# TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

By:

_____        _____

Laura Fineman            John Martin

August 27, 2010

APPROVED:

_____

Prof. David S. Adams, Ph.D.
WPI Project Advisor

# ABSTRACT

This Interactive Qualifying Project (IQP) examined the controversial topic of transgenic animals, and described its effects on society. The first two chapters focused on the technology itself, describing how transgenic animals are created and screened, and categorized the transgenic animals made to date. The last two chapters went beyond the technology to investigate the ethics, as well as legal and regulatory issues surrounding the controversy. Based on the research performed for this project, the authors concluded that most types of transgenic experiments should be continued, provided that IACUC and FDA supervision continues to balance the animals suffering with reasonable societal needs. Patenting of transgenic animals has the potential to provide incentives to perform this type of research, however the current system is lacking.

# TABLE OF CONTENTS

# PROJECT OBJECTIVES

The objective of this project was to examine the topic of transgenic animals, and to discuss the effect of this controversial new technology on society. The ability to create transgenic animals has come from a long history of scientific progress, chapter-1 explains what transgenic animals are and what methods exist to create them, while chapter-2 goes further and documents and categorizes the types of transgenic animals created to date, and describes their benefits to society. As transgenic research has expanded quickly, new moral and ethical questions have been brought to light, so chapter-3 examines the ethics surrounding this controversial technology, and chapter-4 examines the legal and regulatory landscape, including the idea that animals are patentable. The result of this project should be that the reader is informed of the key relevant information surrounding the transgenic debate, so they will be able to draw their own conclusions. The authors will also present a researched opinion at the end of the report.

# CHAPTER-1:  TRANSGENIC ANIMAL TECHNOLOGY

*John Raymond Martin*

An animal that is transgenic has been intentionally altered at the genomic level to include foreign DNA.  These changes create organisms that would never have existed in nature, and allow scientists new tools for conducting research and finding solutions to many of the world's greatest problems.  This first chapter will focus on the major transgenic techniques which include manipulation of a male pronucleus in a newly fertilized egg, and the manipulation of embryonic stem cells.  The efficiency of these methods is not very high, so additional techniques for selecting positive transgenic organisms that have cloned correctly will be discussed as well.

## BIOLOGY'S CENTRAL DOGMA

The main building blocks of life can be divided into three distinct molecules: DNA, RNA, and proteins.  DNA, or deoxyribonucleic acid, is the central repository of the genetic code. When an organism needs to make a compound, DNA is transcribed into RNA, or ribonucleic acid, which acts as an intermediary.  The RNA is free to travel from the nucleus to the cytoplasm where it is then transcribed into proteins which make up the bulk of the functional units of living organisms.  This one way progression of useful information from DNA to protein is called the central dogma and is essential to understanding transgenic animals.

The nucleic acids are made up of a phosphate and sugar backbone, and a coding core of base pairs (**Figure 1**).  This coding core matches purines: adenine (A) and guanine (G), with pyrimidines: cytosine (C), thymine (T), and uracil (U) to give the genomic code from which the organism eventually makes proteins.
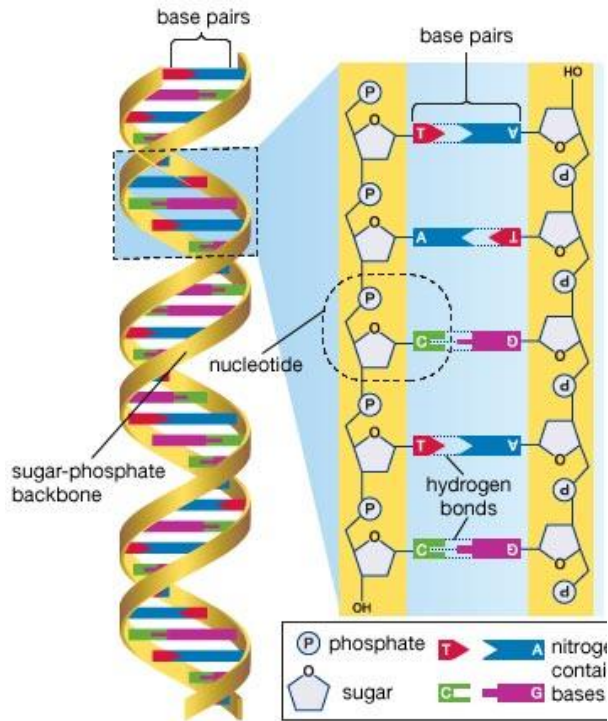
**Figure 1: Structure of DNA.** The left shows the double helix nature of DNA, and the right shows the various components. (Human Genome, 2010)

The two halves of the DNA molecule are held together by hydrogen bonds between the base pairs (**Figure 2**). Due to precise structural fits between the bases, only specific pairings occur: A with T or U, and C with G. When DNA is copied to either form new DNA, or is transcribed into RNA, these hydrogen bonds are broken and then reformed once copied. The genetic information stored in the DNA, in the form of the *order* of the bases, is directly responsible for all of phenotypical traits observed as part of the organism.
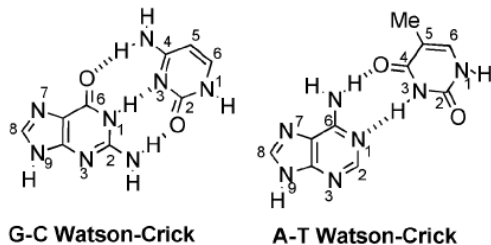


**Figure 2: Hydrogen Bonding in DNA.** The dashed lines between key hydrogen and oxygen residues show the hydrogen bonds between purine-pyrimidine base pairs (Farthalla et al., 2009).

The idea that the phenotype or characteristics of an organism could be driven by some sort of genotype that was affected by mating was first observed by Gregor Mendel in the mid

19th century.  His experiments with peas led him to make two conclusions; first that during gamete formation the allele pairs separate, and second that this segregation of alleles is independent for every allele (Mendel, 1866).  This laid the groundwork for further study into genetics, such as that published by Watson and Crick showing that DNA has the chemical properties that Mendel laid out for genetic information.  They showed that the structure of DNA was such that it would follow a semi-conservation mechanism of replication that Mendel and others after him had predicted (Watson and Crick, 1953).  However, it was not until techniques for manipulating DNA were discovered that the advent of transgenic animals could come to fruition.

**RECOMBINANT DNA**

The recombination of DNA involves using several methods to rearrange, add, delete, or edit a section of DNA making it recombinant (rDNA).  This rDNA can then be inserted into an animal and expressed *in vivo* as if it were the animal's own DNA, giving the animal new properties.  The procedure generally uses restriction enzymes to cut sections of DNA, and DNA ligase an enzyme that can seal the DNA back together.

The ability to recombine DNAs from two different organisms was made possible by the work done on restriction enzymes in 1970; at last scientists could not only isolate and sequence DNA but also make changes to it.  Restriction enzymes make cuts to DNA at specific locations and in a specific way.  For example, the restriction enzyme EcoRI cuts at the sequence GAATTC (between the G and the A) to produce DNA fragments flanked by EcoRI sites.  And because the cut is not straight through both strands of the DNA molecule, it creates single-stranded "sticky ends" that can anneal to other DNA fragments cut with the same EcoRI enzyme.  Thus, DNAs

from different organisms cut by the same type of restriction enzyme will produce DNA fragments that can be annealed to each other, and then sealed by DNA ligase to produce rDNA.


**DNA CLONING**

Once the rDNA has been formed, it then needs to be cloned to make large numbers of it, and inserted into a living cell.  With respect to DNA cloning, the newly created rDNA is usually inserted into a plasmid DNA or virus to amplify it, and to allow subsequent screening of successful positives.  Plasmids are circular DNAs normally found in bacteria (and some eukaryotic cells) that form high copy numbers in the cytoplasm.  rDNAs inserted into them also get replicated to high copy numbers.

With respect to cloned DNA uptake, a number of techniques have been developed to allow this to happen efficiently with microbes, but when making a transgenic animal it is much harder to get a eukaryotic cell to uptake and express foreign DNA.  Both of the animal techniques discussed below in detail use microinjection of rDNA, the most reliable method; however there are other techniques that work as well to deliver the cloned DNA inside a cell, including using chemicals, electroporation, or viruses.  Chemicals such as calcium chloride or strontium chloride have been used for decades to allow bacteria to become competent to take up cloned DNAs.  For eukaryotic cells, delivery techniques often use a positively charged polymer which encapsulates the DNA, then binds to the cell surface and allows the DNA into the cell. Another method is electroporation which uses electricity to pull the DNA through the cell membrane.  Both of these methods rely on the fact that DNA is a negatively charged molecule because of its phosphate backbone.  Another method is the use of a virus, as was done by Jaenisch and Mintz (1974) to make the first transgenic animal.  This method uses the more

complex mechanism of a virus to infect cells to integrate their DNA to make a transgenic animal, and is the only method for creating transgenic animals after they have been born. This makes viruses especially useful in gene therapy research.

## THE FIRST TRANSGENIC ORGANISMS

The new rDNA and cloning technology lead to the creation of the first transgenic organism in 1973. A group led by Stanley Cohen was able to add a gene to the bacterium E. coli from another bacterium (Cohen et al., 1973). The creation of this first recombinant prokaryote was relatively simple compared to modern transgenesis of animals. The first transgenic animal was a mouse created by Rudolf Jaenisch and Beatrice Mintz, which contained SV40 viral DNA sequences inserted into the mouse genome by microinjection into a blastocyst, although the rDNA was not expressed (Jaenisch and Mintz, 1973).

In 1980, a new technique was developed to allow the microinjection of rDNA into the pronucleus of a newly fertilized egg (Gordon, 1980). But again the rDNA was not expressed. It was not until 1982 that a team would finally be able to express a rDNA gene of interest to produce a phenotypic change in a eukaryote, in this case a mouse (Palimiter et al., 1982). In this experiment, the transgenic mouse, later dubbed supermouse, expressed growth hormone from another species, and grew larger than his non-transgenic littermates.

Since this landmark experiment, research in this transgenic field has exploded, and most research facilities have thousands of transgenic animals to perform research on. More recent techniques have also overcome some of the efficiency issues which earlier required many defective animals to be created. With the technology now standardized, the emphasis has mostly shifted from creating transgenic animals to performing experiments on them.

**TWO MAIN METHODS FOR CREATING TRANSGENIC ANIMALS**

In order to create a transgenic animal, the altered rDNA must be inserted into the animal. There are two main approaches to this: pronuclear manipulation and embryonic stem cell manipulation, each of which has its own benefits and drawbacks which will be discussed.

*Approach 1: Pronuclear Manipulation*

One of the oldest and most widely used method for creating a transgenic animal is by manipulation of the male proucleus in a newly fertilized egg. In order to do this, eggs are removed from a female which has typically been induced to super-ovulate by the injection of hormones. The harvested egg is then fertilized by *in vitro* fertilization (IVF). However, before the fusion of the male and female pronuclei takes place, the male pronucleus is microinjected with the cloned transgene of interest (**Figure-3**). The male pronucleus is used specifically because it is larger than the female and can be located easier. The zygote is then allowed to grow in culture until the blastocyst stage, then is implanted into a female uterus who has been dosed with hormones to be pseudo-pregnant (Cozzi et al., 2009).
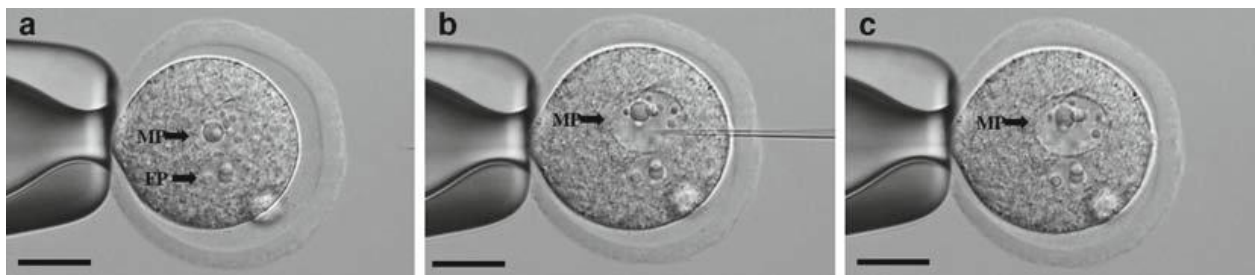


**Figure 3: Microinjection of the Male Pronucleus.** (a) Photograph of a rat zygote prepared by *in vitro* fertilization before cell division. The zygote is held in place with a suction pipette (left side). Arrows denote male (MP) and female (FP) pronuclei. The male pronucleus is slightly larger. (b) Microinjection into the male pronucleus with micropipette (right side). (c) Enlarged male pronucleus after microinjection. 400X magnification. (Cozzi et al., 2009).

There are many benefits to this pronuclear microinjection method, including that its overall reliability has been proven with decades of results. Assuming the gene incorporates

correctly into the zygote, all cells of the new animal will contain the transgene, including the gametes so the transgene will also be passed to the progeny. However, in this process, the transgene does not incorporate in a known location in the genome, and may do so in a location that affects the functionality of the cells. This may result in the embryo not surviving. In general, this procedure must be done many times to get only a few transgenic animals. This procedure has been replaced in many cases by somatic nuclear cell transfer (SCNT), in which a skin fibroblast cell nucleus is microinjected instead of an IVF male pronucleus, however the technique is still essential in cases where somatic or stem cells have been hard to culture (Verma et al., 2007).

*Approach 2: Embryonic Stem Cell Manipulation*

The second approach in making a transgenic animal is to manipulate embryonic stem (ES) cells instead of the pronucleus. The beginning part of the procedure is the same, as an embryo is formed by IVF, but this time it is immediately grown about 5 days to the blastocyst stage. The ES cells are found on the inner cell mass of the blastocyst, and some are removed using a needle similar to the one used in the pronuclear microinjection. Once harvested, the ES cells are then cultured *in vitro* in a medium that inhibits differentiation while preserving their pluripotent nature. The ES cells can then be manipulated to take up the target transgene DNA using one of the techniques discussed previously (microinjection into the nucleus, viruses, chemicals, or electroporation). The ES cells are then screened to make sure they are expressing the gene of interest (discussed below) prior to being further used. The transgenic ES cells are then returned to the blastocyst which is inserted into a pseudo-pregnant female.

The biggest benefit to using this technique is that many more methods can be used to insert the target gene. Some of these methods, especially homologous recombination, can insert DNA into a known location in the genome, which is beneficial in many circumstances. However, since only some of the blastocyst's ES cells were manipulated to take up rDNA, the resulting animal is chimeric, with some of the cells being transgenic and other cells being wild type. But so long as the gametes are transgenic, the chimeric animals can be bred to create pure transgenic offspring.

*Issues with Microinjection*

These techniques, when used correctly, can be very effective at producing a transgenic animal. However, the location in which the gene of interest inserts into the host animal is not controlled when using microinjection. This can result in unwanted pathologies and other unintended results, and it is best to avoid this if at all possible. Most higher-order organisms have large genomes which are divided into multiple chromosomes and generally are diploid, which means they have two sets of the chromosomes. These chromosomes undergo a process called homologous recombination during meiosis which allows them to transfer DNA between the chromosomes. This allows for a solution in which the transgene is targeted to a specific location. By flanking the gene of interest with DNA from the mouse chromosome itself, the natural process of homologous recombination will take place, replacing the DNA of the host chromosome with that of the gene of interest (Bronson and Smithies, 1994).

*Somatic Nuclear Cell Transfer (SNCT)*

Most transgenic animal lines have been created using one of the first two techniques discussed above, however a third powerful method has recently been created that allows the genetic manipulation of an adult cell's nucleus. Somatic refers to the cells of the body, not the gametes, this technique uses nuclei from body cells, and transfers them along with the transgene into an egg in which the nucleus has been removed. Electricity is then used to induce the egg to divide, and it is then implanted in the same manner as the other two techniques. This results in a clone with the same DNA as the original cell from which the nucleus was taken. This technique is highly beneficial because body cells can be cultured, and only nuclei that have taken up the transgene will be inserted into eggs. This results in fewer embryos being used, and fewer animals being born with unintended defects (Fulka et al., 1998).

## TRANSGENIC ANIMAL SCREENING

Both of these transgenic processes have very low efficiencies, meaning that they must be done many times to obtain the desired result. Techniques for screening these animals have been created to test whether the transgene has integrated. This can be done in a number of ways, and following the central dogma, the presence of the transgenic sequences in any or all of DNA, RNA, or protein can be used for screening. These include the use of Western Blots, Southern Blots, and polymerase chain reaction assays.

*DNA – Southern Blots*

The first method, the Southern Blot, has many applications for testing DNA. The technique was invented by Edward Southern as a means for detecting specific DNA fragments in

a mixture of fragments (Southern, 1975). It can be used to test how the transgene has integrated into the DNA and calculating how many copies have integrated. Normally a transgene integration of between 5 and 10 copies per genome is preferred. In order to perform a southern blot, first DNA is isolated from a very small piece of tail tissue. Then restriction endonucleases are used cut the DNA into smaller fragments, and the pieces are electrophoresed on an agarose gel with electric current applied, to separate the DNA fragments by size. The DNA is then transferred to a membrane, and a labeled DNA probe (complementary to the transgene), is hybridized to the membrane to visualize any DNA fragments containing the transgene.

*RNA – RT-PCR*

A variant on the DNA amplification method of polymerase chain reaction (PCR) can be used to amplify a signal from RNA. First, RNA is isolated from a tissue of interest. Then reverse transcriptase (RT) is used to synthesize complementary DNA (cDNA) from the RNA. Then PCR primers specific to the transgene are used to amplify a signal from the RNA. If a signal is observed, the transgene has been expressed into RNA in that tissue. The biggest benefit of this test is that it is very easy to do with a thermocycler machine doing most of the work. Also smaller samples can be analyzed than with Southern blots, because the signal is amplified during the procedure.
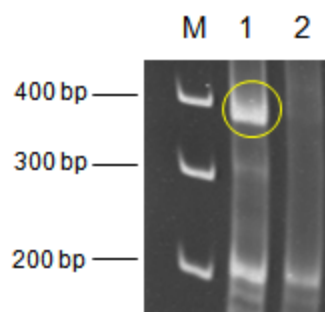


**Figure-4: Example RT-PCR Screening of Transgenic Alzheimer's Mice.** Lane M shows marker bands in basepairs. Sample in lane-1 shows a positive transgenic mouse with an RT-PCR signal at 377 bp (yellow circle) representing the APP transgene. Lane-2 shows a wild-type negative. (Adams, Personal Communication)

*Protein – Western Blots*

The Southern Blot shows that the transgenic DNA has integrated into the animal's genome, however it does not determine whether the transgenic protein is being produced. Since this was the intention of inserting the transgene in the first place, it is important to check for the presence of the protein. The Western blot test itself is very similar to the Southern Blot, except protein is isolated from the tissue being tested. The protein is electrophoresed, then blotted to membrane, and the membrane is incubated with an antibody against the transgenic protein. If the antibody binds to the membrane in the correct location, the protein of interest is being made in that tissue. However, Western blots are time intensive, and antibodies do not exist to all proteins being studied. Often, creative techniques are used to get around these issues, such as tagging the transgenic protein with a series of amino acids for which an antibody already exists, however Westerns can be one of the hardest and most expensive of the three tests discussed.

In the next chapter we discuss applications of these transgenic techniques to create and screen transgenic animals, focusing on their benefits to society as a prelude to discussing their ethics. Transgenic applications are nearly endless; however the ethical and legal dilemmas are great, and will also be discussed in upcoming chapters.

**Chapter-1 References**

Bronson SK, Smithies O  (1994)  Altering mice by homologous recombination using embryonic stem cells. *J. Biol. Chem*.  **269**: 27155-27158.

Cohen SN, Chang AC, Boyer HW, Helling RB (1973) Construction of biologically functional bacterial plasmids in vitro. *Proc Natl Acad Sci U S A*. **70**(11): 3240-3244.

Cozzi J, Anegon I, Braun V, Gross A, Merrouche C, and Cherifi Y. (2009) Pronuclear DNA Injection for the Production of Trangenic Rats. *Methods in Molecular Biology* **561** 73-88.

Fathalla M, Lawrence C, Zhang N, Sessler J, Jayawickramarajah J. (2009) Base-pairing mediated non-covalent polymers. *Chemical Society Reviews* **38** 1608-1620.

Fulka Jr  J, et al (1998) Cloning by Somatic Cell Nuclear Transfer.  *Bioessays*  **10**: 847-851.

Gordon JW, Scangos GA, Plotkin DJ, Barbosa JA, Ruddle FH  (1980)  Genetic transformation of mouse embryos by microinjection of purified DNA.  *Proc. Natl. Acad. Sci USA*  **77**: 7380-7384.

Hirabayashi M, Hochi S. (2010) Generation of Transgenic Rats by Ooplasmic Injection of Sperm Cells Exposed to Exogenous DNA. *Methods in Molecular Biology* **597** 127-136.

Human Genome (2010) *Encyclopædia Britannica, Inc.* Retrieved July 15, 2010. http://www.britannica.com/EBchecked/topic/1377262/human-genome

Jaenisch R and Mintz B (1974) Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA. *Proc. Natl. Acad. Sci. USA* **71**: 1250-1254.

Malakoff, David (2000) The Rise of the Mouse: Biomedicine's Model Mammal.  *Science* **288**: 248-257.

Mendel G.  (1866) Versuche über Plflanzenhybriden. *Verhandlungendes naturforschenden Vereines in Brünn, Bd. IV für das Jahr1865, Abhandlungen,* 3–47.

Palimiter RD, Brinster RL, Hammer RE, Trumbauer ME, Rosenfeld MG, Birnberg NC, and Evans RM (1982) Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes.  *Nature*  **300**: 611-615.

Southern EM (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98** (3): 503–517

Verma V, Gautam SK, Palta P, Manik RS, Singla SK, Chauhan MS (2007) Development of a pronuclear DNA microinjection techniques for production of green glourescent protein-expressing bubaline (Bubalus bubalis) embryos. *Theriogenology* **69** 655-665.

Watson JD, and Crick FHC (1953) A Structure for Deoxyribose Nucleic Acid.  *Nature* **141**: 737-738.

# CHAPTER-2: TRANSGENIC APPLICATIONS

*Laura Fineman*


Now that we have described in the previous chapter what transgenic animals are and *how* they are created, we now turn our attention to *which* ones have been created. The purpose of this chapter is to describe the main categories of transgenic animals and to provide examples within each category. Special attention will be paid to their benefits to society as this will be important in our subsequent chapter on transgenic ethics.


## DISEASE MODELS

Transgenic disease models are animals that have been genetically modified to mimic human diseases for observation and the possible development of drugs or cures. This category of animals is especially important when the disease being modeled has no existing cure. Moreover, since most types of disease research requires testing on animals before continuing to human clinical trials, if no animal model exists for a particular disease it is beneficial to society to create one. Examples of some disease models are the AIDS mouse, Alzheimer's mouse, Oncomouse, and Parkinson's fly.


### *AIDS Mouse*

Acquired immune deficiency syndrome (AIDS) is caused by the human immuno-deficiency virus (HIV). HIV infects humans, but does not infect animals, so a disease model would facilitate research on HIV treatments. Simian immunodeficiency virus (SIV) infects monkeys, and has taught us about this class of virus, but SIV is not HIV. Before the AIDS

mouse, little was known about the events that occur directly after systemic HIV infection due to the lack of a suitable small animal model.

The first AIDS Mouse was a SCID-hu mouse containing human fetal thymus or lymph node implants inoculated with HIV (Namikawa et al., 1988). The SCID mouse lacks an immune system, so the human lymph node and thymus implants are not rejected. The implants are used to allow HIV infection of a human tissue inside the mouse. Incisions were made to expose the implanted human tissue, and HIV doses were injected directly into the incisions. The model showed that HIV drains to lymph organs during infection, and the developing thymus is a target in perinatal infants. The lymph organs were found to have infectable immune cells, such as CD4 T-cells and monocytic cells. When the thymus was examined, it was found that over 70 percent of the infected cells were in the medulla (organ center) as opposed to the cortex. These observations are congruent with what occurs in humans, making this a successful model (Namikawa et al., 1988).

Since the original AIDS Mouse was created, other types of mouse and rat models have been created, including inserting co-receptors for HIV entrance, and inserting key host proteins required for HIV replication. The rats turned out to be equally good disease models in portraying the disease. Their increased size allows for bigger blood samples and greater organ mass for analysis, and some of their host proteins facilitate HIV replication unlike their mouse counterpart (Reid et al., 2001).

*Alzheimer's Mouse*

Alzheimer's Disease (AD) is a progressive brain disease that is the 7th leading cause of death in the United States (Alzheimer's Association, 2004). People with this disease have brains

containing numerous amyloid plaques surrounded by dystrophic neurites. Amyloid plaques are composed of amyloid β-peptide (Aβ) which is a 40-42 amino acid fragment of the β-amyloid precursor protein (APP). Aβ is highly neurotoxic and destroys nerve cells. The Alzheimer's Mouse, created by WPI professor Dave Adams and his colleagues at the former Transgenic Sciences Inc., was the first model to express high levels of human mutant APP which mimics a family in Indiana with early onset AD (Games et al., 1995). This mouse model produces high levels of toxic Aβ in the same areas of the brain that AD patients produce it. It shows extensive Alzheimer type neuropathy after approximately 6-8 months. The mouse was generated by inserting the gene for mutated (Indiana type) human APP with a platelet-derived growth factor promoter to drive its expression in the correct areas of the brain. A transgene splicing that permitted expression of all 3 isoforms of human APP contributed to the high levels found and its successful AD-like pathology. This model also provides strong evidence for the importance of APP (and Aβ) expression in the neuropathy of AD (Games et al., 1995).

A few years after the original Alzheimer's Mouse was created, Elan Pharmaceuticals used it to create the world's first vaccine for the disease (Schenk et al., 1999). The immunogen was a synthetic human $A\beta_{42}$ (a major component of β-amyloid plaques). Almost all the mice immunized with $A\beta_{42}$ developed and maintained significant amounts of serum antibody concentrations against the toxin, and the clearance of the toxin almost completely prevented senile plaque deposition. Thus, the systemic injection of $A\beta_{42}$ and the formation of antibodies against it appears to reduce the levels of the toxin; $A\beta_{42}$ antibodies either prevent Aβ deposition or enhance its clearance from the brain (Schenk et al., 1999).

*Oncomouse*

Oncomouse was one of the first transgenic animals ever produced, and was the first transgenic animal patented. This disease model contains a human oncogene and develops human type tumors, enabling it to serve as a model for understanding cancer formation and for screening anti-tumor drugs. It was developed for cancer research and actually consisted of 13 different strains of transgenic animals, collectively referred to as Oncomouse. Oncomouse contains a fusion transgene (MTV/*myc*) containing a human proto-oncogene *c-myc* with most of the upstream promoter region replaced with a hormonally inducible mouse mammary tumor virus promoter (MTV). The expression levels of the transgene varied among the female progeny, but all of the females that inherited the transgene developed mammary adenocarcinomas during their pregnancies. It was deduced that the regulated *myc* gene acts as a heritable, predisposing factor favoring the accelerated development of tissue-specific adenocarcinomas (Stewart et al., 1984). The patent was awarded four years after being filed, on April 12, 1988 (Leder and Stewart, 1984).

*Parkinson's Fly*

In 2000, a model for Parkinson's Disease (PD) was made; not with a mouse, but with the fruit fly (*Drosophila*) (Feany et al., 2000). PD is a neurodegenerative syndrome characterized by loss of dopaminergic neurons in the substantia nigra. Dopamine is a neurotransmitter that helps regulate neuromuscular transmission, so its loss leads to abnormal muscular control. The cause of PD is unknown, but in some genetic cases it is caused by mutations in the α-synuclein gene; this mutation triggers α-synuclein to accumulate in Lewy bodies and Lewy neurites causing their degeneration. In this disease model, mutant (A30P) α-synuclein and mutant (A53T) α-synuclein

genes were inserted into transgenic flies. The flies' nervous systems formed appropriately, but after 30 days the dorsomedial dopaminergic neurons were absent. The model accurately represents the three key features of the pathology: adult onset, involvement restricted to the nervous system, and anatomical specificity. Some of the flies also experienced retinal degeneration in addition to neurodegeneration, proving that α-synuclein-related degenerative changes show relative rather than absolute specificity for dopaminergic neurons (Feany et al., 2000).

**TRANSPHARMERS**

Before the technology of transpharming was developed, biologically important proteins such as human growth hormone or insulin were harvested from human cadavers or from slaughtered pigs, respectively. However, research is currently being performed on the production of human pharmaceuticals in genetically modified farm animals (Biotech Info Series, 1995). This transpharming technology was first implemented in mice, but is now moving to larger animals such as sheep, goats, and cows due to their larger milk production.

*Transpharmer Mice*

A group in Framingham, MA created a mouse that was genetically modified to produce human tissue plasminogen activator (t-PA) in it's milk. t-PA is a protein that functions as a clot dissolver, so it can be used for treating heart attacks and strokes. The t-PA gene was fused to the murine whey acid protein (WAP) gene. WAP is the most abundant protein in mouse milk, so by using its promoter the t-PA gets expressed only in the milk. The level of WAP RNA in the

mammary gland increases about 340-fold in lactating females. This model proved the feasibility of producing human proteins in the milk of animals for commercial use (Gordon et al., 1987).

*Transpharmer Sheep*

Four sheep were successfully genetically engineered in Edinburgh, Scotland by pronuclear injection of a fusion transgene derived from a sheep milk protein β-lactoglobulin (BLG) promoter (to drive expression in the milk) and a human anti-hemophilic factor IX (a drug used to treat specific clotting disorders). Not only did the two ewes secrete human factor IX in their milk, but both gave birth to lambs with the same trait (Clark et al., 1989).

*Transpharmer Goats*

Recently, GTC Biotherapeutics Corp. received FDA approval to market ATryn®, which is the world's first FDA-approved transpharmed drug. The drug is a recombinant form of human antithrombin (ATIII) for the prevention of peri-operative and peri-partum thromboembolic events in hereditary antithrombin deficient (HD) patients. The transgenesis was accomplished by using cell fusion of a transgenic cell to an enucleated egg. The fusion gene consisted of a milk protein promoter and the ATIII gene. The company is also developing other recombinant forms of additional human plasma proteins, such as albumin and alpha-1 antitrypsin (ATryn®, 2008).

*Transpharmer Cows*

Perhaps the most famous transpharmer is a Dutch bull named Herman, born in 1991. Herman was the world's first transgenic cow. The gene for human lactoferrin (hLF) (an antimicrobial agent present in normal mother's milk but not in cow's milk or in synthetic

formula) was microinjected into immature oocytes, then the embryo was implanted by non-surgical transfer. Twenty-one pregnancies and 19 calves later, two calves tested positively for the lactoferrin gene. The female was a mosaic; the gene was found in the placenta, but not the blood or ear tissue. But the male was transgenic in all three tissues, and was later named Herman (Krimpenfort et al., 1991). He later went on to father at least 8 calves in 1994, all containing the lactoferrin gene (Biotech Notes, 1994).

## XENOTRANSPLANTERS

Ever since the introduction of immuno-suppressor drugs, transplantation has become the preferred treatment for advanced organ failure. However, a very limited supply of human organs is leading to an alternate approach of using animal organs. This process is called xenotransplantation (transplantation between species). The primary animal of choice is the pig, due to its relatively close physiology with humans, ethical considerations, and its relatively compatible organ size. The major barrier of using pigs is the presence of terminal [alpha]-1,3-galactosyl (Gal) epitopes on the surface of pig cells. Because humans have lost the corresponding galactosyltransferase activity during evolution, we lack Gal epitopes on the surface of our cells. This causes its presence in pig cells to be viewed as foreign by humans, leading to hyperacute rejection of the pig organ. Several solutions have been suggested, including Gal antibody removal or competitive inhibition, although these methods are not 100 percent effective. An alternative way has been to genetically knockout the transferase gene that encodes the enzyme that adds on this "foreign" sugar using embryonic stem cell technology (Lai et al., 2002). The first knockout pigs were shown to have a successful knockout of the gene, and

they are all heterozygous for the knockout. The next step is to create homozygous knockout pigs and to test their organs in transplantations (Lai et al., 2002).

Pig heart valves are currently used for heart valve transplantations, so there is already a basis for transplanting animal tissues into humans, but there are several concerns about transplanting entire animal organs. Most of the concern is of pig viruses crossing the species barrier, whether the viruses represent new infectious agents or asymptomatic viruses in pigs that become active in humans (Catez, 2005). However, the organ waiting lists remain miles long, and people on the lists die every day awaiting transplants, so there is a desperate need for organs, and xenotransplantation just might be a good enough answer.

## TRANSGENIC FOOD SOURCES

Transgenic animals have also been created to potentially provide new sources of food. In the cases of superpig and superfish, growth hormones were inserted to create animals that mature faster on less food to commercialize them as food sources.

*Superpig*

This animal contains an ovine metallothionein-1α (oMT1α) promoter (to allow strong zinc-induced expression in a variety of tissues) fused to ovine growth hormone (oGH) (to facilitate rapid growth of the animal) (Pursel et al., 1997). The fusion gene was microinjected into 400 pig zygotes; from this, 15 piglets were born. Of the 12 assayed, 5 contained high levels of oGH, 1 contained low levels, and 6 had none. A dietary supplement of zinc increased the plasma oGH of the piglet with low levels by 20-fold. Although the incorporation of the oGH transgene was successful, there is still a need of improved transgenic methodology in pigs;

microinjection of growth hormone into mice and sheep is far more successful in terms of percent born being transgenic (Pursel et al., 1997).

Although the goals (increased rate of gain, increased feed efficiency, and decreased carcass fat) were achieved, significant suffering of the animals occurred after about 6 months of age. Some problems observed were that of the kidney and liver, lethargy, uncoordinated gait, degenerative joint disease, gastric ulcers, various heart diseases, and pneumonia; this lead to the euthanization of the animals. Because of these severe health effects, the scientific community imposed a moratorium on performing growth hormone experiments in mammals (Rollin, 1996).

*Superfish*

Although growth hormone experiments in pigs were a disaster, similar experiments performed in fish for aquafarming purposes proved far more successful. Several companies, including Aquabounty Technologies, are near to getting FDA approval to aquafarm transgenic salmon and trout (Aquabounty Technologies, 2009). Aquabounty specifically has created an AquAdvantage® fish, an advanced-hybrid salmon, trout, and tilapia hybrid designed to grow faster than traditional fish. This fish is designed to reach marketable size twice as fast as traditional fish, a compelling economic benefit to farmers. The fish is also reproductively sterile, eliminating the threat of interbreeding amongst themselves or with native populations if they are accidently released (Aquabounty Technologies, 2009).

Although being sterile has its benefits to preserving native fish populations, there are still other concerns about what might happen if the containment pen breaks, including concerns about the released fish competing with native fish for food, or being able to survive better with climate

changes or less food. The released fish could also spread to other areas to become an invasive species (Stokstad, 2002).

## SCIENTIFIC MODELS

These scientific models have been created to teach us something about the function of a newly discovered protein by overexpressing the gene or by knocking it out.

### ANDi the Monkey

In 2001, a green fluorescent protein (GFP) gene contained in a retroviral vector was injected into the perivitelline space of mature rhesus oocytes. The GFP transgene was used as a *marker* to prove that transgenic primates can be created, although its physiology would not be altered. The transgenic oocytes were then fertilized *in vitro*, grown to the blastocyst stage, then implanted into the uterus of a pseudopregnant female. Three males were born, and one of them was successfully transgenic; his name was ANDi (inserted DNA spelled backwards). There was GFP direct fluorescence in his toenails, hair, and placenta; however, ANDi does not glow (Chan et al., 2001). At the moment, it is unclear if his sperm contains the transgene as well; if so, he will be able to pass it on to his offspring and more research can be done. The development of ANDi also raises many concerns about creating transgenic primates, (Begley, 2001) which will be discussed in Chapter-3.

### Smart Mouse

According to Hebb's Rule in 1949, learning and memory are based on modifications of synaptic strength among neurons that are simultaneously active. This rule implies that better

synaptic coincidence leads to better learning and memory. The NR2B subunit of the NMDA (N-methyl-D-aspartate) glutamate receptor (the synaptic coincidence detector) predominates when the brain is forming, and is thought to act as a graded switch for memory formation. This hypothesis is what a group at Princeton set out to test, and it proved to be right (Tang et al., 1999). Mice over-expressing the NR2B subunit of the glutamate receptor were seen to have better long term memory than their non transgenic littermates. One hour after they were introduced to objects and taught new ones, both sets of mice did equally well; however 3 days later only the smart mice remembered their training. After one week though, neither remembered (Tang et al., 1999).

*Supermouse*

Supermouse was created using the same transgenic growth hormone technology used later to create Superpig and Superfish.  Supermouse was the world's first transgenic animal in which the transgene actually produced a visible phenotypic change. Slightly earlier models were transgenic, but did not express the transgene. The promoter used was mouse metallothionein-I (a very effective strong promoter) which was fused to the structural gene of a rat growth hormone. The transgene was then microinjected into the pronuclei of newly fertilized mouse eggs. The fertilized eggs were grown to the blastocyst stage, then implanted into the uteri of pseudopregnant mothers. Of the 21 mice born, seven carried the transgene and six were much larger than their littermates. Like Superpig, the mouse diet contained zinc supplements to help drive expression of the growth hormone to enhance their growth. Although when this model was first created in 1982, it had the potential for being a model for gigantism and for correcting genetic diseases affecting height (Palimiter et al., 1982), the growth hormone technology was

subsequently dropped after the 1997 Superpig fiasco. However, the growth hormone technology originated with this animal is currently being used commercially for transgenic fish, as mentioned previously for Superfish.

*Youth Mouse*

In 1999, mice were created carrying the entire coding gene of murine urokinase-type plasminogen activator (uPA) linked downstream from the promoter of lens-specific αA-crystallin gene (Miskina et al., 1999). These α-MUPA mice live about 20 percent longer than normal mice by eating less. These mice have a reduced body temperature, higher plasma corticosterone, maintain a young look, and for the most part resemble healthy dietary restricted mice. Although overall they seem healthy, they do show a high frequency muscle tremor in all legs when placed in a non-stable position, such as sustained by their tail. However, this is only seen in homozygous α-MUPA mice, not heterozygous, and their other motor behaviors appear normal (Miskina et al., 1999).

## Chapter 2 References

1. Disease Models

Alzheimer's Association (2004) "What is Alzheimer's Disease?"
http://www.alz.org/AboutAD/WhatIsAd.asp

Feany MB and Bender WW (2000) A Drosophila Model of Parkinson's Disease. *Nature* **404**: 394-398.

Games, Dora, David Adams, et al. (1995) Alzheimer-Type Neuropathology in Transgenic Mice Overexpressing V717F β-Amyloid Precursor Protein. *Nature* **373**: 523-527.

Leder, P and Stewart, T. (1984) "Transgenic Non-human Mammals, The Harvard Oncomouse. US Patent and Trademark Office. Patent #4,736,866. Cambridge, MA.

Namikawa R, Kaneshima H, Lieberman M, Weissman IL, McCune JM (1988) Infection of the SCID-Hu Mouse by HIV-1. *Science* **242**: 1684-1686.

Reid W, et al. (2001) An HIV-1 Transgenic Rat that Develops HIV-Related Pathology and Immunology Dysfunction. *Proc. Natl. Acad. Sci. USA.* **98**(16): 9271-9276.

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. (1999) Immunization with Amyloid-β Attenuates Alzheimer-Disease-Like Pathology in the PDAPP Mouse. *Nature* **400**: 173-177.

Stewart TA, Pattengale PK, and Leder P (1984) Spontaneous Mammary Adenocarcinomas in Transgenic Mice That Carry and Express MTV/myc fusion Genes. *Cell* **38**: 627-637.

2. Transpharmers

ATryn® (2008) Recombinant Human Antithrombin. *GTC Bioterapeutics, Inc.* http://www.gtc-bio.com/products/atryn.html

Biotech Notes (1994) Herman Becomes a Father. U.S. Department of Agriculture. http://www.accessexcellence.org/AB/BA/Herman_the_Bull.html

Biotechnology Information Series (1995) Pharmaceutical Production From Transgenic Animals. http://www.biotech.iastate.edu/biotech_info_series/bio10.html

Clark AJ, Bessos H, Bishop JO, Brown P, Harris S, Lathe R, McCenaghan M, Prowse C, Simons JP, Whitelaw CBA, and Wilmut I (1989) Expression of human anti-hemophilic factor IX in the milk of transgenic sheep. *Bio/Technology* **7**: 487-492.

Gordon K, Lee E, Vitale J, Smith AE, Westphal H, and Henninghausen L (1987) Production of human tPA in transgenic mouse milk. *Biotechnology* **5**: 1183-1187.

Krimpenfort P, Rademakers A, Eyestone W, van der Schans A, van den Broek S, Kooiman P, Kootwijk E, Platenburg G, Pieper R, Strijker R, and Herman de Boer (1991) Generation of transgenic dairy cattle using 'in vitro' embryo production. *Biotechnology* (NY). **9**(9): 844-847.

3. Xenotransplanters

Catez, Steven (2005) Xenotransplants: Are Pig Cells in Humans the Answer? http://allthings2all.blogspot.com/2005/03/xenotransplants-are-pig-cells-in.html

Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL et al. (2002) Production of Alpha-1,3-Galactosyltransferase Knockout Pigs by Nuclear Transfer Cloning. *Science* **295**: 1089-1092.

4. Food Sources

Aquabounty Technologies (2009) http://www.aquabounty.com/products/aquaadvantage-295.aspx

Pursel VG, Wall RJ Solomon MB, Bolt DJ, Murray JD, and Ward KA (1997) Transfer of Ovine Metallothionein-Ovine Growth Hormone Fusion Gene into Swine. *Journal of Animal Science* **75**: 2208-2214. http://jas.fass.org/cgi/reprint/75/8/2208.pdf

Rollin BE (1996) Bad Ethics, Good Ethics, and the Genetic Engineering of Animals in Agriculture. *Journal of Animal Science* **74**(3): 535-541.

Stokstad, Erik (2002) Engineering fish: Friend or Roe of the Environment? *Science* **297**: 1797-1799.

5. Scientific Models

Begley, Sharon (2001) Brave New Monkey. Newsweek, January 22 issue, pp. 50-52.

Chan AW, Chong KY, Martinovich CC, Simerly C, Schatten G (2001) Transgenic Monkeys Produced by Retroviral Gene transfer into Mature Oocytes. *Science* **291**: 309-312.

Miskina R, et al (1999) Alpha-MUPA Mice: A Transgenic Model for Increased Life Span. *Neurobiology of Aging* **20**: 555-564.

Palimiter RD, Brinster RL, Hammer RE, Trumbauer ME, Rosenfeld MG, Birnberg NC, and Evans RM (1982) Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* **300**: 611-615.

Tang YP, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ (1999) Genetic Enhancement of Learning and Memory in Mice. *Nature* **401**: 63-69.

# CHAPTER-3:  TRANSGENIC ETHICS

*John Raymond Martin*


At all times in human history, great progress has coincided with great atrocities.  As transgenics makes great strides, it is important that we do not commit the crimes of our past and that we evaluate the consequences of this emerging technology, one that is hardly comparable to any in our collective histories.  The benefits to society of doing transgenic animal experiments, discussed in the previous chapter, could be substantial, however many see this type of technology as having stepped into a realm man was never meant to exist in.  For many people, altering organisms at the core level of DNA is akin to playing god.  They see using animals for any kind of research as cruel and immoral.  Others feel that animals are here to service us, and transgenic experiments are just one addition to the laundry list of current uses.  Some people only see using this technology on humans as wrong, while accepting it on animals.  Some transgenic animals live comfortably for their entire lifespan, while other suffer and die early.  This large disparity in beliefs, and the significant disparity in the transgenic animals themselves, means that an answer cannot be derived for the entire subject.  For that reason this chapter goes beyond the transgenic applications previously discussed to focus on ethical perspective to help elucidate whether the benefit to society is truly worth it.  This chapter will initially be written in a position-neutral manner, because these disparities make transgenic ethics an especially difficult area.  At the end of each application a litmus test will be used by the author to put that application into perspective.  This test will look at three areas: (1) harm to the animal, (2) benefit to society, and (3) whether it crosses the line of mainstream morality.  At the end of the chapter, the author's main conclusions and opinions will be presented.

## ANIMAL ETHICS

Several systems exist for evaluating the ethics of a situation, the two classical systems are utilitarianism and deontology. Utilitarianism has just one criterion: utility. This results in a balancing act in which the greatest difference between good and bad is encouraged. Deontology does not look at just the consequences of an action but also moral principles. However, this can also result in a complex balancing act, especially as the principle of not harming animals is weighed against saving cancer patients. A third system, that of the "network model", constitutes six principles: beneficence (promoting the well being of the animal), non-malfeasance (not harming the animal), justice (equal treatment of similar animals, and fair distribution of good and evil between the human and animal), integrity (physical and genetic), irreversibility (the idea that risk should be minimized because of the irreversible nature of biotechnology), and verifiability (that there should be a public debate on the regulations which govern the technology) (Boer et al., 1995). In general, the integrity principle will be taken to mean physical integrity, as all of the animals discussed in this project lack genetic integrity by being transgenic. These three systems will be balanced when using our litmus test for each application. There exists the possibility that one system may disapprove of a particular animal while still be considered acceptable by the author because the balancing leans more heavily towards the use of the animal.

## TRANSPHARMER ETHICS

Transpharming is the practice of using animals as bioreactors capable of producing some kind of pharmaceutical product. The standard way of doing this is to engineer the animal to express the foreign protein in the milk because it results in high yields while doing little to no

harm on the animal itself. Another benefit of production in the milk is that milking an animal is a long-standing industry with significant technology to extract the product in a way that does no harm to the animal. Since the animals live full lives without any behavioral sign of the transgenesis, it is hard to have a significant argument against this type of transgenic application. Another key benefit to transpharming is that the animals are portable; they can be brought to other areas of the globe needing drugs such as antibiotics or HIV medication, including areas without electricity. As far as our litmus test: there is no detectable animal suffering as long as there are no defects in the transgenesis process, and the benefit to society producing life saving medicines is great, without putting mainstream morality at risk.


**XENOTRANSPLANTATION ETHICS**

It comes as a shock to most people to think about humans having their blood cleaned by a pig; however the next step in this field is using animals to grow human organs and then transplant them back into humans (Butler, 2002). The lack of human organs to serve critically ill patients awaiting human organ transplants is one of the primary reasons this technology is being pursued. Even when there are organs available, they are not always histocompatible with the patient. This means that there are receptors on the organ that the patient's immune system recognizes as foreign and mounts a response against it. Even patients that are histocompatible must take immunosuppressive drugs that can stop the immune system from reacting to other real infections, and these drugs have powerful side effects. Since organ transplantation is major surgery and involves the insertion of a foreign organ that potentially came from a donor that is infected by a virus, even at small amounts of immunosuppression, the presence of a virus can be very dangerous. Xenotransplanters however are transgenic animals that have had the receptors

that cause this immune reaction removed.  They have also been grown in a sterile environment, which means the risk of infection after surgery is greatly reduced.  There is the concern that people with animal organs could incubate any virus present, and cause animal diseases to jump to humans.  Since the animal organ would be inside the human the virus would have a chance to evolve in an environment (a mixture of human and animal cells) beneficial to crossing species.  This risk can be reduced by testing for known viruses, and maintaining sterile conditions while the animal is raised.  However, even normal individuals always have the possibility of coming into contact with a pig virus and creating a new human virus that could spread to the population.  Mainstream ethics which says it is acceptable to raise a pig to be eaten (which involves animal death), makes it very hard for a mainstream rejection of this kind of transgenic animal on the basis of animal cruelty.

However, the future of this technology includes many more ethically ambiguous areas such as their use in cosmetic surgery.  Growing a new face on a pig then transplanting it onto a human is ethically ambiguous; however it is a future whose ethics must be considered today.  The idea of using a pig organ as a last resort when a human organ is not available is not nearly as ambiguous, as it would be a life or death situation that most would see as a necessity if available.  This difference is evident in the justice principle, in the case of the heart transplant a pig dies to save a deathly ill human, while in the cosmetic case the pig dies so that the human could look better.  Our litmus test shows that there is little suffering to the animal (as much as with any other farm animal) and the benefits to society are great, however some say our morality is at risk if humans take xenotransplanting too far.

**TRANSGENIC DISEASE MODEL ETHICS**

The area of disease models is where the real ethical concerns, for most, start to appear. Not all of the animals discussed in Chapter 2 live full or pain free lives. All of the animals in Chapter 2 have the potential to be of great benefit to society, although there was little balancing needed for the first two applications, the benefit must be weighed against the harm to the animals.

*Alzheimer's Mouse*

First produced by this project's adviser, Dave Adams, the Alzheimer's mouse does not suffer in any detectable way. Alzheimer's research is key to curing a disease that affects millions, and has no effective treatment. These mice are used as part of research which includes collecting samples, testing new drugs, and eventually sacrificing the animal so that its tissues can be tested. The litmus test here shows that there is little mouse suffering, and while during research the lifespan may be shortened significantly, there exists a moral imperative to find a treatment for this devastating disease.

*Parkinson Fly*

This model system benefits from being in a lower order animal, however this brings out different objections. While there are no significant animal welfare concerns to this type of insect research, there are objections to making such a portable animal containing transgenes for fear that it might escape. While larger animals are easy to track, and only produce small litters, flies are small and produce very large amounts of offspring. An accident in 1957 resulted in African "killer bees" being released into North America which allowed cross breading with native bee

species.  Such an accident according to the irreversibility principle must be done in a laboratory that does not allow the chance for the flies to escape or breed with other non-transgenic species. The litmus test here shows no concerns for the animal itself, but concerns that society could be affected in unknown ways if the fly were to escape.

*AIDs Mouse*

Since HIV cannot naturally infect mice, a transgenic solution was needed to create an animal model for testing potential treatments.  These mice have been made so that their engrafted human tissues are not rejected by using the SCID knockout to compromise their immune system. This leaves the mice unable to fight off infections, and they essentially suffer from the "bubble-boy" syndrome and must be kept in a sterile environment to stop them from dying.  This transgenesis causes suffering on the part of the mouse that is not insignificant.  The seriousness of the diseases' prevalence however makes it a moral imperative that it be researched and treated.  This is a case where the litmus test shows animal suffering, however because of the massive scale of AIDS, the moral imperative, and the overall benefit to society such research will bring, mainstream ethics says that this is important to do.

*Oncomouse*

Cancer kills millions of patients per year, and current treatments in many areas are not adequate.  Although HIV crossed species from chimpanzees in this century, cancer has been with the human race since its inception.  Most cancer develops later in life, and kills at a fairly slow rate, although some cancers affect children in early life and cause many early deaths.  Oncomice show most of the symptoms of a person who has cancer, and are used to learn about what

initiates cancer, and for screening potential drugs. But tumors grow which cause pain, and the mice can die early. This model for some people gets murky. With respect to the litmus test, there is animal suffering, although strong regulative oversight from animal care committees usually requires the use of pain killers and sacrifice of the mice prior to advanced tumor formation. However cancer, one of the top killers in the world, pushes many people (including the author) to find it a worthwhile endeavor to use these mice to do research with continued strong oversight from institutional animal care and use committees (IACUC).

**TRANSGENIC FOOD SOURCE ETHICS**

Transgenic farm animals seek to increase the speed of the breeding programs that have been going on for many centuries to bring about animals that provide more food faster. These animals produce hormones at an accelerated rate that causes them to mature at a significantly faster rate. Because of the increased growth rate, these animals require less food per pound of meat produced than their non transgenic littermates. However, there is general public distain at the idea of genetically modified food, and people worry that the hormones increased in the animals will be ingested. Parents are especially cautious of giving anything that has been genetically modified or has been given growth hormones to their children (Environmental News Service, 2000).

Ideally animals in this realm should follow the "principle of conservation of welfare" which was proposed by Bernard Rollin, and states that "genetically engineered animals should be no worse off than the parent stock would be if they were not so engineered, and ideally should be better off" (Rollin, 1996). Efforts to increase efficiency have resulted in significant suffering that cannot be balanced with a societal concern. It is true that there are people without food in parts

of the world; however this has just as much to do with economic, transportation, and political issues than the inability to produce enough food. As of 2009, the FDA has finally put a regulatory mechanism in place for the approval of transgenic food products and drugs (FDA, 2009). The author believes that transgenic animals that will be eaten must meet very high standards, and that the FDA should evaluate the use of these animals carefully for the safety to the human population as well as the welfare of the animal population.

## SCIENTIFIC MODEL ETHICS

By over-expressing a specific gene, or by knocking it out, scientists are able to discover its purpose. This type of research is often in the category of expanding scientific knowledge, and is often hard to measure ethically because there is no pressing societal issue pushing for the research to be done. However, often times this type of research is the most "pure" because it is the least influenced by businesses trying to make money, it is done in the pursuit of knowledge for knowledge sake.

*ANDi the Monkey*

ANDi was created as a proof of concept that transgenic monkeys could be made. Such animals may eventually serve as human disease models. ANDi was created with an "inert" transgene, green fluorescent protein, which only served as a marker for transgenesis, but created no symptoms. ANDi is different from the other disease model animals because his genome is so close to that of a human which makes this research directly applicable to transgenesis in humans, although the researchers say that they would never support doing transgenesis in humans. They

especially want to distant this type of research from the public image of a society in which everyone had the option of swapping in or out genes for their children (Begley, 2001).

The litmus test on ANDi is hard because while ANDi himself is a scientific model, the research was done so that more monkeys could eventually become disease models. ANDi himself does not solve any societal issues, but the technological development will allow for future disease research, which in turn could prove useful for disease such as HIV which is very hard to study in other animal models. Monkey research is often the tipping point for people in their ethical struggle. People have always been turned off by research in primates because of their close relation genetically and morphologically. It is this resemblance however that can make monkey research that much more fruitful, and when done in a controlled manner it is possible that the ethical balance should allow for it.

*Other Scientific Models*

In general, based on the literature, most of the transgenes chosen for use in scientific models are advantageous to the organism. For example, "youth mouse" lives longer than normal mice, "supermouse" is bigger than normal mice, and "smartmouse" learns faster than wild type mice. However, there are often unintentional consequences of the experiment. While "youth mouse" lives longer, researcher noticed that its legs quiver more than usual (Miskina et al., 1999). While this may not seriously affect the mouse, it brings about important questions about using animal models. Unlike humans, animals are unable to communicate the 'little pains' that transgenesis brings to them. Instead, scientists look for signs such as eating, reproductive behavior, noises, shaking, how playful they are, and other measures to determine the animal's quality of life and whether they are suffering.

**CHAPTER-3 CONCLUSIONS**

While considering the ethics of a developing subject, it is import to do so in the light of Gresham's law which says that "bad money will drive good money out of circulation". This implies that in any new situation when no consensus exists, the outliers will be what is brought to the surface. While society is being significantly benefited by the advent of transgenic techniques, at every step in the path our discussion must evolve. When *in vitro* fertilization first allowed people to have babies that initially could not because of medical issues, the same ethical issues surfaced, the concern that society would change to a race of genetically modified part-humans; and this remains valid in many minds. And in some ways it should be, as corporations realize that there is great profit in the life sciences, the same companies that today make cosmetics and breast implants could tomorrow be offering full body transplants.

However, it is important that the discussion of transgenesis does not move away from the documented good of what is going on. I like to think of Greshman's Law as an equation such that increasing the good money (ethics) will out compete the bad money (profit driven negative experiments). It is the author's conclusion that most of the transgenic research being done today is in the right. Curing HIV, Alzheimer's, and cancer are clear social and moral imperatives. These diseases detract from society in immeasurable ways, which should attract many to promote transgenic research. The next chapter of this project will take an in-depth look from a legal and regulatory perspective on the use of transgenic animals.

**CHAPTER-3  REFERENCES**

Begley, Sharon (2001) Brave New Monkey. *Newsweek*, January 22 issue, pp. 50-52.

Boer IJ, et al (1995) An Ethical Evaluation of Animal Biotechnology: The Case of Using Clones in Dairy Cattle Breeding. *Animal Science* **61** : 453-463.

Butler, D (2002) Xenotransplant Experts Express Caution Over Knockout Piglets. *Nature* **415**: 103-104.

Curran G, and Koszarycz Y (2004) Animal Transgenesis and Cloning: Scientific, Religious, and Ethical Considerations.
http://dlibrary.acu.edu.au/research/theology/ejournal/aejt_3/Curran_Koszarycz.htm

Environment News Service (2000)"Franken Foods: Promise or Peril"? February 23, 2000.
http://www.wired.com/science/discoveries/news/2000/02/34507

FDA to Regulate the Use of Transgenic Animals (2009) *Nature* 457: 371.
http://www.nature.com/news/2009/090116/full/news.2009.36.html

FDA.gov (2009) Guidance for Industry Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs-Final Guidance.
http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf

Jarvis, Audrey, et al (2006) Transgenics: A Perspective from the Interchurch Bioethics Council.
http://www.casi.org.nz/issues/science/Transgenics%20A%20Perspective%20from%20the%20Interchurch%20Bioethics%20Council.htm

Loma Linda University (1997) Seventh-day Adventist Guidelines on Genetic Engineering.
http://www.llu.edu/central/bioethics/llethge.page

Miskina R, et al (1999) Alpha-MUPA Mice: A Transgenic Model for Increased Life Span. *Neurobiology of Aging* **20**: 555-564.

Rollin BE (1996) Bad Ethics, Good Ethics, and the Genetic Engineering of Animals in Agriculture. *Journal of Animal Science* **74**(3): 535-541.

# CHAPTER-4: TRANSGENIC LAWS

*Laura Fineman*

In the previous chapter, the ethics and controversies of producing transgenic animals were discussed. In this chapter, the focus will be on how society regulates the use of this interesting technology, especially through animal patenting. Animal patenting is a highly controversial topic; how much is society is willing to patent, and where should it stop?

## NEW PATENT APPLICATIONS

When applying for a new patent, inventors must fall into one of three categories: utility, design, or plant patent. *Utility* patents are granted to those who invent or discover a new and useful process, machine, article of manufacture, or composition of matter, or any new and useful improvements. *Design* patents are granted to those who invent a new, original, ornamental design for an article of manufacture. *Plant* patents are granted to anyone who invents or discovers and asexually reproduces any distinct and new variety of plant. In addition, any new invention or discovery must be novel as well as non-obvious. (United States Patent and Trademark Office, 2005)

## ANIMAL PATENTS AND FDA APPROVAL

In 1980, following the 1970s landmark case of *Diamond vs Chakrabarty* to allow the patenting of microbes (discussed below), the Supreme Court ruled that novel living microbes can be patented. However, with respect to animals, after the original 1984 Harvard Oncomouse patent received backlash from many animal rights groups (discussed below), the government self

imposed a moratorium on animal patents for several years (Andrews, 1993). After the moratorium was ended, several transgenic mice received patents, including two more patents for a different oncomouse (Leder and Stewart, 1992, 1999), a mouse carrying a non-infections HIV genome (Jolicoeur, 1994), a mouse model for Kaposi's sarcoma (Lira and Yang, 2000), and a mouse expressing amyloid beta to be used as an Alzheimer's model (Stern and Yan, 2000).

In addition to the government's Patent and Trade Office (PTO) controlling patenting, the government's Federal Drug Agency (FDA) plays a big part in the control of transgenic products. After a decade of fierce debate, the FDA finally decided how genetically engineered animals will be evaluated to benefit the public. Such animals will be regulated under the Federal Food, Drug, and Cosmetic Act; the recombinant DNA in the animal will be the 'drug', and its safety and environmental impact will be investigated (FDA to Regulate the Use of Transgenic Animals, 2009). This landmark decision on how to regulate transgenic animals was soon followed by the first FDA approval of a biological product produced by genetically engineered animals (FDA.gov, 2009). The product is called ATryn®, an anticoagulant used for the prevention of blood clots in patients with hereditary antithrombin deficiency. ATryn® is produced in the milk of transgenic goats, maintained at a farm in Charlton, Massachusetts (GTC Biotherapeutics, 2006). Although these new guidelines provide a key step for evaluating transgenic products for FDA approval, it is still a new idea, and a potentially disastrous one to both the FDA and biotechnology companies.

**DIAMOND VS CHAKRABARTY (First US Patenting of a Microbe)**

In 1972, microbiologist Ananda M. Chakrabarty filed a patent application for a genetically engineered bacteria that was able to break down crude oil. This bacterium was

derived from the genus *Pseudomonas* and contained two different plasmids, each with a separate pathway for crude oil degradation. This organism was intended to be used as a treatment for oil spills; no naturally occurring bacteria is able to do this. The patent included three claims: (1) the process for producing the bacteria, (2) for an inoculum comprised of a carrier material floating on the water, and (3) the bacteria themselves (Diamond vs Chakrabarty, 1980).

After examination, a patent examiner accepted the first two claims, but not the third under the reason that living things are not patentable. The case was appealed to the Supreme Court, where it was eventually ruled that Chakrabarty's microorganism constituted a new 'manufacture' or 'composition of matter' because the trait is not nature's handiwork. The court decided that microorganisms can indeed be patentable subject matter under patent law. *Diamond vs Chakrabarty* became a landmark court case, and the bacterium became the first living thing to be patented in the United States (Diamond v. Chakrabarty, 1980).


**ONCOMOUSE LEGALITIES**

Although the Oncomouse was not the first life ever patented, it was the first transgenic animal patented. The patent was awarded on April 12, 1988 to Harvard and DuPont's mouse containing a recombinant activated oncogene (Leder and Stewart, 1984). The patent covers all mammals, and more specifically the ancestors of the animal that first received the oncogene (Leder and Stewart, 1984). Two subsequent oncomouse patents followed the original; for the method for providing a cell culture from a transgenic non-human mammal (Leder and Stewart, 1992), and for testing methods using transgenic mice expressing an oncogene (Leder and Stewart, 1999).

For the original patent, there is some argument about what is legally covered. While the oncomouse itself is an entirely new entity, the *technique* for oncogene insertion was not new yet is part of the patent. The patent also covers oncogene insertion into any mammalian species, an arbitrary boundary that goes far beyond what was actually invented. Awarding a narrower patent could open up doors for competition and independent development as well as better ideas (Stallman, 2002). However, currently DuPont is becoming more assertive about asking United States researchers to obtain licenses to use the Oncomouse (Marshall, 2002), and the company is asking institutions to enforce the licensing agreements. Some institutions have adhered to these requests, but Massachusetts Institute of Technology and the University of California have not yet done so (Marshall, 2002).

## ONCOMOUSE IN CANADA

Although the Oncomouse patent was approved in the United States, in Europe, and in Japan, it was denied in Canada (Check, 2002). On December 5, 2002, the Supreme Court of Canada rejected the patent application for Oncomouse. The court agreed with the Canadian Council of Churches and the Evangelical Fellowship of Canada who both had arguments against the commodification of life (Mitchell and Somerville, 2002). According to Canada's Patent Act, higher life forms are not deemed patentable because they are not a 'manufacture' or a 'composition of matter.' However, the claims on the *process* used to make oncomouse were granted a Canadian patent (Check, 2002).

**Benefits of Patenting Animals**

Over the last several decades, technology and technological innovation has gradually replaced manufacturing and agriculture as the main economical drivers (Blaug et al., 2004). For example, the transgenic industry has grown with inventions of things like protein secretion in milk, disease models for drug testing, and faster growing food sources. However, such research is expensive. Patent protection for transgenic animals could be a good reward for companies willing to undertake the risky and expensive research, development, and manufacture. Many people believe that biotechnology holds the key for finding cures of diseases, improving food quality, making pharmaceuticals more cost-effective, and above all, that the benefits outweigh the possible costs (Walter, 1998). So with respect to benefitting society, the protection of biotechnology inventions may not necessarily be a bad thing; by protecting new discoveries that may provide cures for diseases, you protect the industries making those discoveries.


**Potential Detriments to Patenting Animals**

Although there are many benefits associated with patenting animals, there are also some concerns. For example, there are moral and religious concerns, animal rights concerns, and environmental concerns.  It is important to note various religious beliefs and their views on how to treat animals. Many Jews and Christians follow the Old Testament's teachings in that all life, including animal life, is sacred.  Hindus also see divinity in all living creatures, that animals are a form of re-incarnation, and that cows are sacred.  Therefore, they would be against things such as transgenic cows (Curran and Koszarycz, 2004).

Several animal rights groups also protest transgenic animals and animal experimentation. One of the most visible of these groups is People for the Ethical Treatment of Animals (PETA).

PETA is predominately concerned with animals in labs and their rights, and are attempting to end all animal testing. The *Animal Welfare Act* requires labs to report the number of animals used in experiments; however, this does not include mice, rats, and birds which are used in 80 to 90 percent of all experiments. However, a big factor going against PETA's war opposing animal testing is the FDA requirement that all drugs need to first be tested on animals (PETA.org, 2009).

With respect to environmental concerns, if a genetically engineered animal is released, or escapes into the wild, what is the environmental harm if the animal breeds with native species? So strong oversight is needed to mandate such animals are handled only in licensed vivariums. And with respect to economics, farm animals altered to grow faster or bigger may result in small family farms being unable to compete (Walter, 1998).

There are also some concerns with the patenting process itself being too broad; the Oncomouse patent is a prime example of this (Stallman, 2002). Although university collaborations with research companies are beneficial to both sides in terms of creating new inventions, there is an unclear boundary for balancing academic access to information and the created strains versus the company's rights to patent their technology. This disagreement is currently happening with Oncomouse, as DuPont has become very aggressive in enforcing licensing and collecting royalties from academics for its role in the invention (Marshall, 2002; Blaug et al., 2004). Some argue that an extreme industry position of broad patents could severely inhibit disease research in smaller research facilities that may not be able to pay the licensing fee. In the end, society would lose out.

# Chapter-4 References

Andrews, Edmund (1993) U.S. Resumes Granting Patents on Genetically Altered Animals. *The New York Times*. 03 Feb. http://query.nytimes.com/gst/fullpage.html?res=9F0CE3DD1139F930A35751C0A965958260&sec=&spon=&pagewanted=all

Blaug S, Chien C, Shuster M (2004) "Managing Innovation: University-industry Partnerships and the Licensing of the Harvard Mouse." *Nature Biotechnology*, Vol. **22**: 761-763. June 2004.

Check, Erika (2002) Canada Stops Harvard's Oncomouse in its Tracks. *Nature* **420**: 593.

Curran G, and Koszarycz Y (2004) Animal Transgenesis and Cloning: Scientific, Religious, and Ethical Considerations. http://dlibrary.acu.edu.au/research/theology/ejournal/aejt_3/Curran_Koszarycz.html

*Diamond vs Chakrabarty* (1980) 447 US 303-322, 1980. http://digital-law-online.info/cases/206PQ193.htm

FDA to Regulate the Use of Transgenic Animals (2009) *Nature* **457**: 371. http://www.nature.com/news/2009/090116/full/news.2009.36.html

FDA.gov (2009) "FDA Approves Orphan Drug ATryn to Treat Rare Clotting Disorder". http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm109074.htm

GTC Biotherapeutics (2006) "About GTC" http://www.gtc-bio.com/about.html

Jolicoeur, Paul (1994) "Transgenic Mouse Carrying a Non-infectious HIV Genome." US Patent and Trademark Office. Patent #5,574,206.

Leder P and Stewart T (1984) "Transgenic Non-Human Mammals, The Harvard Oncomouse." US Patent and Trademark Office. Patent #4,736,866. Cambridge, MA.

Leder P and Stewart T (1992) "Method for Providing a Cell Culture From a Transgenic Non-Human Mammal." US Patent and Trademark Office. Patent #5,087,571.

Leder P and Stewart T (1999) "Testing Method Using Transgenic Mice Expressing an Oncogene." US Patent and Trademark Office. Patent #5,925,803.

Lira S and Yang T (2000) "Transgenic Mouse Model for Kaposi's Sarcoma." US Patent and Trademark Office. Patent #6,610,905.

Marshall, Eliot (2002) Dupont Ups Ante on Use of Harvard's Oncomouse. *Science* **296**: 1212-1213.

Mitchell A and Somerville J (2002) The Mouse that Made the Lawyers Roar. Life: Patent Pending. Canadian Council of Churches. http://www.ccc-cce.ca/english/biotech/index.htm

PETA.org (2009) People for the Ethical Treatment of Animals. http://www.peta.org

Stallman, Richard (2002) Are U.S. Patents Too Broad? *Science* **297**: 336.

Stern D and Yan S (2000) "Transgenic mice over-expressing amyloid-beta alcohol dehydrogenase (ABAD) in brain as model of Alzheimer's disease and uses thereof." US Patent and Trademark Office. Patent #6,891,081.

United States Patent and Trademark Office (2005) General Information Concerning Patents. http://www.uspto.gov/web/offices/pac/doc/general/index.html#whatpat

Walter, Carrie F (1998) Beyond the Harvard Mouse: Current Patent Practice and the Necessity of Clear Guidelines in Biotechnology Patent Law.   http://www.law.indiana.edu/ilj/v73/no3/walter.html

# PROJECT CONCLUSIONS

This IQP research allows for conclusions to be made by the authors. The overall conclusion is that animal suffering should be tightly regulated and balanced with reasonable societal needs. Certain transgenic animal types have been agreed upon by the authors as acceptable (see below); however, the authors disagree on others. Strict regulation by Institutional Animal Care and Use Committees (IACUC) and by the FDA should be continued to maintain the strong oversight required with this technology. It is also agreed that while transgenic animal patenting is beneficial, changes need to be made to the patenting system to allow for the greatest innovations.

Transpharmers are considered by the authors to be the best type of transgenic animal due to the great medical benefits to society, with no apparent health problems for the animal. Although there is some worry about xenotransplantation, with the right precautions for euthenasia, this area can be also very beneficial for saving lives with no unusual harm to the animal, except for the harvesting of the organs. The authors also believe that, as long as strong regulation is placed upon the transgenic creation of food sources to eliminate animal suffering, this will also be a good use of transgenic technology. For scientific models, for now the benefits appear to far outweigh the detriments, but there is some concern about future research on these models, particularly with creating transgenic primates, as primates are very closely related to humans. The last type of transgenic animals, disease models, is where the authors of this IQP begin to disagree. Although the authors agree that all the models discussed in this report (Alzheimer's Mouse, Parkinson's Fly, AIDS Mouse, and Oncomouse) have great societal benefit, there was not a consensus among the authors that this benefit outweighs the suffering of

two of the models (AIDS Mouse and Oncomouse).  Due to the great suffering that can be inflicted while studying the diseases, it seems like researchers should look for a better way to conduct the research if at all possible.  And IACUC committees should ensure that pain killers and early euthenasia are used whenever possible.

The authors agree that the patenting of transgenic animals has great potential, and provides a means of rewarding the companies that take the risks. However, both authors agree that the current patenting system is not quite developed enough for biotechnology at this point. Changes need to be made in the system before patents will fully unlock the potential of this growing field. However, the recently approved standards for FDA evaluation of genetically engineered animals are on the right track; one transpharmer drug (ATryn) has already been approved, and many more drugs will come.  It is agreed that once patenting changes have been made, the benefits of transgenic animal patenting will far outweigh the risks.