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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

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ABSTRACT

Transgenic animals are animals that have a new non-native sequence inserted in their DNA in order for the animal to mimic some human trait. Two main methods are used to create a transgenic animal, and because these methods have existed since 1980s, many animals on this planet have a transgenic twin. In this IQP are discussed the technologies for creating a transgenic animal, their various applications in medicine and industry, and their positive and negative impact on society, shown through their ethical and legal concerns.

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EXECUTIVE SUMMARY

In the late 1970s, scientists first created chimeric mice, a great development in the field of biotechnology. By inserting a foreign DNA into the murine genome, the first transgenic animal was introduced to the world. The development of the transgenic animals had a broad use in the fields of medicine, biotechnology, and industry. They have been used as disease models, for testing chemical substances in the chemical industry, for producing human pharmaceuticals in farm animals, and for making organs for transplantation, etc. The main points discussed in this IQP are the methods used for creating transgenic animals (i.e the technology), transgenic animal classification, and the effects of the technology on society with transgenic ethics and legalities.

When talking about the different methods used, the oldest and most used are the microinjection of DNA into the male pronucleus of a zygote, injection of DNA into embryonic stem cells, and homologous recombination. The pronuclear microinjection is used most commonly and is the oldest. After these methods, follow less used technologies including the use of viruses and gene guns to deliver DNA. The newer techniques are more efficient and increase the possibility that the transgene will be incorporated into the cell's genome.

Transgenic animals have very broad uses and therefore they are classified in several groups. There are transgenic disease models, transpharmers, xenotransplanters, transgenic food sources, and transgenic biological models. Disease models are transgenic

animals that mimic specific aspects of human diseases, and are used in medical research. Such models include the Alzheimer's Mouse (that develop senile plagues), Oncomouse (which contains human oncogenes in its genome and therefore develops tumors), and AIDS rat (that develops partial HIV cellular infections). Transpharmers, also known as biopharmers, are animals engineered to secrete human pharmaceuticals into the milk. Examples are the Tufts-Genzyme Goats (that produce clot dissolvers) and Baby Annie (a cloned cow). Xeno-transplanters are animals that have been genetically modified for transplanting organs into humans. Transgenic food sources are transgenic animals or plants that have a transgene in their DNA that allows them to produce more meat with less food intake, or to be more disease resistant. Examples are the superpig, superfish, and the antifreeze protein that is inserted in salmon fish. Biological models are used for learning the biological function of some protein found in the transgenic animal, such as Doogie smart mouse that has an enhanced learning and memory gene.

Ethics have always been an issue whenever science has been controversial. In this IQP, transgenic ethics were discussed from both their pro and con sides. We agree with strong oversight to alleviate animal suffering with painkillers whenever experiments necessitate advancement to a painful state. Ethical issues were discussed concerning Alzhimers mouse (which does not appear to suffer), and Oncomouse (which definitely can suffer at advanced stages of tumor formation). Religious aspects were also discussed, including the Buddhist stance that all cows are sacred, and the stance that experimentation needs to save human lives. We conclude that most transgenic experiments have been proven to be more useful than deadly.

Transgenic legalities were also discussed, especially the Oncomouse case in which it was first determined that animals can be patented. Oncomouse was filed for patent in 1984 and went trough many trials of appeal because of the animals' rights laws and animal protection groups. It was first granted in the United States in 1988, in Europe in 1992, in Japan 1994; however, it still has not been granted in Canada, where they argue animals can not be patented. Another important transgenic legal case involved Beagle dogs used for fighting fungal lung infections. The dogs were exposed to radiation and other painful research methods, so that research was banned in 2004.

Transgenic animals have opened the doors to whole new research methods and approaches in medical research, and have improved the economical state of the biotech industry. The IQP authors agree that the benefits of most transgenic animals outweigh negative factors, so long as animal suffering is minimized.

PROJECT OBJECTIVE

The Transgenic Animal IQP Project is designed to research and explore the scientific and ethical observations of this highly controversial new technnology, and do demonstrate the impact of that technology on society. The objective is to provide the reader with concrete knowledge about the many facets involved. In Chapter 1 the reader will discover how transgenic animals are made through detailed pictures and explanations of various procedures that are practiced. After an understanding of how these animals are made, the authors use Chapter 2 to present the different transgenic animals created, and to discuss what benefits they each. Although transgenic animals have shown great potential for saving human lives, not all things come with out a cost however; in Chapter 3 the ethical topics surrounding transgenic animals are described to the reader. The final stage in providing the reader with a clear understanding of what is going on in the transgenic community are discussed in chapter 4, the laws and patents which pertain to transgenic animals.

CHAPTER 1: TRANSGENIC ANIMAL TECHNOLOGY

Background on DNA

DNA is the reason cells act the way they do, produce the proteins they do, and are differentiated as they are. DNA is a polymer found in the nuclei of almost all cells in the body (Figure-1).

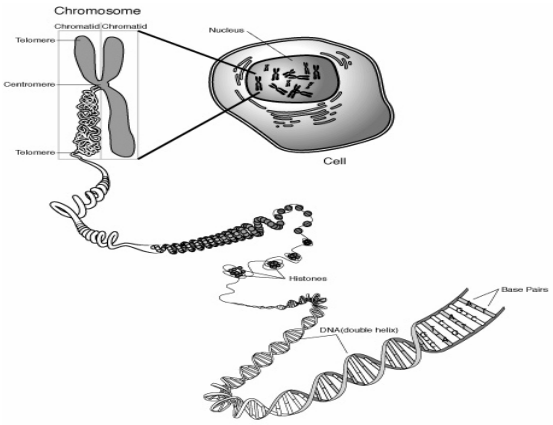


Figure-1: Diagram of DNA in the Chromosomes of Nuclei. A typical cell is shown in the right of the diagram. The nucleus is the large spherical object inside the cell. The enlargement on the left side of the diagram shows an enlargement of a chromosome containing the DNA polymer unraveled from one of its ends. (Mount Sinai Hospital, 2006)

The polymer is made from the assembly of many monomer units (nucleotides) of a five-carbon sugar (deoxyribose) a nitrogen containing base, and a phosphate group (Figure-2).

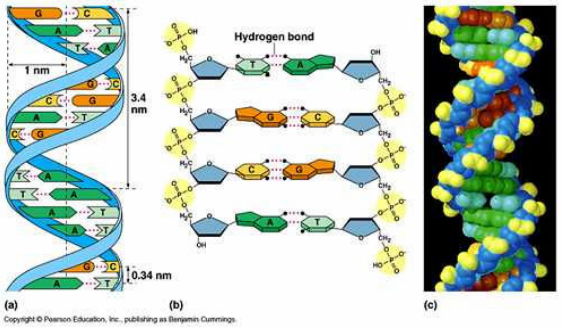


Figure-2: The Structure of DNA. The four kinds of nucleotides are represented by blue, green, orange, and yellow colors in the center of the diagram (DNA Structure, 2006).

The nucleotides can be found in four different structures depending on the base that they contain. Therefore there are two purine bases and two pyrimidine bases. The purines are

adenine and guanine, and the pyrimidines are thymine and cytosine; adenine pairs with thymine and guanine pairs with cytosine. Because of this type of pairing, the secondary structure of DNA is a double helix (Figure-2, left side), and when this double helix is packed with a specific type of proteins called histones, the product is a chromosome.

The chromosome is located in the nucleus of the cell, and never leaves it (although the nuclear membrane disipates during cell division). Genes represent specific locations on the DNA with specific sequences of nucleotides. These genes are the hereditary factors. When they are transcribed or expressed they make different proteins. If mutational changes happen to the sequence, the results can vary from malfunctioning proteins to deadly diseases.

For one individual, the DNA sequence is same in every cell. The reason cells have different functions is because the genes that lie on the DNA strand are expressed differently. The parts that are transcribed are called exons, and the parts that are not transcribed are called introns.

Transgenes

Animals that contain externally introduced foreign genes in their DNA are called transgenic animals. The new genes in their DNA are introduced into a newly fertilized egg by using a vector that can usually be a virus or a plasmid. In order for the transgene to be expressed into a protein, the transgene has to be transcribed into mRNA, and then the mRNA is translated by ribosomes into the protein. Transcription is a complicated process with three parts, initiation, elongation and termination. To start the initiation process, a promoter sequence (Figure-3, blue box) has to be added to the transgene. The

promoter sequence dictates in which tissue the transgene will be expressed. If a casein promoter is used on the transgene, the protein will only be made in the milk. Transcription termination is induced by inserting a poly(A) termination sequence downstream from the transgene (Figure-3, yellow box).

Figure-3: Diagram of a Transgene. The blue region denotes the promoter sequence used to control in which tissue the protein gets produced. The transgene is represented by the red box. The yellow box denotes the poly(A) termination sequence that stipulates the end of the RNA to be made from the transgen.(Genetically Modified Animals, 2006)

Creating a Transgenic Animal

The recombinant DNA, the DNA from two or more sources (i.e. human transgene and bacterial plasmid) combined into a single molecule, is grown in bacterial or mammalian cells to create many copies, then inserted into a newly fertilized egg or embryonic stem cell using one of several techniques: microinjection of DNA into the pronucleus of a zygote, microinjection into embyronic stem (ES) cells in a blastocyst, homologous recombination, somatic cell nuclear transfer, or retrovial-mediated transfer. Each of these techniques is discussed below.

Microinjection of DNA into the Male Pronucleus of a Zygote

The most commonly used technique for inserting a transgene is microinjection of linear DNA sequences into the nucleus of *in vitro* fertilized oocytes. This was first successfully applied on a mouse in 1980 (Gordon et al., 1980) and has been frequently used from then on. The reason for inserting the DNA into the nucleus before the embryo

has fully developed is because the animal has to be born with the new information in every cell. Thus microinjections are typically performed even before the first cell division, while the nuclei are still pronuclei. The male pronucleus is used most frequently for microinjection since it is slightly larger (Figure-4).

Figure-4: Diagram of Microinjection of DNA into a Male Pronucleus. A suction pipette (right side of figure) is used to hold the newly fertilized egg in place while the needle (right side) is inserted into the male pronucleus (UNMC, 2002)

After the pronuclei have merged together, the embryo is usually cultured to the blastocyst stage to increase its vigor, then implanted into the uterus of the foster mother, and left there to fully develop into a healthy offspring. The newborn animal will hopefully have the foreign genes into its genome and will produce the transgene product. Figure-5 shows the microinjection procedure being used to create a sheep that transpharms a protein in its milk.

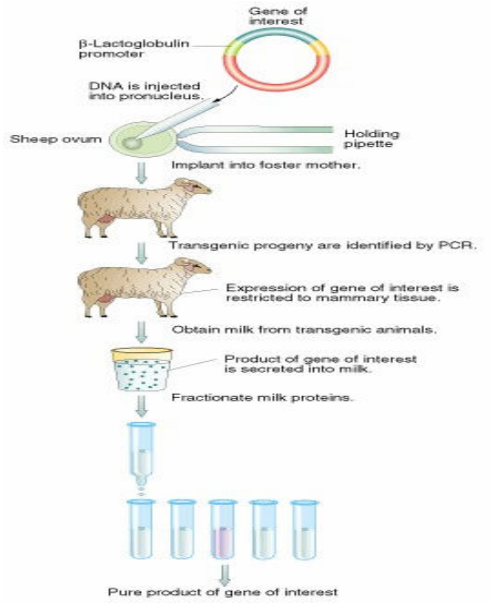


Figure-5: Diagram of Nuclear Injection to Produce a Transpharming Sheep. The transgene is represented by the blue portion of the vector (top of diagram). The vector and transgene are injected into the fertilized egg, and the milk of the offspring screened for production of the protein (BioTeach, 2006)

Injection of Embryonic Stem Cells into Blastocysts

A second method used to introduce a transgene into an animal is microinjection into cultured embryonic stem (ES) cells. This method is similar to microinjection into fertilized eggs, except *in vitro* fertilized embryos are first cultured for 5 days to the blastocyst stage in which ES cells are present (Figures-6 and 7). An ES cell line is created, then they are microinjected with the transgene DNA. The injected ES cells are implanted into another blastocyst, which is then implanted into the uterus, as before. Because some of the embryo's ES cells are un-injected, sometimes the transgene is not incorporated into all cells of the animal, creating a chimeric animal. This method is different from the other methods for creating a transgenic animal because it allows testing for insertion of the transgene prior to injecting the ES cells, so this technique can be more efficient.

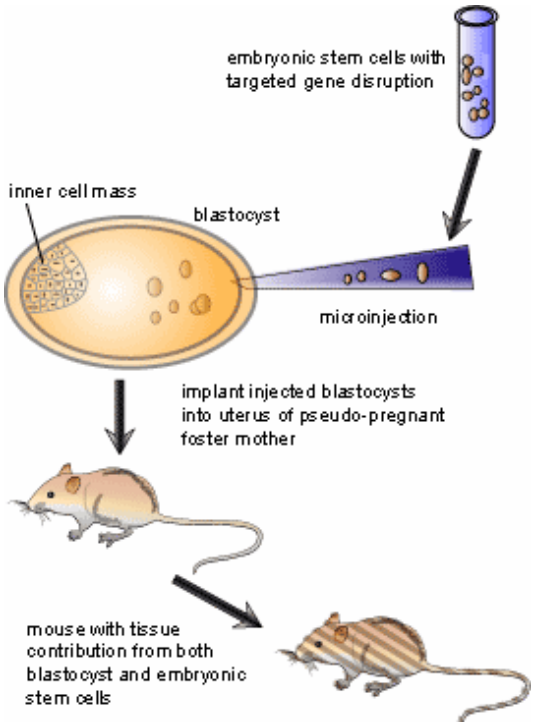


Figure-6: Microinjection into Cultured ES Cells for Making a Transgenic Animal. Cultured ES cells (upper right) are injected with transgene, or targeted for homologous recombination, then injected into a blastocyst containing other ES cells. The blastocyst is implanted into the uterus (SCQ, 2004).

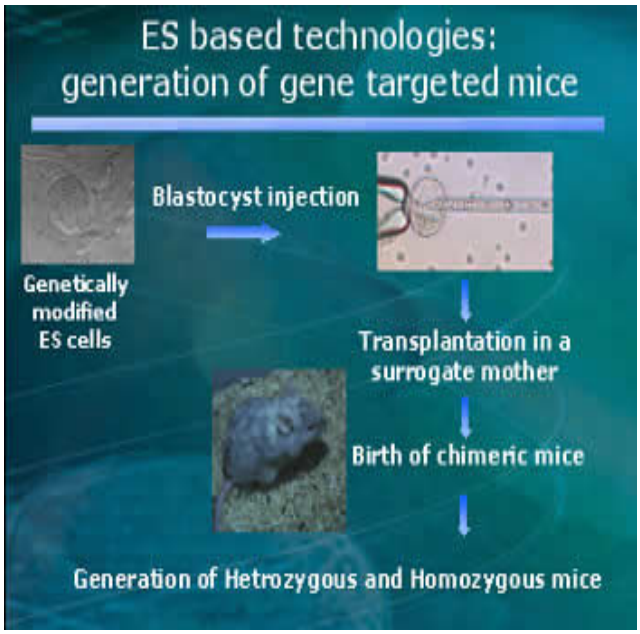


Figure-7: Diagram of ES Cell Manipulation to Make Chimeric Mice. Genetically modified ES cells (left side) are injected into a blastocyst (upper right), which is translanted into a surrogate mother. (Genoway…2003).

Homologous Recombination

Homologus recombination, also referred to as gene-targeted knockout, is a technique where a transgene is introduced to a targeted location on the native DNA. The targeted gene is usually prevented from expression (is knocked out) (Figure-8).

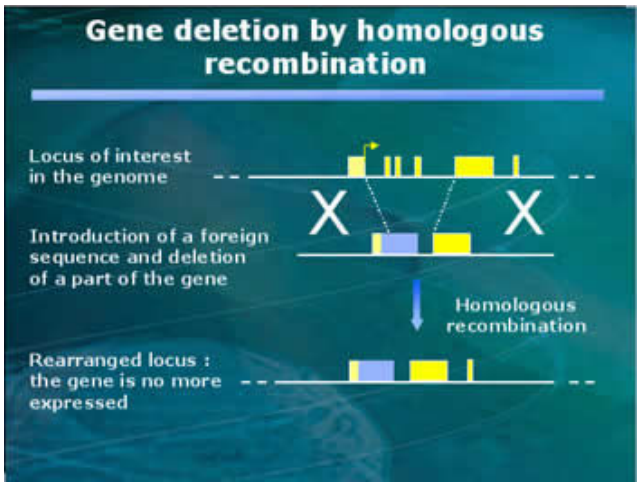


Figure-8: Diagram of Gene Deletion by Homologous Recombination. The upper line represents the normal gene to be deleted. The middle line represents the "stuffer" DNA (blue) used to knock out the normal gene. It contains flanking sequences identical to those flanking the normal gene. The flanking sequences recombine (shown as "X" in the diagram), replacing the normal gene with the stuffer DNA (lower line) (Genoway…2003).

When a transgene is used instead of the stuffer DNA shown in Figure-8, a transgenic animal is produced in which the transgene replaces a specific targeted gene (Figure-9). The transgene can be incorporated by electroporation- a process using electrical current to create small pores on the membrane of the cell allowing DNA entrance. This technique is also known as the knock in gene because it introduces a new gene (the transgene) into the host DNA. After the new gene is introduced it will be able to use the promoter sequence to begin production of the wanted protein or gene product. If the transgene is from a human DNA and is inserted into an animal, the process is known a humanization of the animal hence the animal is being humanized.

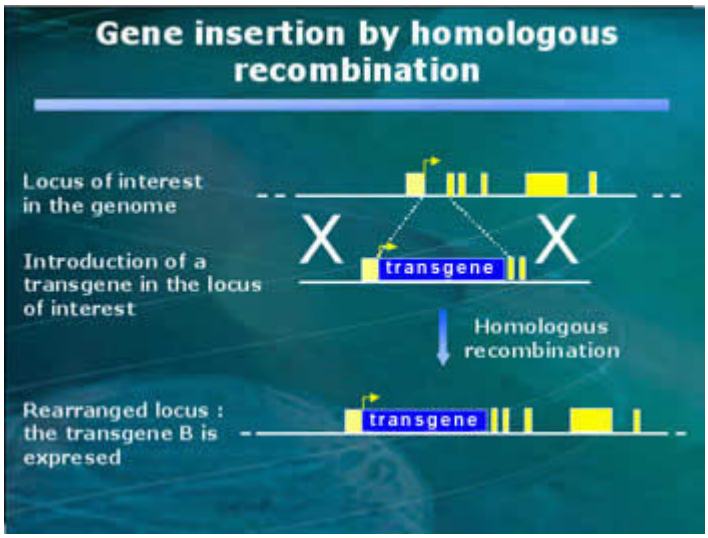


Figure-9: Diagram of Transgene Insertion by Homologous Recombination. The upper line represents the normal gene to be deleted. The middle line represents the transgene (blue) used to knock out the normal gene. It contains flanking sequences identical to those flanking the normal gene. The flanking sequences recombine (shown as "X" in the diagram), replacing the normal gene with the transgene (lower line) (Genoway… 2003).

The advantage of this homologous recombination system is that only a single copy of the transgene is incorporated into the new cells, so it is easy to control copy number. Also, the site of integration is highly controlled, which can be important since we want no oncogene to become activated in the animal. Unfortunately, the system is

very time-consuming, in that the flanking DNA sequence must be known in order for the transgene to be constructed.

Retrovirus Mediated Gene Transfer Method

A retrovirus is a virus that carries its genetic material in the form of RNA rather than DNA. Such viruses can be used to efficiently incorporate transgenes since they normally integrate DNA copies of their RNA genomes into the host DNA. A helper cell line is frequently used to help package infectious virions (Figure-10).

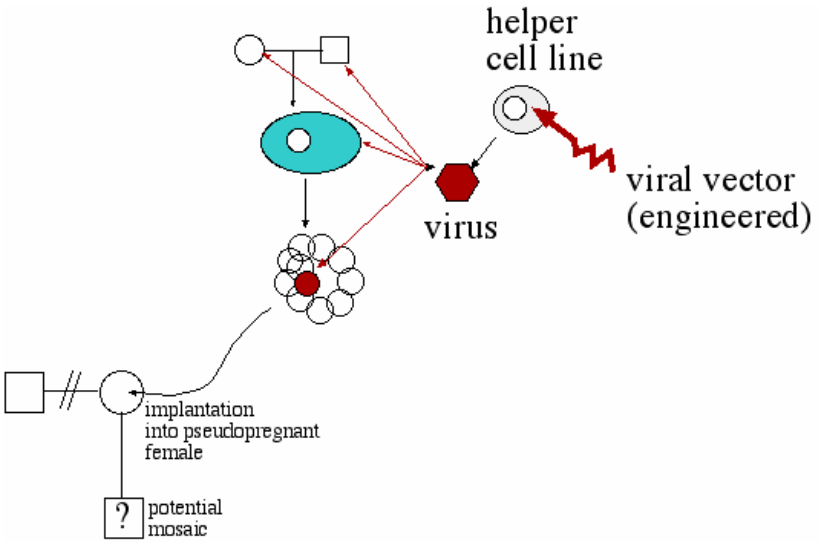


Figure-10: The Use of a Helper Cell Line to Package Infectious Retroviruses for Inserting Transgenes. A genetically engineered transgene is inserted into a helper cell line (upper right) that expresses viral RNA that is packaged into infectious virions (red hexagon). The virions are used to infect a target cell (center of diagram) which is then implanted into a pseudopregnant female (lower left) (Transgenic Animals, 2006).

The genetically engineered virion is used to infect a target cell line, then the viral RNA genome is reverse transcribed to DNA which integrates into the host DNA carrying the transgene with it (Figure-11).

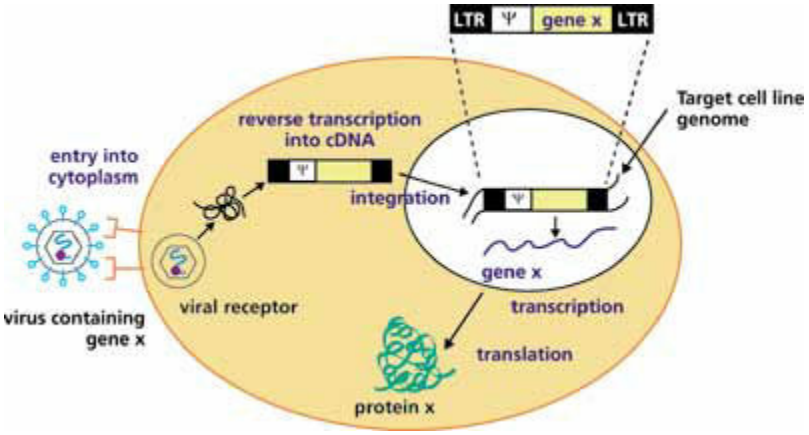


Figure-11: Integration and Expression of the Transgene Using Retroviruses. A genetically engineered virus containing the transgene (left side) is used to infect a host cell. The viralRNA is reverse transcribed into DNA (center of disgram), which integrates into host DNA in the nucleus (right side of diagram). The integrated viral DNA expresses the transgene (lower part of diagram) (Clonetech, 2006).

This method of using retroviruses to introduce transgenes was first used on mice in 1974 when simian virus SV-40 sequences were inserted into mouse embryos (Jaenisch and Mintz, 1974), and the viral DNA was subsequently found in the mice genome. Because RNA viruses go through a lot of mutations, there is a great concern that the transgene viruses may get in contact with the naturally present viruses in the cell and harm the organism; however, few have been observed so far. Future experiments will likely use viruses like AAV-5 that target their genomes to harmless sequences in the host.

Nuclear Transfer Method

The microinjection and embryonic cell transfer methods are the two most frequently used methods for creating transgenic animals, but they have some

disadvantages, including the loss of a large number of embryos. A safer and more efficient way of producing transgenic animals is the nuclear transfer method, sometimes referred to as somatic cell nuclear transfer (SCNT). The nuclear transfer technique uses cell cloning to produce transgenic animals. A nucleus is extracted from a somatic cell, usually a skin cell, injected with transgene, then the nucleus is reimplanted into an enucleated egg. The egg develops into a blastocyst which is implanted into a surrogate mother. Since the newly implanted nucleus has the transegene in its genome, offspring will be 100% transgenic animals.

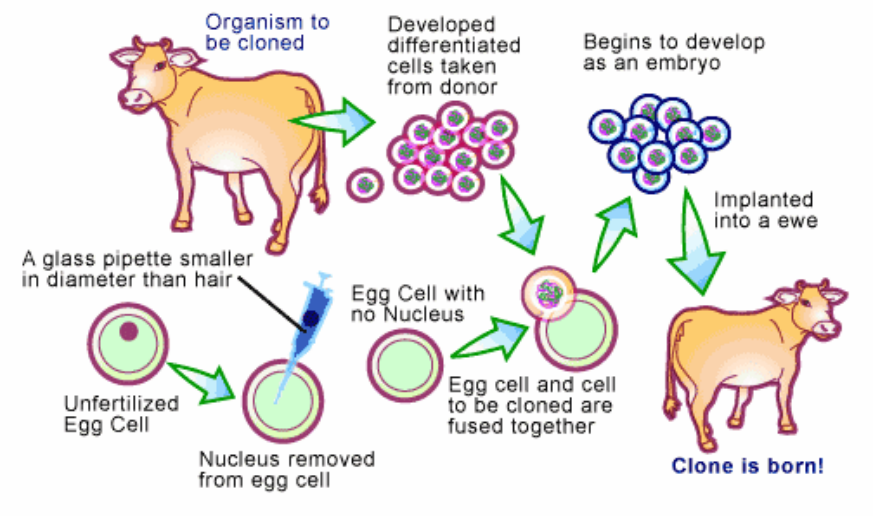


Figure-12: Creation of Transgenic Animals Using Somatic Cell Nuclear Transfer. Skin cells are removed from a donor animal (upper left). A nucleus is extracted (center of diagram) and injected with transgene. The nucleus in injected into an enucleated egg (lower left) that is implanted into a surrogate mother (BioTeach, 2006).

CHAPTER 2: TRANSGENIC ANIMAL CLASSIFICATION AND EXAMPLES

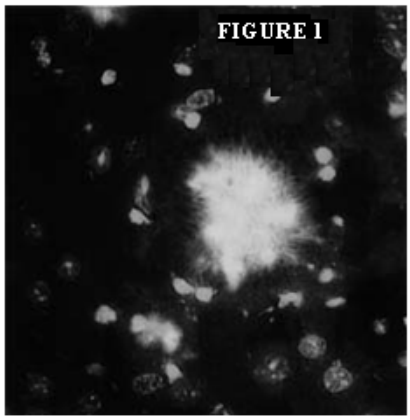
In this chapter the main classes of transgenic animals that have been created will be identified and described. Five main classes of transgenic animals will be explored, including those used to study disease, to create better food sources, and others used strictly to create medications for human consumption. Through the manipulation of the genetic structure of these transgenic animals, scientists have successfully been able to utilize advanced techniques for the betterment of mankind.

Transgenic Disease Models

In order to further understand the causes of a disease and to help develop cures scientists have created transgenic animal disease models. These models contain genes that are introduced into these animals to allow them to mimic certain aspects of a human disease. A transplanted gene must be introduced into the host animal to create a platform for the disease. This creates the same symptoms in the host animal that a human would exhibit, allowing scientists to study the characteristics of medications and cures. By testing on the animals, it can be determined if the medicines would create unwanted side effects, as well as making sure they are effective. If a medicine passes the tests on the transgenic animals they are then ready for human clinical tests, where it is determined if the medication can be released to the general public.

Alzheimer's Mouse

This transgenic animal was created, in part here at WPI (Games et al., 1995), to study why memory and cognitive thought are affected by the disease. The cause of Alzheimer's disease (AD) is thought to be the accumulation and development of senile plaques and intracellular neurofibrillary tangles (knots). These plaques (figure-1) and tangles are focused in the area of the brain associated with memory and cognitive thought, the cerebral cortex (Games et al, 1995). These buildups and deposits make it difficult for the brain to send information and process it effectively, causing the effects commonly associated with Alzheimer's patients. Although the average age of onset for Alzheimer's disease is 70, some families get it in their 40's so have an early onset version. These individuals have mutations that accelerate the processing of amyloid precursor protein to toxic β-amyloid, leading to neurodegeneration.



A large amyloid deposit (lighter color) in the frontal cortex of the novel Alzheimer's mouse. (Photo courtesy of Bruce T. Lamb, Ph.D.)

Professor David Adams from WPI, along with his collaborators at the former Transgenic Sciences Inc, used genetic engineering to mimic an early onset Alzheimer's mutation in mice, so they developed senile plaques after about 6-8 months of age (Games et al., 1995). The animal's brains showed signs of neurodegeneration associated with

plaque formation, so the animal became the first Alzheimer's disease model, but the model did not express neurofibrillary tangles. This model was sold to Elan Pharmaceuticals who have used it for vaccine development to block plaque formation. Although an early vaccine trial in humans was terminated due to brain inflammation in a small subset of the patients (Young, 2002), subsequent Elan vaccine trials have so far been successful, and show a good use of transgenic disease models.

More recently, Frank LaFerla, Associate Professor in the department of neurobiology and behavior at the University of California Irvine, successfully created subjects that expressed both the tangles and senile plaque deposits. This has allowed researchers to test vaccines and antibodies which will remove both the deposits and the tangles. LaFerla and his group were able to perform tests to try to eliminate both the proteins and the tangles by injecting antibodies used to target amyloid beta protein. The injections were only introduced to one side of the brain as a control. What was observed is that the antibodies for the amyloid beta protein eliminated the plaque within 3 days, and the tangles were not present after 7 days (Kingman, 2004).

A test where an antibody for the tau protein was injected produced no beneficial results. The development of the mice exhibiting both the plaques and tangles is a breakthrough in its own, however this does not mean that a successful cure can be made.

Oncomouse

The Oncomouse is a disease model created by Leder and Stewart in 1982 at Harvard University. This groundbreaking mouse is hailed not only as a great model for studying cancer, it is also the first transgenic animal to be patented (Leder and Stewart,

1984). This mouse (Figure-2) was genetically engineered to contain an activated oncogene sequence in its germ cells and somatic cells. An oncogene is a DNA sequence that when inserted into the animal's genome increases the chances the subject will develop malignant tumors (neoplasms).

In order to ensure the gene is present in all of the animal's cells, the oncogene sequence is inserted at an early embryonic stage, traditionally before the 8 cell stage (Leder and Stewart, 1984). Introducing the sequence at such an early stage ensures that all cells in the animal will contain the oncogene, and researchers will have sufficient subjects to test anti-cancer treatments and agents. If tumor formation was low, it would be more difficult to test potential antitumor agents.

FIGURE 2: A Photo of Oncomouse (Leder and Stewart, 1984).

The transgenes for oncomouse are the V-Ha-Ras and the c-myc gene, which are activated by the mouse mammary tumor virus (MMTV) promoter. The cancerous growths form when the V-Ha-Ras gene is mutated, which is crucial to create the

abnormal cell growth (Sinn, 1987). To accelerate this processes Ledger and Stewart observed that when the V-Ha-Ras and the c-myc mouse lines are crossed the resulting oncomouse exhibits accelerated neoplasm growth.

Previously to cause cancerous growth in mice it was necessary to put large amounts of carcinogens in their living spaces. This method was very dangerous to the researchers because the amount of material required to create effects on the mice was potentially sufficient to induce cancer in the researchers themselves. With the onset of the crossed highly reactive / fast acting cells, very small amounts of cancer causing elements could be used. This makes it easier to perform more conclusive and safe studies. As a result researchers are learning more about the causes of cancer, and its potential cures (Marshall, 2002).

AIDS Rat

The human immunodeficiency virus (HIV) was the discovered in 1983 to be the cause of the greatest epidemic to plague the world, AIDS (Acquired Immune Deficiency Syndrome) (Baylor Scientists, 2001). HIV is known to attack cells that have human CD4 genes, namely T-cells and B cells. The virus envelops itself inside these cells and begins to replicate (Bunce and Hunt, 2004). AIDS disease is known to cause skin lesions, problems in lymph node tissues, malignancies, and cellular and immune irregularities (Kohn, 2001).

Currently there is no cure, and the only treatment slows the effects of AIDS. Although monkeys get simian immunodeficiency virus (SIV) infections that mimic AIDS, primates are an extremely expensive model to study in the lab, so scientists have

created a mouse line to carry HIV. However, it is difficult to study their small organs, and mice provide only 2 to 3 millimeters of blood for testing. Joseph L. Bryant, head of the Animal Model Division of the University of Maryland's Institute of Human Virology, with his team have successfully created a rat which they successfully infected with HIV-1. This rat HIV model is preferred over a mouse model due to its larger size which eases organ studies, and because the larger animal produces 30 to 40 millimeters of blood (Kohn, 2001). The rats were genetically engineered to contain the human CD4 gene, which allows HIV to enter T-cells and do its job. The rats successfully developed symptoms typical to humans such at weight loss, cataracts, and atrophy of skeletal muscle, neurological abnormalities, and respiratory difficulties (Reid et al., 2001). Also severe skin lesions, kidney disease and cardiac disorders were present, which eventually lead to early death.

Having the ability to learn how HIV causes problems and disrupts normal organ activity it is possible to start thinking of ways to stop the progression of the retrovirus. The research which has been completed on transgenic mice has also proved very useful in identifying direct causes of many symptoms associated with AIDS, and host proteins engaged by the virus. Without having the ability to examine and research HIV replication in these small animal models, progress towards a cure would be slower.

Transpharmers

A transpharmer is another type of transgenic animal used to produce pharmaceuticals. In a field know known as "biofarming" scientists engineer the DNA of a specified animal to secrete pharmaceutical drugs naturally. This is done by

manipulating the promoters of genes of abundant proteins in milk, urine, saliva, or blood to produce a drug of interest in one of those fluids. The most common approach due to ethical and health issues is to extract the desired pharmaceutical compound from milk which is abundant, healthy, ethically sound and high in protein. No animal sacrifice is needed to obtain the product, nor does the product affect the health of the animal. It is important to have a high protein level to facilitate extraction of the product from other milk proteins.

The preferred animals used in milk biofarming are cows, goats, sheep and rabbits (Ziomek, 1998). The animal is chosen with consideration of the time it takes to milk, volume of milk and amount therapeutic proteins required. Figure 3 (below) shows different values associated with production times and amounts of product per year.

Figure 3:
Animal selection options for transgenic milk expression

Animal	Gestation (Months)	Maturation (Months)	Milk Yield per Lactation (Liters)	Elapsed Months from Microinjection to Milk
Mouse	0.75	1	0.0015	3-6
Rabbit	1	5-6	1-1.5	7-8
Pig	4	7-8	200-400	15-16
Sheep	5	6-8	200-400	16-18
Goat	5	6-8	600-800	16-18
Cow	9	15	ca. 8000	30-33

(Ziomek, 1998)

Tufts-Genzyme Goats

News of a great example of a transpharmer creation came from the Tufts-Genzyme study which successfully produced two transgenic goats. A professor at Tufts University School of Veterinary Medicine, Karl M Ebert, collaborated with Biotech

company Genzyme in order to create two goats, one male and one female, which carried human genes to the enzyme known as tissue plasminogen activator (tPA) (Ezzel, 1991). tPA is known to prevent heart attacks by dissolving blood clots associated with myocardial infraction, a major cause of heart attacks.

To create the Tufts-Genzyme goats, Ebert had to surgically remove eggs from a host female goat, then he microinjected DNA containing the gene for human tissue plasminogen activator (tPA) a clot dissolver drug. The success rate for this experiment was not high, 200 fertilized eggs were inserted into 36 surrogate mothers, and 29 offspring were born, however only two of the goats carried the transgene. The female goat produced 3 to 4 grams of milk daily, containing 3 micrograms of tPA in one milliliter of milk (Ezzel, 1991), this yield is sufficient to be of commercial interest since it is greater than 1 μg/ml. After refining the technique, Ebert's group produced a female goat which can produce a thousand-fold higher yield of 3 mg/ml.

Baby Annie

Not all transpharmed animals are engineered to produce pharmaceuticals; baby Annie (Figure 4) is the first cloned cow which will be used to improve an agricultural problem. Mastitis disease causes a loss in profits for farmers up to $1.7 billion dollars yearly, due to an inflammation of the mammary glands (Suszkiw, 2001). Approximately 30 percent of reported mastitis cases are caused by bacteria known as Staphylococcus aureus, these bacteria target and destroy milk-releasing cells in mammary glands. Currently the only method to cure cows infected with the bacteria is to inject antibiotics, which is only successful in 15 percent of recipient cows. If mastitis could be successfully

erased through transgenic means, the general public would benefit through the reduction of antibiotic and antibody uptake in the milk, and the farmers would make larger incomes.

Developed by University of Vermont researchers and members of Geneticist Kevin Wells team from the U.S. Department of Agriculture's (USDA) Research Service, baby Annie produces lysostaphin which is a protein that prevents mastitis disease in cows. As an embryo Annie's zygote was injected with lysostaphin genes, a protein tag, and a sheep promoter gene called beta lactoglobulin which will direct the expression of the lysostaphin into the milk (Suskiw, 2001).

Figure: 4

Annie, born March 3, 2000, is a clone of a pure-bred Jersey calf whose cells were modified with genes for producing **lysostaphin** a protein that kills *Staphylococcus aureus* bacteria, a leading cause of mastitis disease in dairy cows. She is the first transgenic cow clone engineered to resist mastitis, which costs the U.S. dairy industry $1.7 billion annually.

(Suskiw, 2001)

Xenotransplanters

One of the largest causes of preventable deaths across the world is a lack of transplantable organs for those in need. The American Heart Association in 1997 reported that only 2,300 out of 40,000 Americans who needed a new heart received one

(Mooney, 1999). The lack of transplant organs is also present for all other vital organs, including kidneys and livers.

Responding to this growing lack of transplant organs, scientists are trying to perfect a procedure known as xenotransplantation. A xenotransplanter is an animal which has been genetically altered to produce organs that will be transplantable to humans. Until recently only primate organs could be successfully transplanted into human beings. This is because other animal's cells contain sugars or proteins on their cell surfaces that when introduced into the human body triggers a response that sends the body into hyperacute rejection, also the immune system will kill the organ by destroying the cells (Kaiser, 2002).

The expenses of using primates, and the low number of primates available, have caused researchers to turn their focus towards using pigs as organ donors. The benefit of using pigs is the ability to create an organ of adequate size, creating large numbers of organ hosts, and the presence of similarities in physiology. The drawback however is that the endothelial cells which line the vessels of the donor organ contain enzymes that release sugars which the human body recognizes as foreign causing rejection as explained above. The enzyme has been identified as alpha-1,3-galactosyl transferase and is the trigger for release of the sugar alpha-1,3-galactosyl, which is responsible for causing the organs to be rejected by primate and human recipients (Butler , 2002). Based on this in 2002, a team directed by researchers from the University of Missouri, Colombia created four cloned piglets that did not produce the rejection causing enzyme in its cells. However the animals contain two separate copies of the gene, and after analysis of the

cell structure it was discovered the pigs only lacked one of the copies of the malicious gene (Kaiser, 2002). However, there is great cause for enthusiasm in this field due to the potential to harvest transplantable human organs from a pig host. These animals could be farmed for their meat, and instead of discarding their internal organs they could be used to save human lives.

Transgenic Food Sources

A transgenic food source is a genetically modified plant or animal containing a foreign gene which is intended to provide enhanced nutritional characteristics. There are many commercially sold transgenic fruits and vegetables in the global market today. These fruits and vegetables are designed to provide important nutrients, grow larger, and have the ability to grow in non-native climates. Transgenic animals however are not found in commercial markets, due to ethical issues and uncertainties regarding safety to health, yet they still are produced and studied (Harper, 2006).

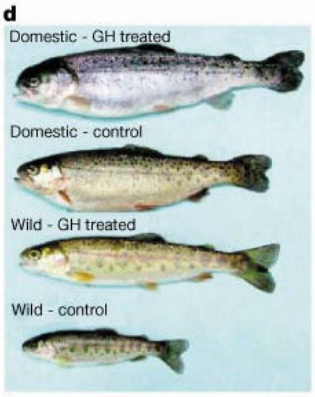
Superpig

The development of food sources derived from pigs and lambs has not produced a viable model that can be used for commercial sale and human consumption. The goal is to produce transgenic animals which take less time to mature, consume less food, and grow to larger sizes. To accomplish this scientists microinject the gene for human growth hormone into newly fertilized eggs which causes the transgenic animal to develop quicker, grow larger, have lower body fat and consumer less food (Rexroad and Caird, 1994).

Unfortunately there are also a number of negative side affects which make this practice not feasible for commercial sale and human consumption for mammals. Superpig exhibited painful health problems caused by the genetic manipulation; the pig was plagued with arthritis, gastric ulcers and stomach lesions, a general lack of coordination, and severe muscle weakness (D'Silva 1998). Following the failure with the Beltsville pig, biologists placed a voluntary moritorium on performing any kind of growth hormone transgenesis with farm animals.

Superfish

During the struggles scientists had producing "enhanced" food sources from mammals, salmon were found to react well to enhanced growth techniques. Salmon were given an additional growth hormone gene. These transgenic salmon can grow at a rate six times faster than native farmed salmon, see figure 5. Additionally these animals can convert their food to fishmeat at a 20% faster pace, allowing increased production for a smaller cost (Stokstad, 2002).



d, Phenotype of growth-hormone-treated domestic and wild strains of rainbow trout (Devlin RH, 2001)

Figure 5: Comparative Sizes of Normal and Transgenic Trout.

Antifreeze Protein

A startling new discovery was made one February morning in 1975 when ,Choy Hew, Ph.D, at the time Assistant Professor in the Biochemistry Department at the Memorial University of Newfoundland, discovered his research laboratory's cod tank full of frozen to death cod. To his surprise, the winter flounder in the tank were not frozen. After insisting on learning how they managed to survive, he discovered they contained a protein later named antifreeze protein, which kept these fish alive in the extremely cold temperatures (Hew et al., 1995). This protein is normally not activated until the fish encounters extremely cold temperatures at which point the protein is activated by the antifreeze gene and the tissues resist freezing. Transgenic scientists have isolated this gene and discovered that if it is inserted into salmon in an activated state it causes growth hormone to be released at accelerated rates (Hew et al., 1995). The transgenic salmon have provided many models, that when perfected can be used to produce unlimited supplies of transgenic food which will reduce over-fishing and provide more food.

Transgenic Biological Models

Biological models are transgenic animals that teach us something new about the biological function of a specific protein.

Doogie the Smart Mouse

Memory is one example. Joe Tsien, Neurobiologist at Princeton University, along with members from MIT and Washington University has created a strain of mice, named Doogie, that possess enhanced memory and learning activity. These mice were observed to maneuver mazes better, learn from sounds and objects, and to retain knowledge better than non-transgenic mice. Certain NR2B receptors in the brain that function in memory, prominent in juvenile mice, were over-expressed into adulthood in the Doogie mice (Tang et al., 1999), which is important because juvenile brain characteristics are understood to be able to absorb new information in large amounts (Harmon, 1999).

The Tsien lab (Tang et al., 1999) proved that a gene called NR2B is responsible for controlling learning and memory by creating mice which did not contain that gene in a small part of their brain. The result was mice that expressed learning and memory difficulties. By implanting either additional NR2B genes, or genes with strong promoters in the doogie mouse line, the mice showed increased brain activity resulting in quicker learning and more effective memory capacity. Tsien is now using this technology to discover chemical causes of memory loss, learning deficits, and disorders involving brain activity (Harmon, 1999). It has been proven that a corresponding gene exists in human brains creating an interest for drug makers who might want to use the NR2B gene to help people with learning deficiencies or neural disorders involving learning and memory.

CHAPTER 3: TRANSGENIC ETHICS

Since the creation of the world's first transgenic animal in 1980 (although it did not express the transgene) (Gordon et al., 1980), transgenic engineering has been a focal point for animal rights activists along with religious activists. It has been shown that transgenic animals are crucial for performing tests and gaining understanding of human disease. The controversy arises however when one takes into consideration the quality of life these creatures experience. Some group's feel that man should not interfere with natural selection, while others are afraid changing the genetics of animals can eventually cause abnormalities in humans who consume transgenic products. However more substantial worries include those of controlling animal suffering and fears that genetic animals will escape and alter the natural gene pool in native ecosystems.

The technological barrier that once limited what transgenic scientists can achieve has been broken, resulting in scientists having the capability to manipulate life with no bounds. However this is not the case, ethical considerations are now used to determine whether a desired transgenic animal can be created. This chapter will focus on discussing general pros and cons of making different types of transgenic animals. This will be achieved by discussing the benefits of food sources, advancements in medicine, and our increased understanding in human health. Arguments will be raised that transgenic engineering is wrong due to animal suffering, environmental concerns, and religious issues involving manipulation of life. Specific examples will be discussed in order to evaluate severity of animal suffering ranging from no unhealthy side effects, to early death. In retrospect, examples will also be given of the great health benefits and medical

breakthroughs transgenic models have offered. There is a potential to save hundreds of thousands of human lives across the world, however one must ask "at what point do the negatives outweigh the positives?"

Transgenic Positives

Transgenic animals offer a large number of positive benefits which can be divided into the three extensive categories: medicinal, scientific, and food enhancement. Medicinal models include disease models, transpharmers and xenoplasters (xenotransplanters). Disease models such as the Alzheimer's mouse, Oncomouse and the AIDS Rat have provided a platform to aid in understanding the effects of diseases at a genetic level. From these studies it has been possible to identify the genetic reactions caused by the disease, and researchers are now creating cures and preventative medications (Baylor, 2001). There is a strong need for creating disease models because it would be highly unethical to run such experiments on humans.

Transpharmers produce pharmaceuticals in their genetically altered mammary glands. Pharmaceutical drugs are extracted from the milk, without animal sacrifice. Utilizing this method a larger amount of pharmaceutical drugs can be obtained than was possible in culture dishes. This type of transgenic animal shows no apparent effects of producing the foreign protein in the milk, so shows no apparent animal suffering.

Animals have also been modified to produce proteins that make them resistant to disease; this is common in farm applications where sick animals cause a loss of income (Harper, 2003). This type of transgenic application is an extension of animal cross

breeding practices that have been used for thousands of years to produce stronger livestock.

Another type of transgenic animal is one that is used to harvest human-compatible organs; these are called xenoplasters or xenotransplanters. To make animal organs implantable in the human body proteins must be removed that activate sugars in the transplanted organ which are seen as foreign. If these sugars are not knocked out the human body will reject the new organ, killing it (Kaiser, 2002). There is a great deficiency for healthy organs in the United States to be used for transplantation. In 2005, approximately 6,500 people died in the United States waiting for an organ transplant, this number represents 18 people dying each day. With upwards of 92,000 Americans on the organ transplant waiting list the demand is overwhelming (Edwards, 2006). People waiting for kidneys, livers, pancreases, intestine, hearts and lungs could have their suffering and time waiting relieved instantaneously if there were sufficient organs available, with the onset of successful transgenic techniques, transpharmer sources will become a reality not a dream.

Scientific models are those in which scientists add or remove specific genes to the animal's genetic code. This enables the observer to discover the function of otherwise unknown proteins. For example, the function of protein NR2B was discovered by removing its gene from the DNA of a mouse and observing reduced learning and memory skills. When that same gene (with a strong promoter to increase its expression) was added to a mouse genome, the mouse showed signs of advanced learning and increased memory capacity (Hew, 1995). This has led researchers to try and apply this gene into medications to help humans with learning disabilities and memory deficiencies.

Food enhancement models include transgenic animals such as superfish and agriculture animals. Superfish have been created through two processes, one involving the insertion of growth hormone genes, and another inserted a special "antifreeze" gene which stimulates the host fish to create its own growth enhancing proteins (Hew, 1995). Superfish develop quicker, grow larger, have lower body fat and consumer less food (Rexroad, 1994). Agricultural animals are altered in the same manner to promote faster maturation, lower food consumption and increased muscle mass. As noted above, farm animals can also be inserted with genes to promote disease resistance, offering longer production life spans and added income due to minimization of dead loss (Harper, 2003). Keep in mind however; all these miracles of science are not without their mistakes and unexpected outcomes.

Transgenic Negatives

Science is a field constantly trying new unprecedented experiments to discover that which is not known. As a result they must learn via trial and error. It would be naïve to think all the great of examples of transgenic success culminated without any mistakes. When mistakes are made, or unexpected results are acquired during transgenic animal experiments, animals are often deformed and diseased to a point many find unethical.

Transgenic models such as AIDS rat, and Oncomouse, are intentionally induced with diseases that can cause internal and external physical deformities, which can lead to early death and significantly reduces animal welfare (Society, 2006). Animals injected with growth hormone are made to grow bigger and quicker to produce more meat, but

they also develop severe arthritis, respiratory problems, severe stress and again early death.

Aside from physical concerns there are also environmental concerns, scientists prefer to have cloned specimens which lack diversity because that would ensure consistent results to their experiments. Success for scientists in creating a genetically consistent gene pool could cause disaster because the animal would be more susceptible to diseases and other deformations (Donnelley, 1993). If transgenic animals were to escape into the wild there is large concern the ecological balance would be affected. The worry is that the hybrid animals would replace native species. This could happen if the transgenic animal flourished in the new environment and the native species could be wiped our by contracting foreign disease and the release of unknown pathogens (Donnelley, 1993).

The less accepted arguments (to this IQP author) are those of religious and environmental activists who feel man has no right to manipulate the animal genome (Jarvis et al., 2006). They feel that man has no right interfering with million year old biological checks and balances which allowed animals to evolve naturally, not through the limited knowledge and wisdom of mankind. Other radical advocates fear the technology used on animals will be used on humans one day (Schroten, 1997). Regardless of all the concern, there is still a huge human need for the services of transgenic research, consider this quote from Donnelley,

> "Other ethical theoretical frameworks have been suggested — e.g. the "ethics of Intervention", which recognizes the fact that simply by pursuing our own existence, humankind must intervene in nature, including in animal lives, but insists that we must still regard ourselves as part of nature" (Donnelley, 1993).

Alzheimer's Mouse Ethics

Alzheimer's disease plagues more than 4.5 million Americans annually, and costs nearly $100 billion, while at the same time being the fourth leading cause of death in developed nations, followed by heart disease, cancer, and stroke (Alzheimer's Association, 2004). These stark facts support efforts that lead to the creation of Alzheimer's disease models in mice. The research performed by Professor Adams and his colleagues at the former Transgenic Sciences Inc., to create a mouse model for this disease (Games et al., 1995) that over-expresses a neurotoxic β-amyloid protein that leads to the formation of senile plaques and neurofibrillary tangles associated with loss of memory and cognitive function (Kingman, 2004).

This animal does not suffer from any recordable type of suffering or added stresses; it simply learns slower on a maze test. This is a classic example of a transgenic animal which offers large therapeutic potential, with few negative effects on the animal. Using this disease model, Elan Corporation developed an experimental vaccine, AN-1792, which removed the plaques in the test animals (Schenk et al., 1999). Removal of the plaques also restored cognitive function in the vaccinated animals. When the vaccine was tested in human Alzheimer's patients, in January of 2002, initial results suggested that AN-1792 was safe. However, this was not the case a couple of weeks after the second injection where four of the 97 test patients developed inflammation of the central nervous system causing the testing to be supended.

Later in January of 2002 another 11 patients fell ill, forcing Ivan Lieberburg, Elan's chief scientific officer to halt the testing (Young, 2002). He commented by saying "our decision to first suspend dosing, and now permanently discontinue dosing, remains

in the best interest of the health and safety of patients." Since that first human trial, a second generation vaccine has been administered to Alzheimer's patients with no adverse side effects. He continued to state that they will continue to try and advance the development of a vaccine through other approaches (Young, 2002).

Oncomouse Ethics

Another transgenic disease model is Oncomouse, which has lead to breakthroughs in understanding how cancer affects human tissues and organs. By having a convenient experimental model to closely observe, scientists are able to identify how these diseases are activated, and develop test methods for cancer prevention.
Without this model it would be nearly impossible to gain such understanding without performing unethical experiments on humans.

Traditionally, organizations like the Science, Religion and Technology Project feel that "there is a need for a culture of restraint on the use of model mice to avoid complaint that the mice involved have been reduced to little more than material commodities (Society, 2006)." However in the case of Oncomouse it is generally agreed upon that the seriousness of human cancer is so great that the animal suffering is justified. This example shows how groups are willing to accept the negative impacts due to the severity of cancer in humans. To make sure that scientists don't overstep their bounds, the use of transgenic mice has been strongly regulated. In 1992, David Porter devised a way that animal suffering could be assessed on a measurable basis. He suggested creating a method of assigning scores from 1 (tolerable) to 5 (not-tolerable) which would rate several categories, such as pain, mental stress, immobility, length of experimentation,

etc. Once the evaluation was completed, researchers can then devise a plan to minimize the categories that are rated high. Such methods involve administering pain killers, reducing testing times, and putting the animal to sleep before the worst stages of the disease model set in (Porter, 1992).

Super Pig Ethics

What started as a potential means of producing increased amounts of meat for the American market turned into a large debate and a clear example of how transgenic experiments do not always proceed as hoped. Beltsville Agricultural Research Center located in Beltsville MD, Genetically engineered a pig which produced human growth hormone. This pig was designed to grow at an accelerated rate and produce meat with less fat (Pursel et al., 1997). The pig grew faster and produced leaner meat, yet severe problems arose that overshadowed the successes. Super pig contracted arthritis, developed stomach lesions and gastric ulcers, and exhibited muscle weakness and a lack of coordination (Rollin, 1996). Again in this case the scientists observed that the negative effects to the animal outweighed any benefit it could provide, scientists soon after suspended experiments on mammals involving human growth hormone.

Transpharmer Ethics

Another important example of a transgenic positive are transpharmers, a wide array of pharmaceutical drugs are produced using this technique. By altering genes in the mammary glands of milk producing animals, pharmaceuticals are produced naturally and in large quantities (Ziomek, 1998). This method has also been used to create disease

resistance in animals, Baby "Annie" was genetically altered to produce lysostaphin which prevents mastitis, a disease which destroys mammary glands in turn lowering milk production and causing economic loss for farmers (Suszkiw, 2001).

Herman the bull (the world's first transgenic cow) was given a human gene for lactoferrin, to make cows produce milk more similar to that of humans. Although Herman could not transpharm the lactoferrin himself, his semen was used to inseminate female cows that would be used to birth babies which produce lactoferrin milk. But after studying the affects of the baby cows it was discovered that elevated levels of abnormalities existed. The baby cows expressed increased mortality rates, higher recorded birth weights resulting in the need for caesarean section, and increased stresses to the mother cow (Van Reenan and Blokhuis, 1997).

Scientists need to learn from these mistakes and use the evidence they've gathered to prevent further occurrences of animal suffering. Still the argument will arise concerning the potential to prevent abnormalities that are unforeseeable. There is broad support for the field of transpharming due to overwhelming ability to produce animals that show little to no physical or mental harm while producing lifesaving pharmaceuticals.

Religious activists feel that as long as the pharmaceuticals are being used for therapeutic use, and the animal's integrity is being upheld, then the technology is justified. To support this consider that we have used the production capabilities of the cows mammary glands for hundreds of years to produce milk and cheese products, why shouldn't we adapt the gland for other beneficial causes (Society, 2006). For some it is

felt that transpharming is more ethically correct than killing an animal merely to eat its meat.

Xenotransplanter Ethics

The development of Xenoplasters resulted from the fact that in the United States 13 people die each day while waiting for an organ transplant. Non-primate animal organs cannot be inserted into a human due to a protein in their cells which creates a sugar that triggers the human body to kill all the cells exhibiting the sugar. If scientists could find a way to make it possible to implant animal organs not expressing this foreign sugar, many lives could be saved, but currently the health risks are too high (Carnell, 2000).

The main concern held by activist groups is not about rejection of the organ, they are worried that Xenoplanter organs could pass non-human diseases to patients. They legitimize their concerns by expressing the fact that humans have picked up diseases from domesticated animals and animals used as food sources, the most common being the influenza virus which can infect humans, pigs and birds. Take bird flu as an example, this form of influenza is very virulent and difficult for humans to combat, the scary thing is we don't know what kind of diseases could be created until we actually try to implant an organ into a human (Carnell, 2000).

The Public Heath Service issued guidelines in 2000 which require Xenotranplanters to "Procure source animals from herds or colonies that are screened and qualified as free of specific pathogenic infectious agents, and that are maintained in an environment that reduces exposure to vectors of infectious agents (U.S. Public Health Service, 2001)." Even if Xenotransplanters are created in clean labs, animal rights

activists will still argue that the animals are not kept in their native environment, not condoning the practice of keeping them in "cages".

Super Fish Ethics

The world's fish consumption is rising, and fish supplies are decreasing due to over fishing. Some feel that farming transgenic fish is the solution. Aqua Bounty Farms Inc. of Waltham, Massachusetts has produced Atlantic salmon that are close to getting FDA approval. These salmon are given a growth hormone gene which allows them to produce growth hormones on a year round basis instead of only during spring and summer months. These fish grow to maturity six times faster than unaltered salmon in similar hatcheries; they do not grow any larger. However they would be available for sale a year earlier than conventionally raised fish. Aqua Bounty is currently seeking FDA approval to begin testing the fish for human consumption (Stokstad, 2002).

The potentially positive benefits to society that could be achieved are overshadowed by an overwhelming concern that if these fish were to escape their hatcheries environmental instability could ensue. In December of 2000 a nor'easter hit a fish farm on Machias Bay in Maine, releasing 100,000 transgenic fish into the water (Stokstad, 2002). These transgenic salmon are larger, eat more and tend to act more aggressive than native fish. A largescale escape posses the possibility of the superfish pushing native species from their current habitats. Figure 6 shows a typical salmon farm, from observing the structure it should be easy to see that if a large storm moved into the waters it could draw fish out, or the wind could destroy the perimeter enclosures.

Figure: 6

Yearning for freedom. Fish raised in pens have escaped to join their wild kin.

CREDIT: BRITISH COLUMBIA SALMON FARMERS ASSOCIATION

Researchers are trying to create sterile transgenic salmon to eliminate the fear of ecological disaster caused by superfish running rampant in the sea. The numbers are hard to ignore, boasting increased growth rates and 20% higher efficiency to convert fish food to fishmeat, farmers are seeing increased profits in the future. If these fish are harnessed safely, overpopulated countries could produce ample amounts of food, and decrease the damage being done by over-fishing the seas.

Trangenic Ethics in Religion

The topic of transgenic ethics reaches further than just the concerns of people regarding the ecosystem, and the welfare of humans and animals. There are also spiritual

and religious beliefs that must be acknowledged, most of our ethical and moral fibers have been stitched from our religious beliefs.

Hinduism and Buddhism are two of world's largest religions that are largely spiritual and symbolic. Due to beliefs regarding reincarnation of the human soul, followers treat all creatures as divine beings. They feel animals are essential to aid the soul to its next stage in life. The cow is the most sacred animal in the Hindu faith; their teachings tell them that the sacred cow of the gods, Kaamdhenu, will grant all of their wishes and desires. Considered the mother of all cows Hindu tradition focus's on protecting the cow and living in harmony with it, not using it for its meat (Curran, 2004). This harmony is reached because in return for their care, the cows offer villagers dairy products for food, transportation for people and materials, the cows also offer their dung which is used for household heating and cooking fuel. The cattle require little to no special care, they eat wild grass, and to maintain their diet, villagers survive on a dairy, grain and vegetable diet (Sagner, 2003).

The Islamic religious codes are derived from the Qur'an and Hadith. Islamic people operate under the conception that "whatever god has not forbidden is allowed (as a mark of his generosity), though always within the boundaries of "what God wills" (Curran, 2004)." God's will was transformed into the schools of shari'a (law), which states what things are not legal which are prohibited. Therefore animals are available for human use as long as their sacrifice is for the benefit of mankind. In ancient Islamic civilizations animal sacrifice was practiced regularly to appease the God's, hence benefiting mankind.

The Christian and Jewish religions have been established to look negatively on any cruelty and manipulation of animals. They feel the soul of a human is to be revered as holy and sacred, the soul animals are not seen on the same level as the human soul. The book of Genesis (1:1-2:4) proclaims God bestowed human dominion over animals. Christian scripture explains this dominion should be expressed "with wisdom and love" (Curran, 2004). The overall perspective should be aimed at loving animals within creation, and to provide an ethic of care for all creatures, avoiding cruelty and experimentation.

Chapter Conclusions

This chapter described many negative impacts caused by creating transgenic animals, however their strong medical benefits could save tens of thousands of human lives. Although the public's stance on transgenic animal research has been slowly improving, serious concerns still stand. Many of the predominating religious ideals teach us that as long as man uses the technology for the benefit of human health, and it does not cause significant suffering for the animals, it should proceed. Scientists have traditionally used pain medicine to improve animal welfare and we agree such medication should be used here where warranted. We also agree that the animals should be humanly put down in those experiments that demand some pain formation, such as oncomouse.

Actions like these have helped to create positive support for the science, and provided an opportunity to learn from previous mistakes, and further advance our

understating and capability to produce life saving models. The author feels that the main concern of researchers and scientists is to maintain the integrity of these animals, and ensure transgenic animals will not become an industry that is exploited only for money, its main intention has to remain saving human lives.

CHAPTER 4 - TRANSGENIC LEGALITIES

Because of the ethical issues surrounding the creation of transgenic animals, laws are currently in place regulating their creation, patenting, and use. The debate on whether life can be patented is complex and ongoing, with trials in the U.S., Europe, and Canada leading the debate. As discussed in Chapter-2, oncomouse is a transgenic mouse line engineered to express human oncogenes to provide a model for researching oncogenesis. Oncomouse is the worlds' first patented animal, so its case will be used to introduce various concepts on the patenting of life.

The Oncomouse Case

Oncomouse, also known as the Harvard mouse, has been modified to carry the human *myc* oncogene, which is a gene that makes the mouse develop cancer. It is therefore suitable for cancer research (Figure-1). After its creation in 1984 at Harvard University in Philip Leder's lab (Leder and Stewart, 1984), DuPont and Harvard University signed a memorandum of understanding giving the company exclusive rights to license oncomouse and control its use by researchers (Gene-watch, 2002).

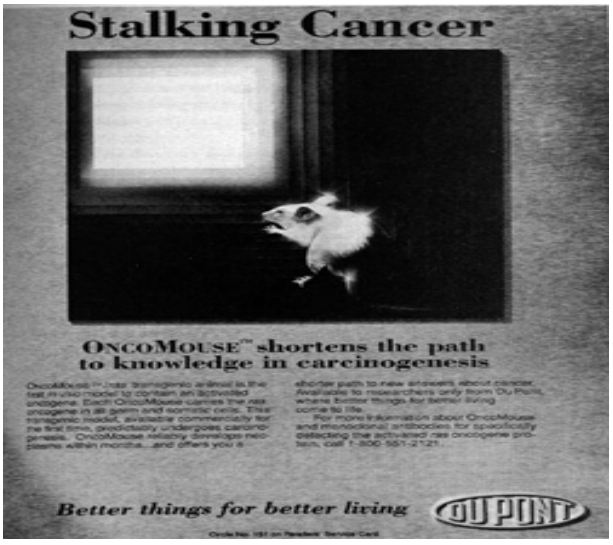


Figure-1: Dupont's Advertisement of Oncomouse. DuPont is the world's first company to profit from the production of a transgenic animal (Vanderbilt, 2001).

When talking about legalities there are two sides of the story. From one point of view oncomouse can be very helpful for curing cancer in people, but on the other side as we discussed in Chapter-3, the animal develops tumors which some consider to be torture. Therefore, with the creation of oncomouse in 1984 and its patent fileing, the world was set for a huge debate on patenting life.

Oncomouse in the United States

In April 1988, the Unites States Patent and Trademark office (USPTO) granted the U.S. patent of oncomouse. The reason why the patent was granted so easily is because the USPTO previously in 1987 approved a rule claiming that non naturally occurring, non human, multi cellular living organisms can be patentable. In 1991, an Animal Legal Defense Fund (ALDF) challenge of the USPTO Rule was dismissed on procedural rather than substantive grounds in a 1991 federal court holding that the ALDF, not being an injured animal or genetic researcher, had no standing to sue (The Endocrine Society,2004).

As a result of the U.S. oncomouse case, three US patents were awarded. US4736866 covered the method for creating transgenic non-human eukaryotic animals whose germ cells and somatic cells contain an activated oncogene sequence introduced into the animal. US5087571 covered the method for creating cell lines derived from transgenic animals. US5925803 covered the methods used to assay transgene expression in transgenic tissues or cell lines (Mitchell and Somerville, 2002). The claims explicitly

excluded transgenic humans, apparently reflecting moral and legal concerns about patents on human beings, and also modification of the human genome (Wikipedia, 2006).

Following the award of the patents, many scientists were concerned that DuPont's licensing of the oncomouse could slow the testing of new therapies since that company would control who receives the animal for research. To overcome this problem, DuPont and the NIH negotiated a deal in 2000 giving non-profit researchers free access to the mouse, with the stipulation that any commercial use must pay for the mice (Smaglik, 2000).

Oncomouse in Europe

European patent application 85304490.7 was filed in June 1985 by "The President and Fellows of Harvard College". It was initially refused in 1989 by an examination of the division of the European Patent Office on the grounds that the European Patent Convention (EPC) excludes patentability of animals per se (European Patent Office, 2002). The explanation for the refusal of the case was explained by saying that it does not agree with Article 53(b), which states: European patents shall not be granted in respect of:

(a) inventions the publication or exploitation of which would be contrary to "ordre public" or morality, provided that the exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation in some or all of the Contracting States;

(b) 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 (European Patent Office, 2002).

However, Harvard University appealed the European decision that was made for the application and it decided that the patent should not be refused, by explaining that the patent is patentable under Article 53(b), because it is refered to as "animal varieties" rather then animals as such. On the 22nd of October 1990, the case was sent to reexamination. In a second decision in October 1991, the examining division granted the "oncomouse" patent as complying with the EPC, commenting that the patent application's purpose - to facilitate cancer research and prevention - was of such importance for humanity as to outweigh any disadvantages such as the suffering of the animals concerned (European Patent Office, 2002). The board of appeal eventually decided that animals such as the oncomouse were not excluded from patentability. The examination division then granted the patent in 1992 (Publication Number EP 0169672) (European Patent Office, 2002). On 13 May 1992 the EPO granted patent EP 0 169 672 to the University of Harvard in respect of its European patent application of 24 June 1985. The European patent was then opposed by several third parties, because it was held to a limited extent. The appeal was made because, the appealers claim, among other things, that the patent does not meet the requirements of novelty and inventive step within the meaning of patent law and are contrary to ordre public and morality (European Patent Office, 2002). After opposition proceedings took place in November 2001, the patent was maintained in amended form. This decision was then appealed. As of May 2004, the proceedings were still pending (Nodeworks, 2006).

Oncomouse in Canada

The Oncomouse case was also filed in Canada in 1985 by the President of Harvard College and his coworkers with the Canadian Intellectual Property Office (CIPO). The application was then refused with an explanation that it did not go along with the Canadian patent act that stipulates that the patent can not be considered for a "manufacture" or "composition of matter". During appeal, The Canadian Court of Appeal considered the oncomose to be a creation of matter. The case eventually made it to the Supreme Court of Canada, who gave eight explanations why the oncomouse patent could not be granted. First, The court rejected the patented animal with the expiation that not everything can be patented and that invension is not just everything useful invented by man. Second, the court argued that the mouse is not merely genetic material to be considered to be a composition of matter, but is a living creature. Third, the patenting of higher life forms raises unique concerns which do not arise in respect to non-living inventions, and which are not addressed by the scheme of the Act (Orpat, 2003). Fourth, the patenting of life forms raises practical, ethical and environmental issues. Fifth, the Court should not intervene absent explicit legislative direction (Orpat, 2003). Sixth, the Act has already distinguished which life forms are patentable (i.e microorganisms) and which are not, so the court will not further argue with that. Seventh, accepting the patentability of higher forms would create further problems such as drawing the line of which higher forms are patentable and which not. And eighth, the fact that Parliament had not legislated patent protection for plants when it passed the Plant Breeders' Rights Act reinforced the Court's view that, if Parliament had wanted to extend patent protection

to plants, it would have done so at the time of enacting the Plant Breeders' Rights Act (Orpat, 2003).

However, on August 3, 2000, the appellate court determined that the oncomouse is indeed a composition of matter, and sent the case back to the CIPO with the direction to grant a patent on the transgenic animal claims. Then it was the government's turn to appeal. In the name of the Commissioner of Patents, the Attorney General of Canada filed an application to seek appeal to the Supreme Court of Canada. On June 14, 2001, the Supreme Court of Canada granted the application for appeal (Case No. 28155) (isb, 2002). On the 5th of December 2002 the Supreme Court rejected the application with a five to four decision. The Supreme Court explained that the oncomouse is not just a "composition of matter" and therefore it doesn't accord with the Canadian Patent Act.

In conclusion, the oncomouse patent was first granted in the United States of America in 1988, then in Europe 1992, in Japan in 1994, but it still has not been granted in Canada.

Other Patented Animals

After the patenting of oncomouse in 1984, over 460 patents have been granted for a large variety of transgenic animals. There are manipulated frogs for testing nerve gas for the needs of the army, cows and pigs that have been genetically modified to produce human insulin for curing diabetes, horses that have been implanted with thymus and liver organs from a human fetus. However, not only have domestic animals been genetically modified, but also pets that people love so much. There are patented dogs and cats, and

even chimpanzees. Unfortunately there are also bad sides to the patenting story, such as the patent on beagles at the University of Texas in Austin (Fig-2), patented to be a model for testing some types of fungal lung infections, and the methods used for doing so were exposing the animal to x-ray radiation.

Figure-2: A picture of a beagle dog that is used in the university labs for studying lung diseases (Stop Animal Patents, 2006).

In February 2004, two non-profit organizations the American Anti-Vivisection Society (AAVS) and the PatentWatch Project of the Center for Technology Assessment (CTA) filed a legal challenge urging the U.S. Patent and Trademark Office (USPTO) to cancel the beagle patent. The patent was re-examined, and on the 27th of May 2004 the patent was cancelled. A conclusion can be drawn that animals are not supposed to be tortured and killed, especially not a higher form of organisms such as the dogs

Groups against Animal Patenting

Many groups oppose animal patenting because of their believes and ideas. The most important groups though are The American Anti-Vivisection Society (AAVS), the Canadian Environmental Law association (CELA) and the People for the Ethical Treatment of Animals (PETA). Some animal-rights organizations, such as People for the

Ethical Treatment of Animals, known as PETA, have accused transgenic researchers of cruelty in experiments. According to these groups the animals should be considered higher forms and therefore they should not be patented. AAVS had a great role in stopping the beagle dogs and the other groups have had some roles in the oncomouse case in Canada and the US even in Europe. These groups have contributed to the animal protection trough protesting (Figure-3) and showered the process of animal patenting; however, the law has made the right move and allowed the reasonable animal patents.

Figure-3: A picture of people protesting for animal rights (All Creatures, 2006)

Chapter Conclusions

The oncomouse case serves as an excellent source of information on patenting animals since all the main debates as to whether life should be patented were discussed in that original case. The fact that the oncomouse patent has not been awarded in Canada to

date serves as a reminder that not all countries accept life as patentable. In the U.S. and Europe, a large variety of transgenic animals have been patented following the legal precedence set by the oncomouse case. Interestingly, the creation of transgenic animals has actually resulted in a shift in the use of laboratory animals -- from the use of higher-order species such as dogs to very useful transgenic lower-order species such as mice -- and has decreased the overall number of animals used in such experimentation, especially in the development of disease models. This is certainly a good turn of events since transgenic technology holds great potential in many fields, including agriculture, medicine, and industry (Action Bioscience, 2003).

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