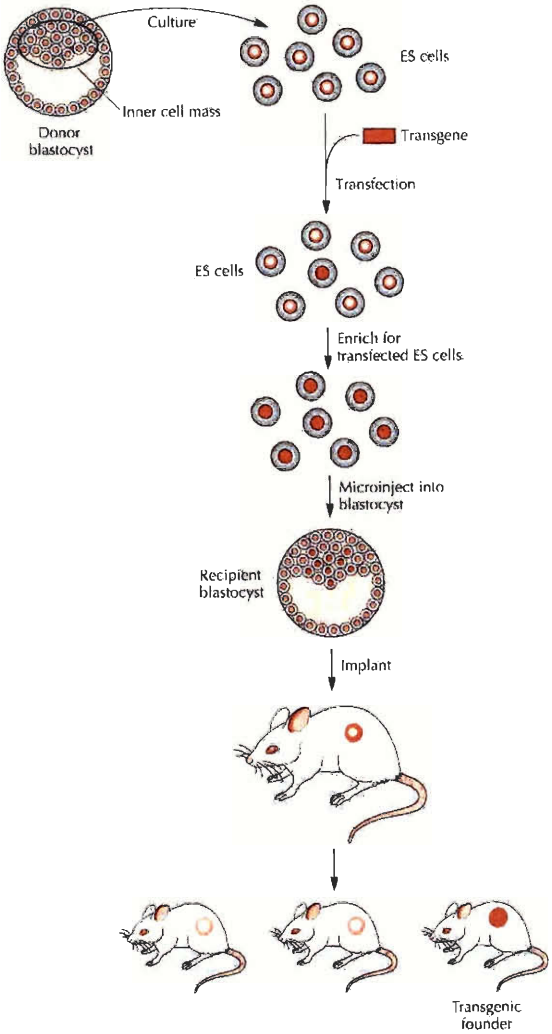

FIGURE 3.7 (Glick and Pasternak, 1998)
Shows the embryonic stem cell method.
The ES cells are transfected with a transgene.

One popular method of detecting the transgene integrated at the target site is by positive, negative selection as seen in figure 3.8. Cells that have vector DNA integrated anywhere in their genomes are positively selected and for cells that have the DNA sequence integrated at nontarget sites negative selection is used. To test for positive-negative selection the targeting DNA vector contains,

1) 2 blocks of DNA sequences (HB1 and HB2) that are homologous to different regions of the target site.
2) The transgene (resistant to G-418)
3) A DNA sequence that has Neo r
4) 2 different genes for thymidine kinase (tk1 and tk2)

If integration occurs at a nontarget site, other than HB1 or HB2, one or both of the tk1, tk2 can be integrated along with the other sequences. On the other hand, if integration occurs due to homologous recombination, the tk1 and tk2 genes will be left out and only the transgene and Neo r gene will be integrated into the genome. Only the positively selected cells that contain the integrated transgene will live when the cells are grown in the presence of G-418, because the cells that lack the Neo r gene will be killed (Glick and Pasternak, 1998).

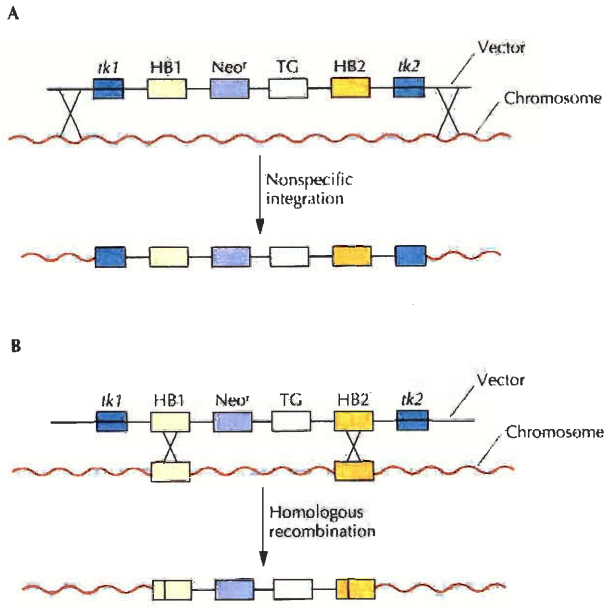

FIGURE 3.8 (Glick and Pasternak, 1998)
Shows negative-positive selection of ES cells.

Once it is clear that the ES cells contain the transgene, they are transferred into the embryo at the blastocyte stage, which results in a chimeric animal. The main advantage is that this method allows precise targeting of defined mutations in the gene through homologous recombination (www.godriva.com/~jkimball/BiologyPages/T/TransgenicAnimals.html). The random integration that is found in microinjection is avoided because a functional transgene can be incorporated at a specific site within a nonessential gene in the genome of ES cells (Glick and Pasternack, 1998). There are three ways in which DNA can be introduced into the ES cells: transfection (which was just described), electroporation, which involves subjecting fertilized eggs to electric pulses in the presence of transgenic copies. High transgenic induction efficiencies have been claimed in some cases and retroviral transfection, which will be discussed next (Watson et al., 1992).

RETROVIRUS METHOD

Retrovirus gene transfer is one method of producing transgenic animals in which gene transfer is mediated by a virus. Viruses are used because they can efficiently transfer genetic material into a cell much in the same way they normally infect host cells. (www.godriva.com/~jkimball/BiologyPages/T/TransgenicAnimals.html). The retrovirus containing the transgene is incorporated into the donor female's mouse embryo at the eight-cell stage of development, before it is implanted into the foster mother. Although this is an effective way of incorporating the transgene into the genome of a recipient cell it has a few major disadvantages: Not all the cells carry the retrovirus so the progeny produced are chimeric, the retrovirus must get incorporated into some of the germ cells in

order for the transgene to be activated, and retroviruses can only transfer small pieces of DNA, which may lack fundamental sequences for regulating the transgene because of the size restraint (Glick and Pasternak, 1998).

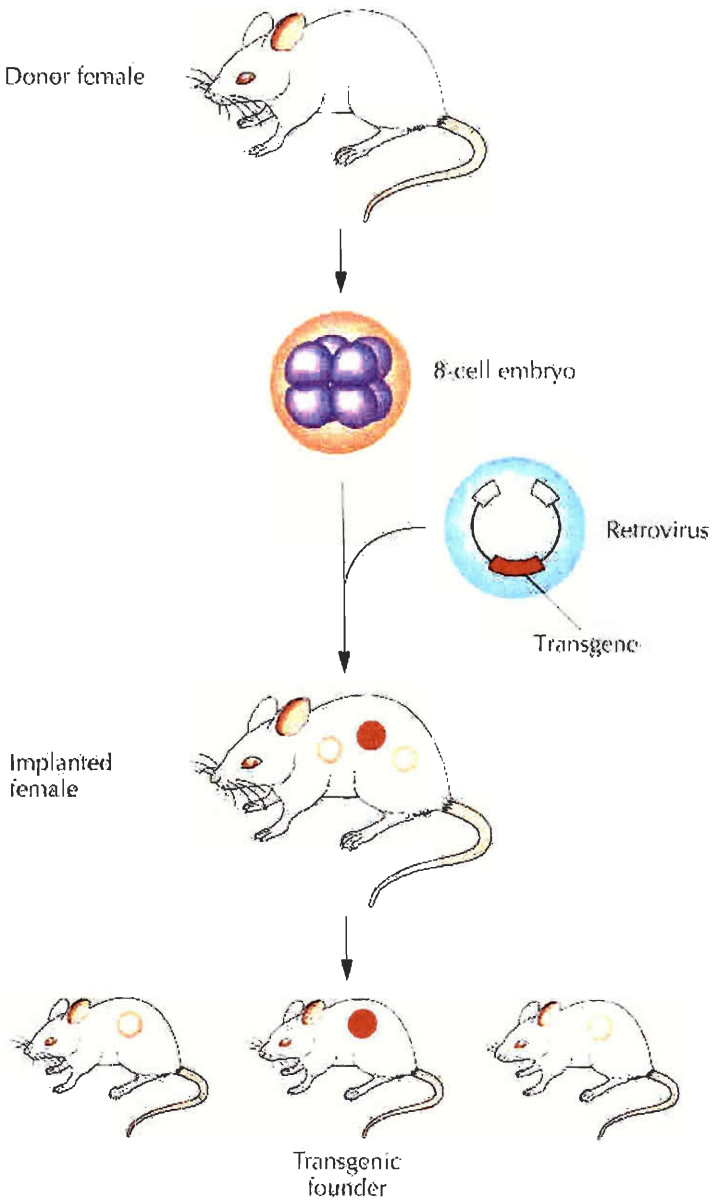

FIGURE 3.9 (Glick and Pasternak, 1998)
Shows the retroviral vector method. The retrovirus carrying the transgene is integrated into the embryo at the eight-cell stage.

EMBRYO IMPLANTATION

The manipulated embryos must be transferred into a surrogated mother so that they can be given an opportunity to become a transgenic mouse. Embryos that are transferred into the reproductive tract of a pseudopregnant recipient should be anywhere from the one-cell stage to the blastocyte stage to complete their development. Manipulated and cultured embryos generally exhibit slightly delayed development to those developed in vivo, which is why the recipient female should be earlier in her reproductive cycle than the donor female (Hogan et al., 1986). In mice, copulation is the only way to prepare the uterus for implantation. The foster mother has been made pseudopregnant by being mated to a vasectomized male, so the male contains no sperm and none of the foster mother's eggs are fertilized. Following uterine implantation of the foster mother delivers her offspring three weeks after implantation of the inoculated eggs.

SCREENING OFFSPRING

Two popular methods in screening offspring are by southern blot hybridization and Polymerase Chain Reaction (PCR). In these two methods, a piece of DNA, such that from the tail of a mouse, is assayed for integration of the transgene. The transgenic mouse is then mated with another mouse to see if the transgene is inheritable, ie. is in the germ line of the founder animal. The positive offspring are then mated to make homozygous transgenic lines.

SOUTHERN BLOTTING

In a southern blot, DNA is isolated from a tail section. It is then cut with restriction enzymes and the fragments are separated by size on an agarose gel. The DNA fragments are then transferred from the gel to a cellulose filter where they bind. A labeled probe, specific to the transgene is hybridized to the DNA molecules bound to the filter containing a complementary sequence. Autoradiography of the filter paper produces a pattern of bands showing the size and number of the fragments that are complementary to the probe. Southern blotting is useful for detecting whether the foreign DNA was incorporated into the mouse. If it detected by the probe then it is present in the germ line of the mouse and the mouse is transgenic and not chimeric (Watson et al., 1992).

POLYMERASE CHAIN REACTION

In 1983 Biochemist Kary Mullis invented the polymerase chain reaction (PCR) technique, enabling scientists to rapidly reproduce bits of starting DNA. Small amounts of DNA are replicated so that there are sufficient quantities to work with. PCR works in a very specific manner, amplifying a desired sequence of DNA potentially billions of times, and it can do this even if you don't know the entire sequence you're amplifying. The recombinant DNA molecule introduced into the recipient mouse must be replicated many times to provide material for analysis and sequencing (Hogan et al., 1986). There are three steps in the PCR process: denaturation, renaturation and synthesis. In order to perform PCR, one must know at least a portion of the sequence of the DNA molecule that is to be replicated. Two primers are synthesized, short oligonucleotides (containing about

two dozen nucleotides) that are precisely complementary to the sequence at the 3'end of each strand of the DNA you wish to amplify. Next the DNA sample is heated to separate its strands and mixed with the primers. If the primers find their complementary sequences in the DNA, they bind to them. Synthesis begins (as always 5' -> 3') using the original strand as the template. The reaction mixture must contain a DNA polymerase that is not denatured by the intense temperature needed to separate the DNA strands, and dATP, dCTP, dGTP, and dTTP, the four deoxynucleotidetriphosphates. (www.godriva.com/~jkimball/BiologyPages/P/PCR.html).

CHAPTER 4: TRANSGENIC LEGAL ISSUES

PATENTS

A patent is a monopoly right of restricted duration which is granted by a country or a group of countries to the holder of the patent for an "invention". It gives the patent holder property rights for 17-20 years, depending upon which country is involved, to use the transgenic animal for commercial gain. The invention must meet four parameters in order for it to be patentable: 1) novelty; the inventions must not have been described as such in the prior art, 2) inventive step; must not be obvious to the person skilled in the art, 3) industrial application; must be able to be "used in some kind of industry" and 4) have commercial application and properly disclosed in the patent specification (Houdebine, 1997). The patent holder has the right to charge others a fee for the use of their patented product and to draw out a royalty on any commercial applications acquired from it. The patent holder also has the power to prohibit an unauthorized third party from using the invention. A patent is not the right to do something, but it is the right to prohibit something (Smith, 1996). In return for this exclusive possession, the patentee exhibits the details of the invention to the public so that at the end of the limited time period, the invention may be worked on without obstruction by the public (Houdebine, 1997).

"Patentable" is defined as any new, industrially useful and inventive product. Patent laws can provide additional means for control. The product, otherwise known as invention, must be described in writing, which is valued by an appointed official. It can be rejected if it is "contrary to morals or public order" (www.ivanhoe.co.uk).

The patent provides rights for stopping other people from copying for commercial gain, not the definite right to use.

> **"The CIPA believes that experimentation on animals should be controlled, but that limiting the areas of patentability is not a suitable way of achieving this. An essential feature of the patenting procedures requires publication of the technical description only 18 months after the earliest filing date. There must also be sufficient detail in the description for those skilled in the particular area to be able to understand and repeat the work." (www.ivanhoe.co.uk).**

In order to qualify as patentable, the inventor must prove that their "object" serves a useful purpose, is something no one ever made before, and it must be something someone has not thought of using prior expertise. With this in mind, the question arises as to whether human genes, cells, tissues, organs or fetuses and embryos should be considered patentable. If an invention meets these three criteria it does not necessarily mean that it is considered patentable. If it is a discovery of nature, such as a chemical element, it cannot be an invention. The PTO has decided that isolation and classification of a gene's purposes and properties is enough to acknowledge it as an invention.

Another question that has arisen asks whether or not chimpanzees are considered patentable even though they share 99 percent of the genetic makeup of a human being. The PTO says yes. This is where the strong debates on the rights and wrongs of patenting life and making transgenic animals come in (Rifkin, 1998).

BENEFITS AND DISADVANTAGES OF THE PATENT SYSTEM	
BENEFITS	**DISADVANTAGES**
The patent holder retains an absolute monopoly on product or process for the period of patent (up to 20 years in some cases).	Knowledge is in public expiry and could be valuable to competitors.
Administration of patent Maintenance once it has been Obtained is relatively easy.	Litigation can be expensive.
	Problems of lack of harmonization of patent laws and other trading blocks not covered by patent could tolerate misuse.

TABLE 4.1 (Smith, 1996)
Shows the pros and cons for
patenting a transgenic animal.

HISTORY

In 1793 Thomas Jefferson wrote the Patent Act of 1793, which depicted lawful subject matter as "any new and useful art, machine, manufacture, or composition of matter, or any new or useful improvement." In 1952, when the patent laws were reclassified, congress replaced the word "art" with "process" but otherwise left Jefferson's language unaltered. The committee reports accompanying the 1952 Act, tell us that congress planned for lawful subject matter to "include anything under the sun that is made by man" (www.ccac.ca/english/transsup.html).

In 1987, the PTO completely reversed its earlier position and issued a ruling that "all genetically engineered multicellular living organisms, including animals are potentially patentable." With that new ruling, it was evident that we were heading into a new era, the Biotech Century. This was a shock to most people, but it was stated that the decision covered all organisms, except human beings. According to the thirteenth Constitutional Amendment, slavery is forbidden. So humans as a whole, cannot be experimented on, but separate pieces such as human genes, cells, tissues and organs are fair game (Rifkin, 1998).

DIAMOND VS. CHAKRABARTY

In 1971, Ananda Chakrabarty, who at the time was employed at General Electric company (G.E.), filed a patent relating to his use of the pseudomonas bacteria that could break down crude oil. It was intended to consume oil spills on the oceans (Glick and Pasternak, 1998). Chakrabarty's patent claims were of three types: 1) process claims for the method of producing the bacteria, 2) claims for inoculum comprised of a carrier material floating on water, such as straw and 3) claims to the bacteria themselves (McCuen, 1985). Sidney Diamond, a patent examiner, allowed the first two claims but rejected the third part, which was the claim for the bacteria. He had two premises for his decision: 1) microorganisms are "products of nature" and 2) the claim saying that bacteria, as living things were not patentable subject matter under the United States federal law under 35 U.S.C.101 (www.2.law.cornell.edu/cg…it_headings/words=4hits_only?). The PTO described the few cases where patents were extended to life forms (in asexually reproducing plants) it

it had taken a legislative act of congress to create a special exception. Chakrabarty and G.E. petitioned the PTO decision to the court of customs and appeals, where they were victorious with a three-two decision, which surprised many observers. The court stated that,

> **"The fact that microorganisms...are alive is without legal significance...patented organisms are more akin to inanimate chemical compositions such as reactions, reagents, and catalysts than to horses and honeybees or raspberries and roses." (Rifkin, 1998)**

The patent office then appealed the case once more, this time to the Supreme Court. The PTO was joined in the case by the People's Business Commision who stated that the case before the court went directly to the question of the intrinsic value and meaning of life. They argued that "if the patent were upheld by court, then manufactured life, high and low, will have been categorized as less than life, as nothing but common chemicals." Their prediction was that a favorable decision in court might allow future patenting of all life forms.

After nine years, in 1980, the justices ruled in favor (five to four) of Chakrabarty and granted a patent on the microorganism, the first genetically engineered life form. The final decision agreed upon by the majority stated that "the relevant distinction was not between living and inanimate things, but whether Chakrabarty's microorganism was a human-made invention." Clearly, the majority found Chakrabarty's microbe to be a "human-made invention."

The court decision pleased many people, such as biotechnological companies and much of corporate America. Soon after the decision, Genetech, a popular South San Francisco biotech company, decided to sell one million of its shares, which by the time they were all sold the shares had nearly tripled. Biotechnological, chemical and pharmaceutical companies were thrilled and began accelerating their research and development work. To them, this patent protection meant big things for commercial gain in the future.

Still others, found problems with the outcome of the famous court case, such as ethicist Leon Kass who said,

> **"What is the principled limit to this beginning extension of the domain of private ownership and dominion over living nature...the principle used in Chakrabarty says there is nothing in nature of a being, not even in the human patentor himself, that makes him immune to being patented." (Rifkin, 1998).**

Kass concluded that a genetically engineered organism was looked at as an invention; the same way computers or other types of machines were regarded, so why make distinctions between lifeless objects and living organisms? He was concerned with the aftermath of this court case. If Chakrabarty's organism could be patented, what genetically engineered organism couldn't be patented next? Kass did not like the idea of life becoming an invention where there seemed to be no boundaries between intrinsic and useful value (Rifkin, 1998).

In conclusion, as decided by the courts, it was not nature's handiwork but that of the patentee. This case has had meaningful implications for the future success of other

genetically engineered organisms, which are now coming forward in ever-increasing amounts for patent consideration (Smith, 1996).

ONCOMOUSE

In 1988, oncomouse was engineered by Harvard geneticist Philip Leder and Timothy Steward. It was created to develop cancer tumors all over the body. They spliced a DNA sequence of human oncogene c-myc, which they were studying, into genetic material from a murine mammary virus and used the combination to make transgenic mice which could pass this material to their progeny (Houdebine, 1997).

The patent office called the Harvard mouse "a composition of matter" and the product of human invention. Therefore in 1989 a patent on the strain was developed.

The summary of the invention states that a transgenic non-human animal, preferably a mouse, contains an activated oncogene sequence, which is introduced into the germ and somatic cells at the one-cell or fertilized oocyte stage of the animal. It is important that the oncogene sequence gets incorporated into the germ cells of the transgenic animal, which means that all of the animal descendants will carry the activated oncogene sequence in all of their germ and somatic cells (www.2.law.cornell.edu/cg…it_headings/words=4hits_only?).

An activated oncogene sequence when fused into the animal's genome, expands the probability of the development of tumors in the animal. A few methods were used by Leder and Stewart to introduce the transgene into the embryo. One method describes using an oncogene whose transcription is controlled by synthetic or viral activating promoter. A second method refers to changing the oncogene or its control sequences

before introduction into the embryo. Lastly, activation results in transfecting the embryo with the gene as it occurs in its natural state, and selecting transgenic animals where the gene was integrated into the chromosome at a locus.

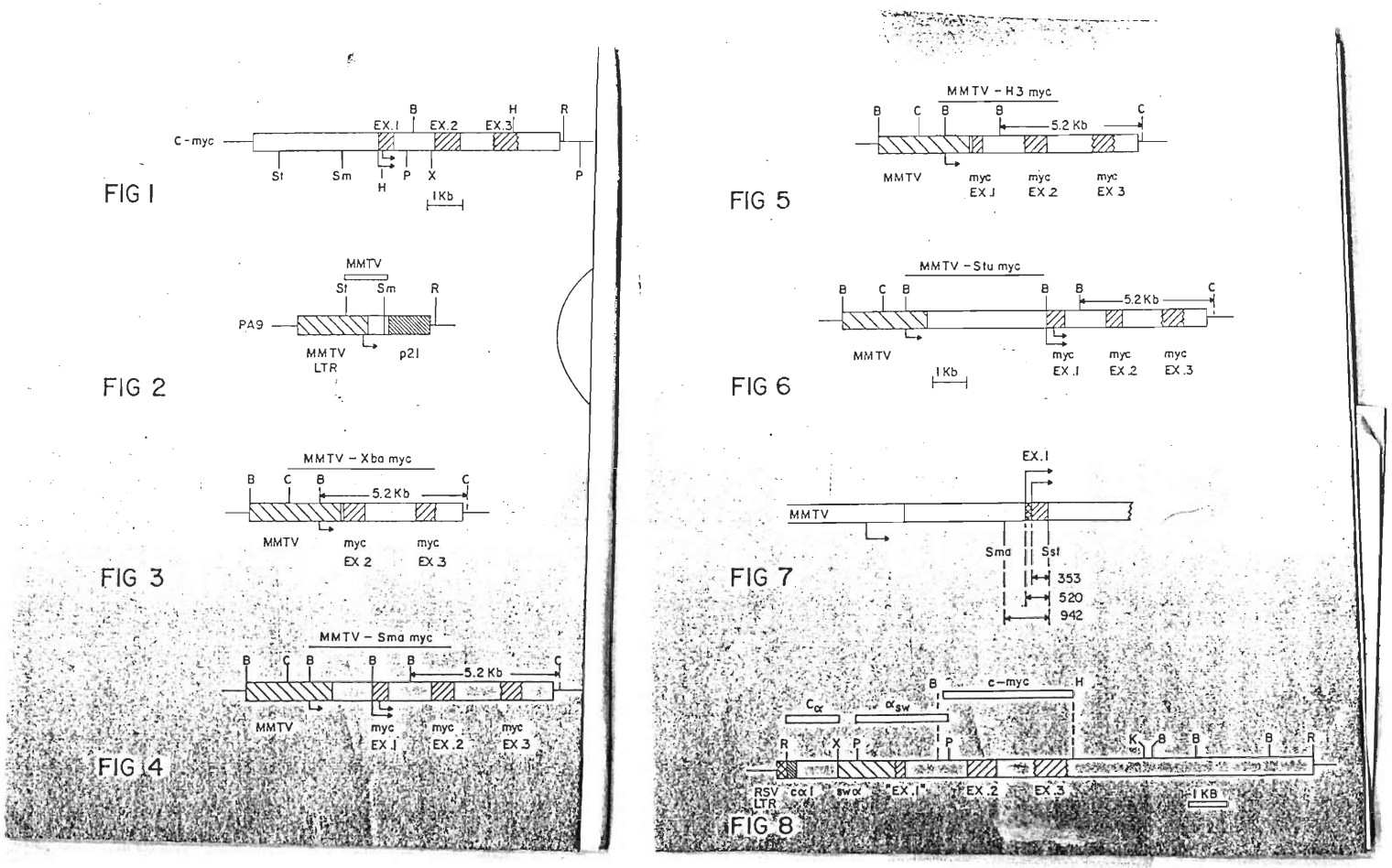

FIGURE 4.1 (www.2.law.cornell.edu/cg...it_headings/words=4hits_only?)
Shows the construction of oncomouse.

Oncomice are used in research in three different ways: 1) they are used to test a material considered to be a carcinogen, by subjecting the mouse to the material and ascertaining the growth of tumors as an indication of carcinogenicity, 2) they are used as a tester for material such as antioxidants like betacarotene or vitamin E that can protect against onset of tumors, and 3) they are used as a source of cells for cell culture because they can positively display beneficial properties of both normal and transformed cultured cells.

There were big objections to this first patented animal. Animal rights and religious groups said higher animals are more than a composition of matter and have purposes other than to serve human needs. Farmers groups and others complained that if all livestock were patented large companies might be able to control livestock production. Still others said it was a get rich quick scheme that would only cost consumers more money.

There were also plenty of supporters of the oncomouse. They thought it would bring the same benefits to animal farmers as it does to grain and other vegetable farmers. Of course, oncomouse would have great benefits to research and biotech companies as well. People need to get more accustomed to the idea and see how the benefits outway the disadvantages (Thro, 1993).

EUROPEAN PATENTS

The European patent law causes concern with some citizens- does it encourage useful developments and why are the patenting laws so different from the United States?

Article 52(1) of the EPC says the monopoly right that a patent holder has can only be granted "for inventions which are susceptible of industrial application, which are new and which involve an inventive step" (Houdebine, 1997). Article 53 in the EPC lists exceptions to patentabilities:

> **"European patents shall not be granted in respect of: 1) inventions the publication or exploitation of which would be contrary to order public or morality...; 2) plant or animal varieties, or essentially biological processes for the production of plants or animals; this provision does not apply to microbiological processes or the products thereof." (Houdebine, 1997).**

This is the basis for the arguments raised by the people opposed to the patentability of transgenic animals in Europe.

The difference between discovery (non-patentable) and invention (patentable) for a biological substance has been touched upon by the EPO. They state,

> **"To find a substance freely occurring in nature is...mere discovery and therefore unpatentable. However if the substance is found in nature and is first isolated from its surroundings and a process for obtaining it is developed, that process is patentable. Moreover, if the substance cant be properly characterized, either by its chemical structure, by the process by which it is obtained, or by other parameters and if it is "new" in the absolute sense of having no previous recognized existence, than the substance per se may be patentable." (Houdebine, 1997).**

The rules of the EPO (Europe) and PTO (United States) have similarities in their exceptions to patentability, but the United States has much less stricter laws. It was not until 1992, that patent cover was granted for the oncomouse at the EPO, after much considerable debate, due to morals and public order. European law allows cover for

microbiological processes but restrains the patenting of animal varieties, unlike that of the United States (www.ivanhoe.co.uk).

CHAPTER 5: ETHICAL ISSUES

PROS FOR MAKING A TRANSGENIC ANIMAL

The main reasons why transgenic animals should be produced are for medical advances and food. In 1987 Dr. John Hasler who is cofounder of an animal biotechnology company, proposed making animals that nature never made. That is what transgenics is all about: creating "custom-designed" animals for various reasons to benefit humans, such as disease models, drug production, environmental cleanup, and food production, to name a few.

FOOD INDUSTRY

In the food industry transgenic animals are a very controversial area of interest. The goal is to create superior animals for food production. Food production is not only done with pigs, but fish, sheep and a large number of other species (Bender and Leone, 1996). Most of us eat food produced by transgenic animals and do not even realize it. Most of the cheese that we eat is made with rennin, which is an animal enzyme that is produced by transgenic bacteria.

Not only could transgenic food increase the world's food supply, but producing transgenic food animals could be meaningful to help the quality of life of both animals and humans, which will lead to greater longevity. The animal's quality of life could improve because their resistance to infectious diseases and their adaptability to environments could expand. Human quality of life could be uplifted because the

accessibility of foods would be enlarged and the safety and quality of animal products could be improved (Bender and Leone, 1996).

The genetically modified animals give the producer increased profits but the consumer also profits as well. Leaner meat and low cholesterol eggs are just two of the benefits produced thus far from transgenic animals. If one feels that a farm animal's main purpose on earth is to produce meat, then you can make the most of it by giving the animals different genes so we can get leaner meat with less fat.

FIGURE 5.1 (Lee, 1993)
Shows a pig born with the bovine growth hormone inserted in the embryo through transgenesis.

MEDICAL PURPOSES

People who support the use of transgenics say there should not be a moratorium on the use of research animals because that could raise costs for the consumer and it could confine the availability of potential new drugs. People suffering from diseases who need these drugs have concerns about these restrictions that could affect their future health.

Biotechnology can also open the doors to new animal strains, growth hormones, and other products that enhance production of pharmaceuticals produced by transgenic animals.

Animals could be created that are resistant to disease. This could be helpful by reducing illnesses of the animals and reducing vaccinations and treatments. An example would be a cow that is resistant to zoonotic tuberculosis. Humans would not have to be exposed to the zoonotic disease or the antibiotics. The lowered food production costs would ultimately lead to lower food prices for the consumer (Bender and Bruno, 1996).

ANIMAL WELFARE

Unlike some animal rights groups, some animal welfare groups actually support transgenic animals. They feel that the use of animals in biomedical research is essential. Some animal welfare advocates support monitored animal use customs whose outcomes will produce prevalent benefits to society. They do not support the use of animals for non-essential practices.

PUBLIC AWARENESS

One of the problems that some people have with the use of animals for transgenesis is their lack of knowledge on the subject. Van Vught says "We are convinced that animal biotechnology can only become successful when society wants it" (Lee, 1993). As soon as people overcome the information gaps between society and science they should be more accepting. It would help if people were allowed to join in the decision-making by public debate.

The public is eager for the benefits transgenic technology has to offer; yet they fear the possible dangers. It has been twenty years since the development of the first transgenic animal and the confusion, controversy, and public uneasiness continues. It is easy to fear something you know little about. One strong viewpoint is there are limits to genetic creation and spread. In other words, we can finesse DNA in a test tube at our convenience but that does not mean the same is true of organisms (Bender and Leone, 1996).

> **"In order for an organism to develop and function effectively, its parts must interact in a coordinated manner, fitting each other like the parts of a smoothly functioning machine. Hence, only the new variants that have sufficiently coherent, balanced set of genes can survive" (Bender and Leone, 1996).**

Another one of the public's fears is that the altered organism could escape into the environment. It would be very hard for such an organism to survive because it suffers great disadvantages in evolutionary competition. Recombination of poorly matched

genes from different species will create organisms that are not very well adapted to their environment (Bender and Leone, 1996). These are not the harmful monsters that some people believe could escape into our outside world, but instead are organisms useful to man that require great care to survive. Organisms that contain foreign DNA material are found in nature. Naturally occurring mutations happen all the time.

CONS FOR MAKING A TRANSGENIC ANIMAL

There are four main ethical issues some people raise as to why transgenic animals should not be created. 1) It is immoral and inhumane, 2) there are various safety problems to both animals and humans, 3) there are environmentalist and religious viewpoints to consider and, 4) it is animal exploitation (animal rights groups views have to be taken into account).

TRANSGENIC ANIMALS ARE IMMORAL AND INHUMANE

In 1993, 48% of Europeans thought that biotechnology was necessary to improve our way of life over the next twenty years. Only 15% of the people thought that biotechnology was unessential. The rest of the people were undecided. This is because of the lack of information. There is an information rift between science, technology, and industry versus the public. People correctly become enraged when animals are used and pain is inflicted upon them (Houdebine, 1997). However, not all transgenic animals suffer. In some cases, the suffering is very mild, and the medical benefits are so powerful as to outweigh the mild suffering. Infecting animals with deadly diseases is sometimes immoral and inhumane. Oncomouse is an example of this (as seen in figure 5.2).

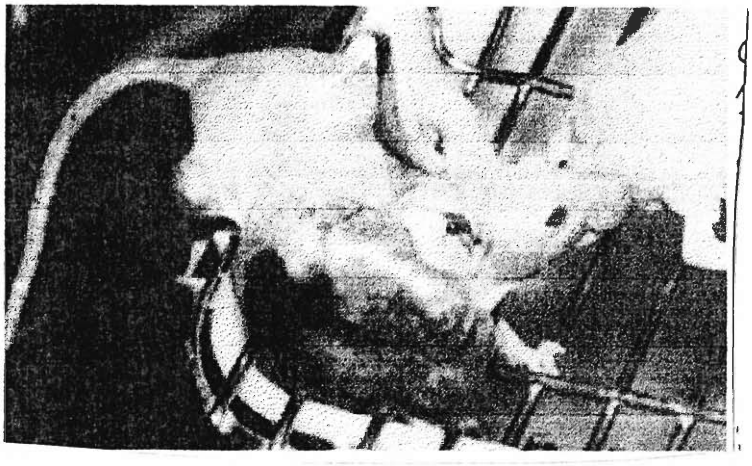

FIGURE 5.2 (www.tierrechte.de/cpn/nopatents_e.html)
Shows a picture of oncomouse- a transgenic mouse inflicted with cancer.

This is a mouse that is rendered susceptible to the deadly, painful disease, cancer. Obviously pain and suffering go hand in hand with the disease. The decisions we all must make concern how to minimize oncomouse's discomfort, while gaining the knowledge about cancer that he will provide. We think people have to look past the vision of the "suffering" mouse and realize the great medical benefits he brings to us. It is sad that some researchers do not treat their animals in a respectful way, and just look at them as things. In those such cases, there should be much stricter laws so the animals do not have to go through unnecessary suffering.

There is "something more" in the animal that needs to be respected. This is because the animal is living and not just a thing, so the "something more" can be termed as wholeness or normality. Normally one would not cut off a dog's paw or a chicken's feet; they are expected to be there as a part of the whole animal. So the idea of inserting a piece of foreign piece of DNA into an animal to make it less "normal" to some people

violates animal integrity. Some scientists may argue that genetic changes are "normal" in nature, but to others, just because it happens in nature is no moral reason for people to do the same. Respect for animal integrity has to do with our responsibility in the way we treat animals. The intrinsic value of animals is the sense that animals have an inborn worthiness, one of their own. In other words, animals are morally relevant. We cannot just do whatever we want with them. Some might say that transgenic animals are a manipulation of nature; very unnatural (Houdebine, 1997).

"We must remember that the mind that views animals as pieces of coded genetic information to be manipulated and exploited at will is the mind that would view human beings in a similar way." Carol Grunewald, *New Internationalist* Jan. 1991 (Bender and Leone, 1996).

In the early 1980's when transgenesis was started, pigs and sheep were made that could create human growth hormone in their blood. Ultimately, the animal was slaughtered to retrieve the hormone. So there were many disadvantages to this method. It was costly and the animal was destroyed before getting the product. However, researchers wanted to find ways to retrieve the drug/protein without destroying the animal "factories" and instead succeeded with transpharming in which the animal secretes the protein into the milk. Although this method negates animal sacrifice, critics have plenty to say about this transpharming method of obtaining proteins. Their worries lie in the moral issues of using transgenics to channel the evolutionary process to using animals for money and efficiency. They say it puts the genetic integrity of different

species at risk. They feel that it is immoral to use the mammary gland of an animal as a bioreactor.

Personally, we feel that animals should be respected and they do have an intrinsic value. This does not mean that transgenesis is necessarily wrong; it should just be used in the proper way. Transgenesis not only helps humans in finding cures for diseases and producing pharmaceuticals, but in the long run it can help make more vigorous animals as well.

TRANSGENIC ANIMALS CREATE SAFETY ISSUES

There are plenty of obvious dangerous safety issues with transgenic animals. In one scenario the pathogens that are in the transgenic animal could change or evolve. This creates problems because the pathogen, which was placed into the animal that was created to be resistant to it, could mutate into a different form. The animal may not be repellent to the new form. If something like this happens it could change the "microenvironment" of the pathogen, which ultimately could produce an end result of danger to humans or to other animals (Shannon, 1997). How far are these researchers willing to go? Predictions have been made by the office of technology assessment (OTA) that in the future we could see "five ton cows and pigs twelve feet long and five feet tall." It seems right now that there are no limits. The creation of monstrous animals could be next. This is a concern of many people opposed to transgenic research (Bender and Leone, 1996).

Similarly, the genetically modified animal could get loose into the environment. One example is in the case of the AIDS mouse. In 1987 an experiment was done by

Malcolm Marlin, in which the entire AIDS virus genome was transplanted into mouse embryos. 10% of the mice incorporated the AIDS virus in their genetic make-up. While many scientists were excited about this new breakthrough, some had reservations. It could be dangerous if any of these mice escaped and mated with "normal" mice. There would be an overpowering and uncontrollable risk of spreading the virus. Of course Martin reported these fears to be ludicrous, but in 1990 some of these fears were put to the test. Gallo, the co-discoverer of the AIDS virus, wrote a paper describing how HIV could interact and combine with the transgenic mouse's natural viruses. This could produce a "super AIDS": a new and possibly more dangerous form of HIV. This new AIDS could target a broader range of cells, and travel by various routes, maybe even airborne routes. This was a scientific probability that scared many people (Bender and Leone, 1996). Another safety issue is that the researchers themselves could be harmed by exposure to a dangerous material while conducting the research (Shannon, 1997). Although this had never happened, it was still a possibility.

As for releasing genetically altered animals into the environment, it has not happened as of yet. It has been about twenty years since the first transgenic animal was created, so we feel that this is not a major area of concern.

Lastly, is the controversy over genetically engineered food animals. One such case is bovine growth hormone (BGH) that is sometimes injected into cows. Although this is not a transgenic animal we can learn valuable information from it. The hormone may have health threats to not only the animal, but to humans as well. People do not want hormones in their milk. If consumers knew that there was BGH in their milk, according to a 1990 poll, 50% of them would not purchase the dairy products. BGH has

been shown to increase the occurrence of mastitis, which is an udder disease that is treated with antibiotics. Not only is this animal cruelty, but also it puts antibiotics, hormones, and scattered pus into the milk we drink. Ultimately, the antibiotics, which are taken by the animals, are absorbed by the person who eats the meat. This can be harmful to our population, as it can promote the development, through adaptive mutations, of new drug-resistant strains of micro-organisms (Bender and Leone, 1996).

The view that we feel most strongly about is the creation of genetically modified food animals. We feel that this has no great benefit to society, thus far. We have not heard any convincing results that come out of using food animals in this way. The animals endure needless suffering, so in our opinions this aspect of transgenesis is unnecessary.

ACTIVISTS POINTS OF VIEWS

Members of various religious groups feel that altering or creating new life forms is an attempt at playing god and it is a revolt against the supremacy of god. They believe that by creating new life forms or changing them in any way endangers the sanctity of life (Seide and Giaccio, 1995). When a coalition of religious leaders met in 1996, they proclaimed in an announcement issued in Washington D.C. that,

> **"The gift of life from god, in all its forms and species should not be regarded solely as if it were a chemical product, subject to genetic alteration and patentable for economic benefit. Moral, social and spiritual issues deserve far more consideration before binding decisions are made in this area." (Sagoff, 1995).**

The phrase "playing god" does not necessarily have to be a religious statement. It can just be a moral metaphor. In muddling with the genetic disposition of living beings, science and technology are encroaching boundaries, which they are not allowed to encroach. Scientists are acting like god by molding organisms into his/her own will. Genetic modification can be taken to be a symbol of pride and arrogance, which can ultimately be harmful to society and the environment (Houdebine, 1997).

Environmentalists are concerned with the release of genetically altered animals. Their fears lie in the theory that releasing these "mutated" animals into the environment could have harmful consequences (like the AIDS mouse). They are also concerned with the effect that a new genetically engineered animal product could have upon the "purity and diversity" of the total ecosystem. Environmentalists worry not just about the effect that transgenic animals will have on the earth, but also about the undirected multiplication of the progeny, such as organisms that replace nitrogen in soil or prevent frost damage to crops (Shannon, 1997).

Back to our non-transgenic BGH example, the farmers could be hurt economically if the drug BGH is continued to be used. Currently, the U.S. is undergoing a milk surplus and increasing the milk production could drive small farmers out of business. We do not need more milk. If we increase milk production, that can lead to an extreme decrease in the price of milk. Studies have shown that there has been a loss of up to 50% of the nation's dairy farms to BGH (Bender and Leone, 1996).

In our opinion, the activist's views are strongly opinionated and one-sided. Religious groups tend to be very traditional. They feel one way, and will not change their

minds for anyone. There is no room for compromise. As for the environmentalists and farmers, they are mostly looking out for themselves.

ANIMAL EXPLOITATION

Animal rights groups are concerned with the humane care and use of animals. They are focused on whether humans have the right to view and use animals as resources and what rights animals are entitled to as living, feeling beings. These rights are determined regardless of human benefits from animal use. Most of these groups are against any form of transgenics on animals and against patenting on animals, since it promotes transgenesis.

They feel the way pain is sometimes inflicted on animals for human benefits is disgusting. One example is the use of BGH on cows. The cows injected with this genetically altered hormone, did produce up to 20% more milk but there were adverse affects such as they undergo a enormous increase in infections of the udders, lameness (to the point where some cows can not walk), reproductive problems, digestive problems, and death in several cases. Recent studies have shown that cows injected with BGH have not only become ill, but have given birth to deformed and deceased offspring. Also, of the offspring that lived, some have given birth to deformed calves, even though they themselves, were not injected with BGH. Thus there is a possibility that genetic damage may have occurred among the cows. BGH is also a step towards genetic diversity in dairy animals, such as cows. Breeding records could become affected and genetic diversity would suffer (Bender and Leone, 1996).

In 1988 a "super pig", was created in the hopes that it would imitate Palmiter's super mouse. The human gene that controls growth was injected into a porcine embryo. They wanted this pig to grow larger and faster than any pig had before. The results they got were not expected: this pig had all sorts of health problems. It was cross-eyed, lethargic, excessively hairy, and its arthritis was so bad that it could barely stand up. Obviously the human growth gene had altered the pig's metabolism in a way, which was unanticipated. These pigs eventually developed slightly different problems. They have enlarged hearts, ulcers, dermatitis, and kidney problems. The only benefit is the slightly leaner meat that they produce but the health risks of eating meat with genetically altered fat content are still unknown. The USDA released a statement saying that although these genetically modified pigs were not any larger than they were supposed to be, their meat would be leaner because of the added muscle mass (Bender and Leone, 1996). Are these the pigs we want to eat? Recently these transgenic experiments were terminated voluntarily by the scientists performing the research. This is an exceptional example of the research community acting to regulate itself.

Animal rights activists believe that animal research is unnecessary. Animal models are being made to mimic diseases such as AIDS, schizophrenia, drug addiction, cancer and heart disease, all of which can develop in response to factors caused by the environment, heredity, and lifestyle. The animals are created to hold a single gene which causes aspects of the disease and they are treated like living "test tubes." They are experimented on to test the effectiveness of different treatments and drugs (Bender and Leone, 1996).

"It is impossible to mimic a chronic human disease in animals because of biochemical, immunological and physiological differences between the species. It is also impossible because researchers cannot understand the spontaneous process of disease by artificially placing it in the bodies of animals." (Bender and Leone, 1996).

The two species most often used on cancer studies are rats and mice. A large sum of money is poured into research with these animals annually. One of the goals is to identify various substances, which could cause cancer in these animals. Studies have proved that almost half of the substances believed to be carcinogenic in mice were safe in rats, so what does that tell us? This has the same outcome as using these animals to forecast cures for cancer in humans. Just because drugs are found effective on animals does not necessarily mean the same is true of humans. They could be useful, or maybe even harmful (Houdebine, 1997).

We tend to agree more with the animal welfare, rather than animal rights groups. The animal rights groups are right with their opinions that making transgenic animals for unnecessary experimentation is wrong, but the animal welfare groups have a broader understanding for the use of animals. They feel that using animals in a way which will benefit society, yet employ no pain or suffering to the animal. This view makes the most sense, because everyone benefits from it.

Overall, there needs to be more public awareness in this area, so there can be more support for necessary medical disease models. People need to know what these animals do for us in terms of medical advances, such as vaccines, pharmaceuticals, and other treatments. On the other hand, unnecessary handing of animals (such as transgenic

farm animals created for food production) needs to be outlawed. Not only is this inhumane and immoral, but personally we do not want to eat animals crippled with disease, or pumped full with hormones and antibiotics.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

In brief, transgenic animals are created for a variety of purposes, all of which are extremely controversial. They are created as transpharmers, disease models, xenotransplanters, in enhancement engineering and for basic biological studies. There are also a wide variety of species used in transgenesis such as mice, rabbits, pigs, goat, sheep, and cows to name a few. All of these various transgenic animals contribute something to the biotechnological field for the future.

The process of creating a transgenic animal can be summarized into numerous steps. The DNA is first prepared and then the eggs are matured and extracted. Next is the in vitro fertilization of the eggs. The foreign DNA is injected into the zygote by one of three methods: pronuclear microinjection, transfection of DNA into ES cells, or retroviral infection of DNA into ES cells or embryos. Once the foreign material is introduced into the cells, embryo collection and then implantation occurs. Lastly, the offspring are screened by southern blotting or PCR.

One of the issues that was covered was the legal issue of whether transgenic animals are patentable matter. In order to be patentable, the inventor must prove that their object serves a useful purpose, has never been made before, and it must not be obvious using prior expertise. Two major controversial issues were the Diamond vs. Chakrabarty court case, in which this matter was first recognized, and the Harvard mouse patent, which was the first transgenic animal patented.

After viewing both sides of the controversial, intense ethical issues in the creation and use of transgenic animals, both of us came to a common positive conclusion about

the controlled use and optimistic future of transgenic animals. Overall, the benefits that transgenic animals bestow upon society outweigh the disadvantages. The most important positive influences transgenic animals give us are medical advantages. If carefully monitored and used in a controlled manner, there is no reason for anyone to believe that the animals are being unnecessarily harmed or are a threat to society. These are the two biggest disputes against making a transgenic animal. Many people are very unaware of the big picture of transgenesis. They feel that it is inhumane, immoral and we are putting the animal's intrinsic value on the line. We agree, that animals should be respected and do have an intrinsic value. This does not mean that transgenesis is necessarily wrong; it should just be used in the proper way. Transgenesis, not only helps humans in finding cures for diseases, but it aids in making medications and pharmaceuticals to treat diseases.

As for the future of transgenesis, there needs to be more public awareness, so there can be more support for the necessary medical disease models. People need to be conscious of what transgenic animals do for us in terms of medical advances, pharmaceuticals and other treatments. On the other hand, in order to ease society's minds, needless usage of animals in research (such as animals created for food production) needs to be banned. Not only is this inhumane, but personally we do not want to eat animals stricken with disease, or pumped full of hormones and antibiotics.

BIBLIOGRAPHY

Bauman D.E, Everett R.W, Weiland W.H, and R.J. Collier. 1999. Production responses to bovine somatotropin in northeast dairy herds. *J Dairy Science* **82**: 2564-2567.

Bender D, and B. Leon. 1996. Genetic Engineering: Opposing Viewpoints. Pp.1-117 Greenhaven Press, Inc. San Diego, CA.

Colman, A. 1999. Dolly, polly and other 'ollys': likely impact of cloning technology on biomedical uses of livestock. *Genet Anal* **15**: 167-173.

Colman, A. 1996. Production of proteins in the milk of transgenic livestock: problems solutions and successes. *Am J Clin Nutr* **63**: 639s-645s.

Dziadek M. 1996. Transgenic Animals: how they are made and their role in production And research. *Australian Veterinary Journal* **73**: 182-187.

Eynon E, and R. Flavell. 1999. Walking through the forest of transgenic models of human disease. *Immunological Reviews* **169**: 5-10.

Fan J, Challah M, and T. Watanabe. 1999. Transgenic rabbit models for biomedical research; current status, basic methods and future perspectives. *Pathol Int* **49**: 583-594.

Fernandez J, and J. Hoeffler. 1999. Gene Expression Systems. Pp. 367-420. Academic Press, CA.

French A.J, Greenstein J.L, Loveland B.E, and P.S. Mountford. 1998. Current and future prospects for xenotransplantation. *Reprod Fertil Dev* **10**: 683-696.

Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell B, Carr T, Clemens J, Donaldson T, and F. Gillespie. 1995. Alzheimer-type neuropathy in transgenic mice overexpression of V717F beta-amyloid precursor protein. *Nature* **373**: 523-527.

Glick B, and J. Pasternak. 1998. Molecular Biotechnology. Pp 479-501. ASM press, Washington D.C.

Gordon J, Scangos G, Plotkin D, Barbosa J, Ruddle F. 1980. Genetic transformation of mouse embryos by microinjection of purified DNA. *Proc Natl Acad Sci USA* **77**: 7380-7384.

Green E.A, and R. Flavell. 1999. Tumor necrosis factor-alpha and the progression of

diabetes in non-obese mice. *Immunological Review* **169**: 11-12.

Guenette S.Y, and R.E. Tanzi. 1999. Progress toward valid transgenic mouse models for Alzheimer's disease. *Neuobiological Aging* **20**: 201-211.

Helmuth, L. 2000. Further progress on a Beta amyloid vaccine. *Science* **289**: 375-377.

Hogan B, Costantini F, and E. Lacy. 1986. Manipulating the Mouse Embryo: A Laboratory Manual. Cold Spring Harbor Laboratory, NY.

Houdebine, L.M. 1997. Transgenic Animals. Pp 11-19; 317-375; 403-455; 465-480. OPA, Amsterdam.

Kunz J, and A. Finkle. 1987. The American Medical Association: Family Medical Guide. Pp. 406. Random House, NY.

Lee H.C, S. Kim, K. Kim, H. Shin and J.Yoon. 2000. Remission in Models of type-1 Diabetes by gene therapy using a straight-chain insulin analog. *Nature* **408**: 483-488.

Lee, T. 1993. Gene future: The promise and perils of the new biology. Plenum Press, NY.

Lewin,B. 1997. Genes VI. Oxford University press, Oxford, NY.

Lewis, R. 2000. Porcine Possibilities. *The Scientist* **14**: 1-10

Maclean, N. 1994. Animals with novel genes. Pp. 1-20. Cambridge University Press, NY.

Manjula B, Kumar R, Sun D, Ho N, Ho C, Rao J, Malavalli A, and A. Acharya. 1998. Correct assembly of human normal adult hemoglobin when expressed in transgenic swine: chemical, conformational, and functional equivalence with the human-derived protein. *Protein Eng* **11**: 583-588.

Marian A.J, Wu Y, Lim D.S, McCluggage M, Youker K, Yu Q.T, Brugada R, Demayo F, Quinones M, and R. Roberts. 1999. A transgenic rabbit model for human hypertrophic cardiomyopathy. *J Clin Invest* **104**: 1683-1692.

McCuen, G. 1985. Manipulating Life: Debating the Genetic Revolution. E. McCuen Publication.

Merlino G. 1991. Transgenic animals in biomedical research. *FASEB Journal* **5**: 2996-3000.

Morcol T, Akers R, Johnson J, Williams B, Gwazdauskas F, Knit J, Lubon H, Paleyanda

R, Drohan W, and W. Velander. 1994. The porcine mammary gland as a bioreactor for complex proteins. *Ann NY Acad Sci* **721**: 218-233.

Niemann H, Halter R, Carnwath J.W, Herman D, Lemme E, and D. Paul. 1999. Expression of human blood clotting factor VIII in the mammary gland of transgenic sheep. *Transgenic Reasearch* **8**: 237-247.

Palmiter R, Gunnar N, Gelinas R, Hammer R, and R. Brinster. 1983. Metallothionein-human GH fusion genes stimulate growth of mice. *Science* **222**: 809-810.

Rifkin, J. 1998. The Biotech Century. Pp. 37-66. Penguin Putnam Inc., NY.

Sagoff, M. 1996. "Animals as Inventions, biotechnological and intellectual Rights" Institute for philosophy and public policy. Vol 16. no 1. http://epn.org/ippp/sagoff1.html.

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao I, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandenert C, Walker S, Wogulis M, Yednock T, Games D, and P. Seubert. 1999. Immunization with amyloid-beta attenuates Alzheimer-disease pathology in the PDAPP mouse. *Nature* **400**: 173-177.

Seide R, and A. Giaccio. 1995. "Patenting Animals" http://www.ipcounsel.com/animal.html

Shannon, T. 1997. An Introduction to Bioethics. Pp. 134; 186-187. Paulist Press, NY.

Smith, J. 1996. Biotechnology. Pp. 206-211. Cambridge University Press.

Taverne, J. 1993. Transgenic mice in the study of cytokine function. *Int J Exp Pathol* **74**: 525-546.

Thro, E. 1993. Genetic Engineering: Shaping the Material of Life. Facts on File.

Ward K.A, and B.W. Brown. 1998. The production of transgenic domestic livestock: successes, failures and the need for nuclear transfer. *Reprod Fert Dev* **10**: 659-666.

Watson D, Gilman M, Witkowski J, and M Zoller. 1992. Recombinant DNA. Pp 255-267. W.H. Freeman and Co, NY.

Wong F.S, Dittel B.N, Janeway C.A. 1999. Transgenes and knockout mutations in animal models of type I diabetes and multiple sclerosis. *Immunological Reviews* **169**: 93-106.

www.2.law.cornell.edu/cg…it_headings/words=4hits_only?

www.ccac.ca/english/transsup.html

www.criver.com/techdocs/transgen.html

www.godriva.com/~jkimball/BiologyPages/P/PCR.html

www.godriva.com/~jkimball/BiologyPages/R/RecombinantDNA.html

www.godriva.com/~jkimball/BiologyPages/T/TransgenicAnimals.html

www.ivanhoe.co.uk

www.tierrechte.de/cpn/nopatents_e.html 1997. "No patents on life"