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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

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ABSTRACT

This project reviewed the emerging technology of transgenic animals and the positive and negative outcomes it has on society. The biology and genetics behind transgenic animals were first examined, including the methods used to create them and the ways in which they have been applied to science and human welfare. The project then went beyond the technology to focus on the ethical and legal issues that surround this controversial technology, presenting the benefits and detriments of using transgenic animals. Transgenic animals can be extremely beneficial to human welfare and society, but their own welfare must also be taken into consideration. Transgenic research should be continued, as long as the advantages to society far outweigh any disadvantages to the animal. Patenting of these animals should also be allowed, as they motivate researchers to continue work in the field. Transgenic animal research has the potential to save millions of human lives.

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PROJECT OBJECTIVES

The objective of this IQP was to research the controversial technology of transgenic animals, and investigate their current and future effects on society. Although the technology is relatively new, there are already numerous methods for engineering transgenic animals, described in Chapter-1. Transgenic animals have been designed for several reasons, many of which are meant to benefit human welfare, and these applications are discussed in Chapter-2. The second part of the project delves into the controversial debate on the ethics and legalities of transgenic animals. Chapter-3 explores the ethical issues, including arguments from both sides of the spectrum, while Chapter-4 reviews legal disputes that question whether transgenic animals should be patented.

CHAPTER-1: TRANSGENIC TECHNOLOGY

Transgenesis is an evolving technology that uses the genomes of two different organisms to create genetic differences that would otherwise be unseen in nature. A transgene encoding a specific desired trait is cloned into a vector (such as a virus or plasmid DNA), and this DNA cassette is inserted into the genome of a host animal in order for the offspring of that animal to express certain desired traits. This technology is used mainly for human benefit; it enables scientists to study human diseases in animal models, to create pharmaceuticals for human use, to create organs for transplantation, to create new sources of food, or to investigate the function of newly discovered proteins. This project will investigate this new technology, describing how it is performed and what animals have been created so far, then we will expand the investigation to discuss whether the animals *should* be created and what laws regulate their use. Chapter-1 will focus on how transgenic animals are created.

A variety of methods have been developed to create transgenic animals, some have had much success and others are being further researched. First, it is helpful to have an understanding of the biology behind the production of a transgene, which stems from an area of genetics known as recombinant DNA technology.

Recombinant DNA

A concrete understanding of recombinant DNA technology is paramount to the study of transgenic animals. DNA – short for deoxyribonucleic acid – is a polymer that makes up a double-helix structure composed of nucleic acid bases and phosphorylated sugars (Cooper, 2007). Four bases are found in DNA, and base paring is essential in the formation of the double

helix. Through hydrogen bonding, the two DNA strands are connected: the base adenine (A) is always paired with thymine (T), and guanine (G) is always paired with cytosine (C). This complementary base pairing provides information regarding the replication of DNA; each strand can act as a template for a new strand (Cooper, 2007). The ability for DNA to self-replicate is essential to the technology of the creation of recombinant DNA.

Recombinant DNA (rDNA) is DNA that has been modified to contain genetic code from two different genomes (Pray, 2008). There are three main attributes in creating rDNA: the host, the vector, and the donor. A vector is a DNA molecule used to transfer a foreign gene into a host cell of an animal that will be expressing that gene. The foreign DNA is also known as donor DNA, as it is coming from another genomic source.

The discoveries of restriction enzymes and DNA ligase provided scientists with a better understanding of DNA and how to manipulate it. Restriction enzymes cleave DNA at specific sequences (Pray, 2008). These enzymes are produced by bacteria to protect themselves against invading viruses, which they do by cutting the viral DNA to deactivate it and prevent it from causing harm (Griffiths, 1999). The discovery of restriction enzymes allowed scientists to cut specific pieces of DNA out of a genome (Pray, 2008). Because restriction enzymes will cut at specific locations, two DNA molecules from different genomes can be cut with the same restriction enzyme, and their fragments will be complementary to one another (Griffiths, 1999). Through hydrogen bonding and base pairing of their matching ends, a new combined DNA molecule can be formed to create a chimera (Griffiths, 1999).

DNA ligase is the second enzyme pertinent to the creation of recombinant DNA, it joins together separate pieces of DNA (Pray, 2008). The cell uses this enzyme to help repair breaks in DNA molecules. In rDNA construction, it acts as a glue to seal the strands of DNA from

different genomes, and works by creating phosphodiester bonds to secure the double helix, as shown in **Figure-1** (Griffiths, 1999).

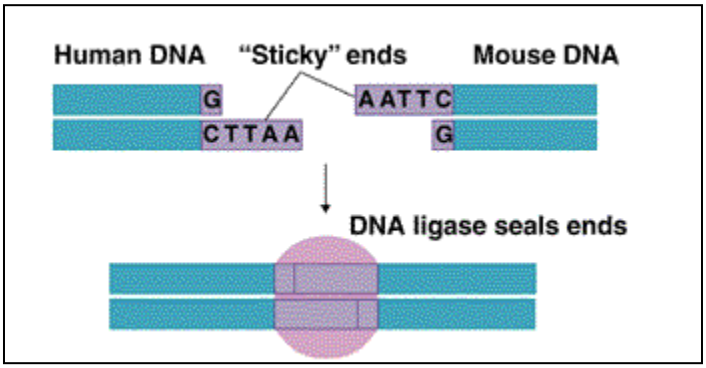

Figure 1: Diagram of DNA Ligation for Sticky Ends. After two strands of DNA are cut with restriction enzymes leaving compatible sticky ends, the two fragments are annealed to each other by base-pairing, then DNA ligase forms phosphodiester bonds between the two strands to create a sealed DNA (Queens.edu).

Without the discovery of DNA ligase and restriction enzymes, recombinant DNA technology would not be possible. Using rDNA technology, the transgene is isolated and inserted into a cloning vector (such as a virus or plasmid) for making copies. A regulatory element of DNA called the promoter is then inserted upstream of the transgene to dictate in which tissue the transgene gets expressed, or to control whether the transgene is on or off (Harper, 1999). In the emerging technology of "transpharming" (discussed in Chapter-2) the promoter is used to prevent transgene expression until desired, and to ensure expression only in the milk of the animal. The final transgene cassette contains the transgene, vector DNA, and regulatory sequences ("Transgenic Animals," 2003).

Transgenesis Methods

The word transgene comes from the term "transferred genetic" material (Charles River, 1991), which describes what occurs in the process of transgenesis. Several methods have been

developed to create transgenic animals. Among the most common methods are the microinjection of a transgene into the pronucleus of a newly fertilized egg, and the injection of transgenic DNA into embryonic stem cells. Other methods use retroviral gene transfer, transposons as vectors, and somatic cell nuclear transfer. Each method for creating a transgenic animal has its benefits and drawbacks, and the technology used in these techniques is continuously advancing.

Microinjection into the Pronucleus

The most common technique for creating transgenic animals is microinjecting genetic material into the pronucleus of a newly fertilized egg. The process involves injecting a transgene (and its cloning vector) into the embryo of a different species. In this technique, animal sperm and egg are united by *in vitro* fertilization (IVF). Before dividing, the newly fertilized zygote contains one male and one female pronucleus. Either the male or female pronucleus may be used for the microinjection, since during the pronucleus stage of development, however because the male pronucleus is larger it is often preferred (Charles River, 1991; Harper, 1999) (**Figure-2**). The injection must be done with extreme care to avoid damaging the zygote. Usually a glass micropipette pulled to a very small diameter is used, a tool that can penetrate the cell membrane without causing harm. Since numerous copies of the transgene are inserted into the nucleus, there is no control as to how many copies will actually incorporate into the genome of the host cell (Harper, 1999). Because the copy number varies so greatly, the extent to which the transgene is expressed will also vary considerably from animal to animal, so screening is sometimes performed to select for animals with the desired level of expression.

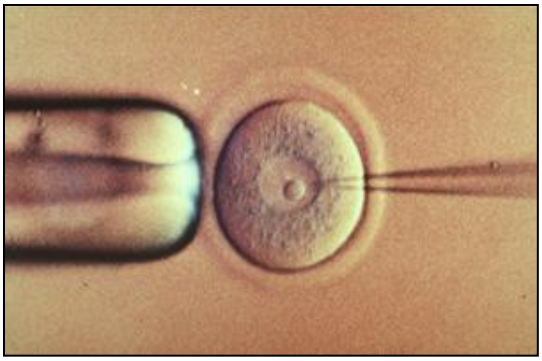

Figure 2: Microinjection of DNA Into the Male Pronucleus. The newly fertilized egg (large circle diagram center) is held in place using a suction pipette (left side), then DNA is microinjected using a pulled glass micropipette (right side). (UCI.edu).

Once the microinjection is complete, the embryo is grown about 5 days to the blastocyst stage to increase its vigor, then it is implanted into the uterus of a psuedopregnant foster mother pre-treated with hormones to mimic pregnancy. The offspring are screened for the transgene, using methods that will be described in detail later in this chapter. Animals testing positive for the transgene are then bred to ensure heritability of the trait and to produce more animals that contain the transgene.

Through this method of pronuclear microinjection, because all cells of the offspring derive from the zygote, if an animal is positive for the transgene it will be present in all of its cells, including the offspring's germ line cells, so it can potentially create transgenic lines. So these are known as germ-line transgenic animals (Harper, 1999). The timing of the injection is extremely important: if the foreign DNA is not integrated into the new genome before the first embryonic cell division, then the new animal may not contain the new genetic code in all of its cells, creating a "mosaic" animal. For mosaics, there will be two genotypically different cell types in the animal, and a transgenic germ line is not necessarily established.

Microinjection into Embryonic Stem Cells

Embryonic stem (ES) cells are cells taken from early embryos which can essentially develop into any other type of cell or tissue (with the exception of the extra-embryonic tissue such as the placenta). Because of this, ES cells are used extensively in gene and cell therapy. ES cells are also used to create transgenic animals. Newly fertilized embryos are created by *in vitro* fertilization (IVF), then grown about 5 days to the blastocyst stage. At this stage, ES cells represent the inner cell mass of the embryo. The ES cells are isolated by a micropipette, and placed in culture under conditions where they grow and remain undifferentiated (Bronson, 1994). The transgene DNA is then inserted into the ES cells by microinjection, viruses, electroporation, or chemicals ("Transgenic Animals," 2003).

Upon exposure to the transgene DNA, some of the ES cells successfully take up the vector, either randomly or through homologous recombination. When the vector randomly inserts itself in the genome, the whole DNA sequence (including cloning vector) becomes integrated into the host genome ("Transgenic Animals," 2003). Random insertion occurs in only a small percentage of cells, so sometimes it is necessary to pre-screen ES cells containing transgenic DNA using antibiotic selection. However, in even fewer cells occurs homologous recombination. Homologous recombination occurs when there are specific pieces of the vector DNA sequence that match up to sequences in the host DNA ("Transgenic Animals," 2003). Because of these two possible outcomes, two types of gene insertion are possible: random and targeted gene insertion, which is shown in **Figure-3**. Random gene insertion, as its name suggests, is completely random, and can in some instances inactivate needed genes in the host cell. The entire transgene DNA and its vector is taken up by the host genome. In targeted gene insertion, a vector is created containing host DNA sequences that will replace their

corresponding sequences in the host cell. This technique requires the host sequences be known surrounding the desired insertion site. This targeted technology is a major advance in science, as it can either restore gene function (knock-in animals) or take out a host gene function (knock-out animals) ("Transgenic Animals," 2003).

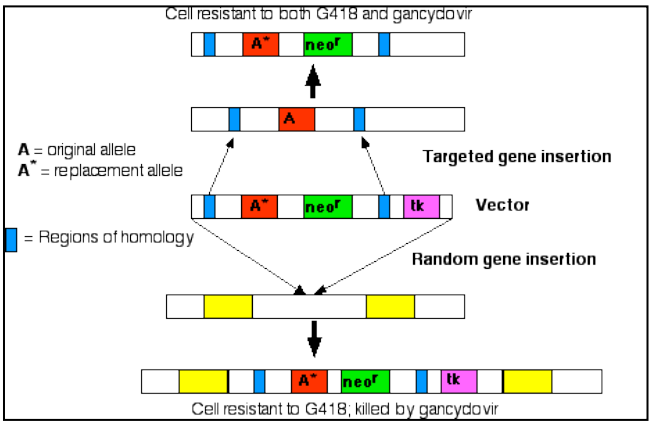

Figure 3: Comparision of Random and Targeted DNA Insertion. In targeted gene insertion (upper half of diagram), regions of homology between the vector and host can be used to remove or replace specific host genetic sequences. Random gene insertion (lower half of diagram) adds the entire genome of the vector to the host genome (RCN.com).

In either type of DNA insertion, once the positive ES cells are screened, they are injected back into the inner cell mass of the blastocyst of the original host animal. Once injected, the embryo is implanted into the uterus of pseudopregnant host ("Transgenic Animals," 2003). As before, pups are screened for transgene insertion, then those containing the transgene are bred to establish a transgenic strain (Bronson, 1994). Because not all of the ES cells of the inner cell mass contain the transgene, mosaic animals will be created using this technique. So mosaic animals are subsequently bred to develop fully transgenic animals. The transgenic offspring will be heterozygous, and with the mating of two heterozygous transgenic animals, there is a 25% chance of a homozygous transgenic animal. The transgenic strain will be established when the homozygotes are mated ("Transgenic Animals," 2003).

Transforming ES cells and injecting foreign DNA into the pronucleus are the two most successful methods for creating transgenic animals. **Figure-4** compares these two technologies. In addition to these commonly used methods, other methods have also shown success, such as the use of somatic cell nuclear transfer and sperm-mediated transgenesis.

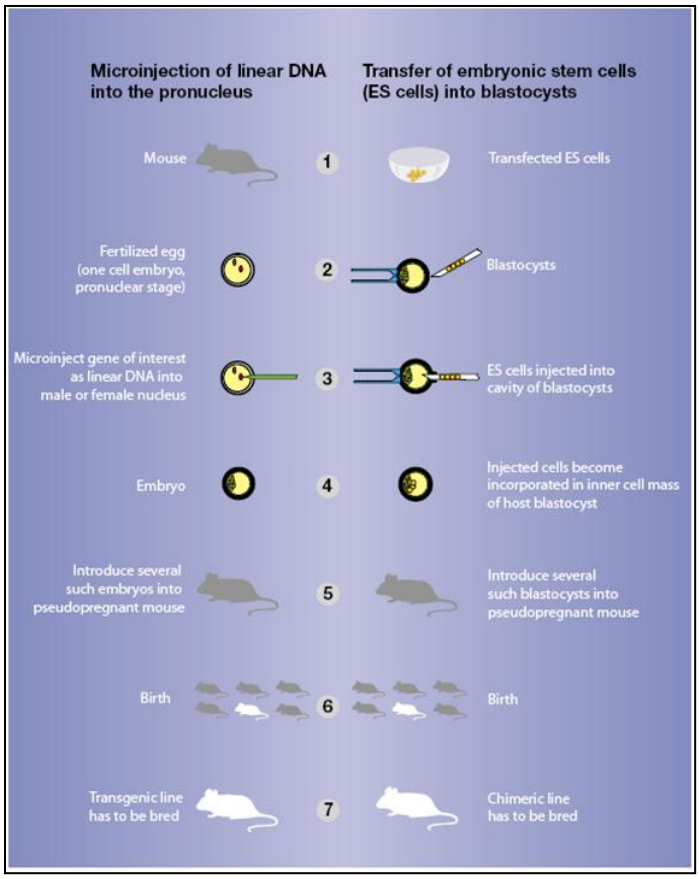

Figure 4: Comparison of the Two Main Methods for Creating Transgenic Animals. The figure compares the microinjection of foreign DNA into the pronucleus (left half of diagram), and injection of transformed embryonic stem cells into a blastocyst (right half of diagram) (biocompare.com).

SCNT

Somatic cell nuclear transfer (SCNT) is an emerging technology in the area of transgenesis. In SCNT, the nucleus of a somatic cell (such as a skin fibroblast cell) and the chromosomes of an egg are each removed. The nucleus is then microinjected into the chromosome-free egg (Markoulaki et al., 2008). Once the embryo develops, it is genetically identical to the animal from which the original somatic cell came from. Dolly the sheep, the first cloned mammal, was cloned using this technique. For a transgenic animal, the nuclear donor cell would have to come from a cultured cell containing a transgene (Bosch et al., 2004). The transgene integrated into the genome of the cultured cells would become part of the embryo clone resulting from the nuclear transfer.

Sperm-Mediated Transgenesis

Another less frequently used technique for making transgenic animals uses modified sperm cells as vectors during *in vitro* fertilization. In sperm-mediated transgenesis, the sperm are placed in culture with the foreign DNA, which binds to the plasma membrane (Bosch et al., 2004). Once the foreign DNA binds, the sperm is implanted in an egg through *in vitro* fertilization. Although this technique is simpler than those that deal with ES cell manipulation, it cannot be used for gene targeting as the sperm no longer undergo homologous recombination. So only random genome integration occurs, and thus the extent to which the gene is expressed will not be known until the offspring are screened (Bosch, 2004).

Methods for Gene Delivery into Cells

In addition to microinjection mentioned above, the most popular method for introducing DNA into cells, two other techniques are also worth mentioning: viruses and transposons. Each of these DNA introduction methods can be applied (with greater or lesser success) to the main transgenic methods discussed above for inserting foreign DNA into newly fertilized eggs, ES cells, or the nuclei of donor cells for SCNT.

Retroviral Gene Transfer

Another method used less frequently than microinjection for gene delivery uses viral vectors. Viruses are an efficient means to insert foreign DNA into cells (Robbins et al., 1998). A virus can only be used for transgenesis and gene therapy if its pathogenic behavior can be stopped so it does not infect the host cell in any unwanted or harmful ways. Although a variety of different types of viruses are used to create transgenes, the retrovirus is most commonly used.

A retrovirus has RNA as its genome. Once it infects a cell, the retrovirus causes its RNA to reverse-transcribe into DNA which then becomes integrated into the genome of the host. This ease of integration into the genome provides a stable means for any sort of genetic modification, and makes the retrovirus an ideal vector for transgenesis. The structure of the retrovirus is important to its efficiency in being used as a vector for transgenesis. Three main viral genes are important for function and replication, and are shared by all retroviruses: *gag*, *pol*, and *env*. These genes are responsible for producing specific proteins that directly relate to viral structure and replication. At each end of the retroviral DNA are long, repeating sequences of DNA known as long terminal repeats (LTRs). These sequences enable the virion to integrate itself (Robbins, 1998). The LTR sequences contain promoter and enhancer sequences that can be modified to

promote expression of the transgene. Location of the transgenic start codon is important, as it determines the extent to which the transgene is expressed (Robbins, 1998).

Transposons

Transposons are DNA sequences that are capable of moving to different locations of a cell's genome (Griffiths et al., 2008). Transposons (mobile DNA elements) can be used as a vector in gene transfer for the production of a transgenic animal. The transposase gene that is responsible for transposon movement is replaced with a foreign transgene. The transposase gene is provided by a separate plasmid (Houdebine, 2002). Upon genomic integration, the plasmid vector degrades, leaving the transgenic transposon (Houdebine, 2002).

Screening Transgenic Animals

Unfortunately, the process of transgenesis is not very efficient. Depending on the species, hundreds of embryos sometimes must be manipulated to create one transgenic line. A majority of offspring are non-transgenic, so offspring must be screened to find the transgenic positives. Different screening techniques will provide information regarding whether the transgene is present in the animal, and if so, the copy number. Southern blot and polymerase chain reaction (PCR) analyses are the two major screening methods used by scientists today.

Southern Blot Analysis

The Southern blot procedure was invented in 1975 by Edward Southern as a technique for detecting specific DNA fragments separated by gel electrophoresis (Southern, 1975). For screening potential transgenic animals, a sample of DNA, usually from a piece of the tail or the

ear, is taken from the animal for testing. The DNA is purified and then cut with restriction enzymes. The DNA fragments are separated by size using gel electrophoresis in which an electric current is placed across the samples loaded onto a separating material such as agarose or poly-acrylamide. With animal genomic DNA, many fragments occur creating a smears on the gel. The potential presence of the transgene is determined by blotting the smeared DNA onto membrane, which is then hybridized to a labeled complementary DNA probe representing the transgene. If the transgene is present in the DNA, the labeled probe hybridizes to it, creating a signal on x-ray film (Griffiths et al., 2008). If the probe does not bind to the membrane, then the transgene is not present in the sample. However, if the probe binds to the membrane, then the test has screened positive for the transgene.

PCR Assay

A polymerase chain reaction (PCR) assay is a relatively easy method for screening for foreign DNA. As before, a sample of DNA is purified from the animal to be screened. As PCR is so sensitive, less DNA is required than with Southern blots, so a sample of saliva is often sufficient for the assay. The animal DNA sample is placed in a solution with a special Taq DNA polymerase to catalyze DNA synthesis at high temperatures (Griffiths et al., 2008). The solution also contains primers complementary to the transgene. Upon heating the reaction in a thermocycler, the DNA strands separate. The temperature is then lowered, and the primers anneal to the transgene if it is present. The temperature is raised to the optimal temperature of Taq polymerase, and new DNA strands are made starting from the primers. The process of denaturation, annealing, and polymerization is repeated about 35 times, until the transgene is

amplified enough to easily see on a gel. This whole process is relatively short, taking less than a day to complete.

PCR and Southern blot screening are the last steps in producing a transgenic animal. Once the animal tests positive for the transgene, it can be bred with another transgenic animal to eventually create a transgenic line.

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CHAPTER-2: TRANSGENIC APPLICATIONS

Transgenic animals have been used for a variety of human benefits, including as models for disease, for producing pharmaceuticals in their milk (transpharmers), as alternatives for human organ donations (xenotransplanters), and also for agricultural benefits and biological models. The purpose of this chapter is to describe some of the transgenic success stories, while categorizing the types of animals created. This information on the benefits of transgenesis to society will serve as an introduction to our subsequent chapter on ethics and whether we should be doing such experiments.

Transgenic Disease Models

One of the most common applications for transgenic animals is to model human diseases. In doing so, certain human diseases can be studied to learn more about the biology behind the disease for developing therapies. HIV/AIDS, Alzheimer's disease, and Oncomouse are just three of the many diseases that have been modeled in animals. In each of these models, tremendous progress has been made in understanding that disease on a biological and genetic level.

Transgenic Mice and HIV/AIDS

Acquired Immune Deficiency Syndrome (AIDS) is a viral disease that attacks the immune system. It is caused by the Human Immunodeficiency Virus (HIV), a retrovirus. AIDS and HIV were discovered in the early 1980s, and have caused millions of deaths. On-going research and experimentation have provided some measures for treatment, but so far there is no cure or vaccine for the disease.

HIV is a virus that predominately infects humans, but few other animal species. This leaves few options for animal studies. Chimpanzees were initially used for experimentation for HIV/AIDS, but they are expensive (costing around $50,000 per animal) and it can take years to get results (Cohen, 2001). Some monkeys have been shown to be infected with a virus similar to HIV, known as simian immunodeficiency virus (SIV); however there are many differences in the way SIV works, and any treatment that may work effectively for SIV might not work in the same capacity for HIV (Pincus, 2004). An ideal animal for studying HIV is the rodent, because they are relatively inexpensive, and results could be seen in just a matter of months instead of years. Rodents such as mice and rats, however, do not become infected with HIV naturally. Scientists have been working on creating transgenic mice that can become infected with HIV so studies can be conducted in a more timely matter.

Numerous road blocks occurred in creating HIV mice. The intial problem focused on getting HIV to enter mouse cells. Soon after the co-receptors were discovered for HIV to infect human cells, mice were engineered to express the human receptor CD4 and CKR5 chemokine co-receptors (Pincus, 2004). This allowed HIV to enter mouse cells, but it could not replicate. Further research showed that the HIV protein tat, which is needed for HIV transcription into RNA, must interact with human cyclin T1 for the HIV to transcribe (Cohen, 2001). Mice were engineered to include this protein, and HIV infection was successful; however, the HIV was not produced to the same extent as in humans. It was later determined that the HIV gag protein p24 which is needed to assemble the viral capsid was being made but was not able to leave the cytoplasm to enter the cell membrane (Cohen, 2001). Thus, HIV still has not been fully replicated in the mouse model. But future research may produce a fully successful model. Once

a mouse model is successful, there is a greater chance of finding better treatments, and possibly a cure or vaccine, for this debilitating virus and disease.

Transgenic Mice and Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder exemplified by a loss of cognitive function, including memory loss, orientation, and judgment (Janus and Westaway, 2001). AD is the most common type of dementia in the later stages of human life (Janus, 2001), and it is the fourth most common cause of death in the industrialized parts of the world (Mineur et al., 2005). There are currently 4.5 million people suffering from AD in the United States alone, and this number is expected to reach 16 million by the year 2050 (Mineur et al., 2005). Although the molecular pathology of the disease is currently unknown, there has been tremendous progression in research regarding pathology. Therapies that could slow down the progression of the disease are constantly being tested.

In AD, neural damage occurs in certain areas of the brain, specifically those areas pertaining to memory and learning (Janus and Westaway, 2001). The two hallmark lesions of AD are senile plaques (composed of β-amyloid deposits), and neurofibrillary tangles (NFTs) (composed of Tau) (Janus and Westaway, 2001). β-Amyloid appears to initiate the disease (Games et al., 1995) while abnormally phosphorylated Tau helps mediate neuronal death (Mineur et al., 2005). Hyperphosphorilated tau cannot perform its normal function of maintaining microtubule morphology, so microtubules are unable to form correctly causing neuronal damage (Mineur, 2005).

Some AD cases are genetic. Early onset AD, familial Alzheimer's disease (FAD) has been shown to be linked to mutations in genes that encode the amyloid precursor protein (APP)

and both presenilins (PS1 and PS2) (Janus and Westaway, 2001). These proteins, when working improperly, have been shown to cause an increase in β-amyloid formation. In the other form of AD that occurs later in life, abnormalities in the ApoE gene have been shown to be a factor in the development in the disease.

Transgenic mouse models have been crucial in AD research, especially in showing which genetic factor impact the progression of the disease. The first successful AD mouse model, created in part by Professor Dave Adams at WPI, produced an early onset version of amyloid precursor protein (APP), and helped show that β-amyloid production initiates AD (Games et al., 1995). Subsequent mice were engineered to express other mutant human genes that are considered to be associated with AD, including PS1, PS2, ApoE, and the tau protein (Janus and Westaway, 2001). Recent studies have shown that transgenic mice with these mutant human genes show an increase in β-amyloid deposits and decreased cognitive function. These mice are currently being treated with various therapies that may stop the disease progression (Janus and Westaway, 2001).

Transgenic Mice and Cancer

Cancer is a debilitating disease caused by genetic mutations of a cell. A mutation in a single cell will give that cell growth advantages and will pass this mutation onto its daughter cells; these cells will continue to replicate to form a tumor (Frijhoff et al., 2004). Further mutations allow for continued cell growth, survival, and replication. These mutations often occur near oncogenes ("cancer genes") and near tumor suppressor genes (Radiloff et al., 2008).

Cancer is also related to apoptosis (cell death) as cancer cells show decreased cell death (Jager, 2007). Mutations in cells can stop the process of apoptosis, which in return enables a cancer cell to continuously reproduce without cell death; the end result of the mutation is a tumor.

In the field of transgenics, mice have played an important role in the study of cancer. Mice have been genetically engineered to have genetic alterations seen in human cancer cells (Frijhoff et al., 2004). There are two ways in which the genome of the mouse can be changed to cause tumor formation. First, an oncogene can be expressed that will cause cell growth, survival, and proliferation. This approach was used in the world's first oncomouse created by Harvard and Dupont that contained the c-myc oncogene (Stewart et al., 1984). Second, tumor suppressor genes can be turned off by creating a knock-out mouse, eliminating the function of the tumor suppressor genes.

Transpharmers

Drug proteins that are produced in transgenic animals are usually done so in the milk of the animal. This is done by using promoters in the transgene that are specific to the mammary gland to ensure secretion of the protein in the milk (Dunn et al., 2005). Although milk is the most common means through which to secrete transgenic-generated proteins, alternative approaches include using promoters that are specific to the bladder or kidney (to express in the urine) (Dunn et al., 2005). One of the benefits of the urine approach is that both male and female transgenic animals may be used, as opposed to only females in the milk method.

One of the major reasons the mammary gland is so successful, is because when the mammary gland is modified through transgenic manipulation, there are no detectable effects on the animal (Karatzas and Turner, 1997). The transpharmed protein is removed from the body

through the milk and does not cause harm to any other part of the body. Prior to transpharming, drug proteins were isolated from human fluids or produced recombinantly in fermentation systems. There is a risk of contamination and disease when isolating proteins from human fluids, and fermentation systems are very expensive and time consuming (Keefer, 2004).

Examples of Transpharmers

The first transpharmer animal was created at Genzyme Transgenics Corp (GTC) (Gordon et al., 1987) who created a mouse producing human tissue plasminogen activator (tPA) in milk. The transgene contained casein promoter sequence, the human tPA gene, and a sequence that induces secretion. Mouse embryos were injected with the foreign DNA, then the pups were bred with other transgenic mice of the same type. The milk of the female transgenic mice was screened for tPA. The experiment was successful, and the milk contained active tPA, which began the field of transpharming.

In 1997, transgenic sheep were made through the method of somatic cell nuclear transfer to secrete a human clotting factor IX (FIX) (Schnieke et al, 1997). FIX is a protein required for proper blood clotting in humans, without it the person has hemophilia. FIX is currently obtained from human plasma, which provides risk of HIV and hepatitis C virus, and is also expensive. Transpharming would eliminate these problems. The transgene implanted into the sheep contained the entire human FIX gene and a sheep promoter sequence for milk protein beta lactoglobulin (BLG) that allows expression in the mammary glands. The offspring of the sheep were screened for the transgene, and three of the six offspring successfully integrated the gene into their genomes.

Cows are another ideal animal to use as transpharmers due to their large milk supply. In 1991, a bull was engineered by the University of Leiden and Gen Pharm International of the Netherlands, named Herman the Bull, who contains a gene for lactoferrin in his genome through the method of microinjection (Biotech, 1994). Lactoferrin is a protein normally found in a human mother's milk, but not cow's milk, that is necessary for proper growth and development (Biotech, 1994). Herman was able to pass this foreign gene onto his offspring, and their milk contained the iron-rich protein. This transpharmer animal was significant in the field of transgenics because the lactoferrin milk could be used worldwide for children who do not get enough of the protein, especially those in underprivileged societies (Biotech, 1994).

Another transpharmer example is the first FDA-approved transpharmed drug in the United States called ATryn. ATryn is an anticoagulant protein produced by transgenic goats (King, 2009). The goats were developed by GTC Therapeutics in Massachusetts in 2009, to produce human antithrombin-α, a protein used for people affected with antithrombin deficiency, a hereditary disease that puts them at a high risk for complications from blood clots (King, 2009). In addition to helping with the antithrombin deficiency disease, it can also be used for people undergoing heart surgeries as a blood thinner to replace heparin if they have developed a resistance to the drug. Heparin is used to prevent blood clots, and it is common for antithrombin levels to decrease during surgery (King, 2009). ATryn is produced in the milk by inserting a human antithrombin-α transgene with promoters for milk proteins as in the previous examples. Both microinjection and somatic cell nuclear transfer methods have been used to create the transgenic animals. The protein appears to have no harmful effects on the goats.

Xenotransplantation

Organ failure is a huge problem in the U.S. resulting in a decreased quality of life and, if untreated, death. Transplant treatments are extremely costly and rare. Most people with organ failure have no other option but to wait for a donor organ to become available. According to the United Nation for Organ Sharing (UNOS, 2010), the US organization that facilitates organ matching and placement, as of July 2010, over 108,000 organs are needed in the United States.

One possible solution to organ shortages is xenotransplantation – using animal organs for human organ transplants (Dwyer et al., 2002). Xenotransplant organs are categorized into two types, concordant and discordant xenografts. *Concordant xenografts* are organs that come from a genetically similar species, such as using primates for human organ substitution. Due to the genetic similarities, the immune response of the recipient's body would not be too dissimilar to the response from a human graft. However, due to ethics and money, primates have not been the animals of choice. The most common animal used for xenotransplants are pigs. Pigs and other animals used for transplants are known as *discordant xenografts* with substantially different genetics. However, pigs can reproduce very quickly, they have large litters, and they can be grown to a specific size to match the organ to the size the recipient needs (Dwyer et al., 2002). Because the recipient immune response much more severe when using discordant animal donors, genetic engineering comes into play to modify the animal's genome to contain transgenes that either aid in the protection of the xenograft or knock-out genes that contribute to rejection (Dwyer et al., 2002).

The first and most severe phase of xenograft rejection is called hyperacute rejection (HAR). HAR is extremely important to prevent because it can result in the loss of the organ function. During HAR, there is a high risk of hemorrhaging, thrombosis, and infiltration by the

neutrophils in the body (Dwyer et al., 2002). Transgenic pigs have been made to express human complement regulatory proteins (CRP) which decrease complement activation (Dwyer, 2002). Uncontrolled complement activation usually results in the formation of a membrane attack complex that puts pores into the lipid bilayer of the cells to which antibodies are bound, causing cell lysis (Dwyer et al., 2002). Human complement regulatory proteins usually prevent this part of the rejection process from happening.

Other methods to help prevent xenograft rejection through genetic engineering involve a sugar called galactose α1,3-galactose (αGal) (Dwyer et al., 2002). αGal is the major pig antigen recognized as foreign by human antibodies. Expression of αGal is very strong in the pig, so transgenic pigs have been engineered to reduce αGal expression by using a competing enzyme so a non-immunogenic functional group is expressed instead of αGal (Dwyer et al., 2002). The Gal transsferase gene has also been knocked out of the genome to prevent αGal expression altogether.

Transgenic Food Sources

Studies have also been done to create transgenic animals with an increased expression of growth hormones. The goal is to create larger animals for food sources. Two of the most common animals engineered for this purpose are pigs and fish. One of the first transgenic animals that had the potential to be used as a food source was "Superpig." Super pig is a transgenic pig that contains a human growth hormone gene. It was created in 1989 by K. Miller et al. in which pigs were engineered to contain either human or bovine growth hormones (Miller et al., 1989). Although the levels of the hGH and bGH expression varied depending on the animal, most of the pigs successfully integrated the genes into their genomes, and one specific

growth factor – insulin-like growth factor-I – was found to increase much greater with the age of the transgenic pigs than with non-transgenic pigs (Miller et al., 1989). The overall goal of Superpig was to be able to create larger pigs that would grow faster and provide larger amounts of food for human benefit. Although this could be accomplished, the over-expression of the growth hormones were extremely harmful to the pig's health. Ulcers, lethargy, and crippling are some of the major health problems that were assessed with Super pig (Pursel et al., 1990).

The transgenic addition of growth factors into animals has also been done in fish, called Superfish. The first experiments with Superfish used mammalian growth hormones, and in some cases, growth enhancement was observed (Delvin, 1998). However, some studies showed the mammalian genes had a greater difficulty expressing themselves in fish cells, so the transgenes were constructed to contain regulatory sequences present in fish (Delvin, 1998).

More success was found in creating gene constructs completely comprised of fish coding regions and regulatory elements. For example, a salmon hormone transgene was engineered to contain a salmon protein promoter which enabled growth stimulation up to eleven times compared to non-transgenic salmon (Delvin, 1998). Although not quite as prevalent as with Superpig, health problems were observed in some of the Superfish, particularly those that grew most rapidly. These health concerns are further discussed in Chapter-3 on transgenic animal ethics.

Transgenic Biological Models

Transgenic biological models serve as a way to further understand transgenesis and to help uncover how certain genes work. Some models do not fall under an earlier discussed category, but they may aid in learning what over- or under-expression of a gene does and its

overall effect on the body. ANDi the Monkey and the Smart Mouse are two biological models that have been successful and have provided new scientific information.

ANDi the Rhesus Monkey

ANDi, which stands for "inserted DNA" spelled backwards, was the first successful genetically modified primate, born in October 2000. ANDi contains a transgene that codes for a green fluorescent protein (GFP) that glows green in blue light (Begley, 2001). The transgene was integrated into the genome of the monkey through retroviral gene transfer, and ANDi was the one monkey born out of 120 successfully fertilized eggs. The GFP gene here was used only as a marker for transgenesis, and did not confer any new property on ANDi other than for his cells to glow. ANDi is considered a landmark in transgenic animal research because he opens the door for further transgenic research using primates. Transgenic rodents and other animals used for human disease models have provided useful information, but are not perfect models (Chan, 2001). ANDi has shown that primates can successfully integrate foreign DNA into their genomes.

Smart Mouse

In 1999, researchers at Princeton University developed transgenic mice that showed great improvements in learning and memory. A transgene was constructed to contain a foreign gene called NR2B, which is essential for the ability to learn (Harmon, 1999). The gene codes for a subunit of the NMDA receptor which binds chemical signals associated with learning and memory. The NR2B subunit predominates in the receptor when the brain is young and learns

easily, so the authors reasoned a transgenic animal over-expressing NR2B might learn faster. The experiment done at Princeton University inserted extra copies of the NR2B gene into some mice, and this resulted in transgenic mice with improved ability to learn and remember using mazes, objects, and sounds. The transgene also enabled the mice to continue to more easily acquire new knowledge and memories even after adolescence by offsetting the gene's natural decline (Harmon, 1999). These smarter transgenic mice have served as excellent biological models for researchers who have been searching for ways to improve the human brain and to further our understanding of diseases affecting learning and memory loss.

Chapter-2 Conclusion

Transgenic animals have been used in a variety of ways for human benefit. Some animals (especially fish) have been engineered to contain growth hormones that make them bigger and better sources for food. Some animals have been made to produce pharmaceutical proteins in their milk that can be used to treat deadly human diseases. Other animals have been genetically modified so their organs can be successfully transplanted into humans. Disease models have provided new knowledge about diseases like AIDS and Alzheimer's. Each one of these applications have had both successes and failures; however even each failure can be viewed as a success in the biological world, as it still provides new information once unknown.

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CHAPTER-3: TRANSGENIC ETHICS

From reading Chapter-1 and Chapter-2, is now evident that there are great benefits of creating transgenic animals, whether it is for finding treatments for human diseases, producing pharmaceuticals in an easier and more cost-efficient manner, or creating organs for transplant. Although there are many benefits in using genetically modified animals, there are also concerns with this technology, thus an ethical debate has risen over the uses and the extent to which transgenic animals should be used for scientific advancement. This chapter will cover the benefits versus costs of transgenic animal research, and will include ethical views from various angles, including those of science, religion, and animal welfare.

Benefits of Using Transgenic Animals

A discussion of transgenic ethics is a balancing act between the benefits to society versus the detriments to society or the animals. We will begin with a reminder of the benefits to society of transgenesis. Genetically modified animals have provided tremendous benefits for human welfare for medical, agricultural, and scientific advances. In chapter-2 we learned that transgenic animals have been used as models for human disease, for producing pharmaceutical proteins in milk, for producing transplant organs, and for agricultural advances.

As disease models, transgenic animals have aided our understanding of many human diseases, such as AIDS, cancer, Alzheimer's, and Parkinson's disease. Although these models do not perfectly replicate a human disease, they do provide information on specific aspects of it. Many scientific roadblocks have been overcome to create at least partial models for specific diseases, and the findings will ultimately help in future treatment, therapies, and vaccines.

Transgenic disease models are perhaps the most challenging yet beneficial application in transgenic animal research.

Transpharmers have the ability to produce pharmaceutical proteins in their milk in an efficient cost-effective manner. This category of transgenic animal rarely show any signs of pain or suffering because the proteins are immediately secreted through the mammary gland without affecting any other part of the body. Xenotransplantation may eventually prove a useful alternative to human organ donation, especially to provide organs to critical individuals awaiting organ transplants for which no human organ is available. Pigs, whose organs are very similar to humans, are the most common proposed donors for xenografts. Transgenic pigs have been engineered to express genes (or to knock out certain genes) that will reduce the complication of graft-rejection. This will be an enormous benefit to human welfare, as the number of organs in need is at an all time high (Dobson, 2007; US Transplantation Data, 2010).

Transgenic animals used for agricultural purposes have also immensely benefited human welfare. Cows, pigs, goats, and other farm animals have been genetically engineered to grow faster and bigger, although in this category only the superfish are still being investigated. Transgenic sheep modified increase wool growth have been developed, and cows have been engineered to produce more milk (Margawati, 2003). This allows faster and increased production of food, clothing, and other agricultural means.

Transgenic animals have had many positive outcomes with respect to human welfare. But each of these applications also presents controversy due to the safety and welfare of both humans and animals alike, which will be discussed next.

Concerns with Using Transgenic Animals

While the benefits of transgenic animal research may make these types of animals seem like superheroes in the scientific world, there is another side of the ethical debate that must be addressed. As in any field of science and research, there are concerns for the welfare of both humans and the animals engineered to express these unnatural traits. People from all ends of the spectrum have varying concerns related to the ethics associated with this type of research. Some people focus mainly on concerns regarding human welfare, while others focus solely on animal concerns. Religious beliefs add additional information to the debate.

Human Safety

Human safety is a key concern in the ethical debate. Vaccines and treatments that have been successful in animals may work differently in humans; the effectiveness of any treatment in an animal model, such as a mouse or a fly, may not last as long in humans or may produce other undesired effects (Almond, 2000). The FDA recently took responsibility for helping ensure transpharmed drugs are safe for human consumption, and in the future may regulate xenotransplantation (Williams, 1996), transgenic food (Krimsy and Murphy, 2002), and all categories of transgenic animals (FDA, 2009; FDA.gov, 2009).

Some individuals are also concerned with the possibility of a transgenic disease model escaping or accidentally becoming released into the natural world (Almond, 2000),as the transgenes that mimic human diseases could be passed on to offspring. Animals currently used by humans for food, clothing, and other uses may not be able to be utilized if an epidemic struck the animal world. This may seem far-fetched, especially if the disease model breeds slower than wild type animals, but it is often brought up regarding human safety. The words “disease” and

"genetically modified" carry a strong negative stigma, and the unknown can cause people to be fearful. So great care must be made to help ensure no escape occurs, especially by mandating transgenic animals be handled professionally inside licensed vivariums.

Another safety factor for humans concerns creating new behaviorally dangerous animals. Toying with the genomes of animals, and ultimately creating a completely new and genetically unique species, could potentially create an animal that is physically more difficult to contain. Although highly unlikely, some people believe that a Jurassic Park-like or other science fiction scenario may become a reality if a transgenic animal escapes into the wilderness (Almond, 2000). Although no drastic changes in animal behavior have been documented yet, fears that a dangerous transgenic animal may be produced from an unanticipated mutation continue.

Animal Safety

Ethical concerns also arise if there is harm caused by these genetic alterations. Cruelty to animals is a major issue in research ethics, especially for groups who work for the protection of animal rights, such as PETA. Animals that are genetically engineered to model human diseases, for example, suffer from these diseases as humans do. As explained in Chapter-2, animals have been designed to mimic some symptoms of the human disease they are designed to model. So in this category, great care must be made to use painkillers whenever possible, and to sacrifice the animal prior to advanced disease state. Some animals previously engineered for agricultural purposes, such as Superpig, suffered greatly. Superpig developed life-threatening health hazards, and had to be euthenized (D'Silva, 1998). More rarely, transpharmers and animals used for xenografts have resulted in complications that cause pain and suffering, so strong oversight

from the Institutional Animal Use and Care Committees (IACUC) are required to help minimize pain, and to sacrifice when needed.

Animal concerns are also raised against the high number of embryos required to create a positive transgenic. The cloning process is inefficient, sometimes requiring hundreds of eggs (Gillespie, 2010). More often than not, the pup born is a "normal" wild type animal, not containing the transgene. And some positive animals born with the transgene do not always express it properly. So this results in a great number of animal lives created without any direct scientific benefit. Some view this as an opportunity to learn which methods do not work, so adjustments in future research can be made.

Religion and Transgenics

Concerns with transgenic animal research also stem from religious beliefs, ranging on one side from the sacredness of some animals, to the other side and the belief that all animals are inferior to humans. Many religions view that animals should be respected, yet they also recognize the importance of the benefits associated with animal research. Christianity, for instance, believes animal experimentation as moral, as long as it pertains to the care of human lives (Ethics, 2009). In a more traditional view, some Christians believe that animals are inferior and were created by God to be used by humans, whether it is for food, clothing, research, or other uses humans may have for them. Christianity continues to preserve its position that animals may be used for research purposes, as long as the animals do not suffer any more than needed. The Vatican is one governing religious body that recognizes transgenic research. They state that the Catholic Church believes that any sort of transgenic expression in animals must be openly discussed, and any pain the animals may experience must be minimized (Correa, 2001).

Jewish beliefs also allow animal experimentation, under similar terms to those of Christianity; the research must be done to benefit human beings, and any pain that may be associated with the research must be minimized (Ethics, 2009). Buddhism has similar views as well, and if the animals are considered to be used for experimentation for a wrong purpose, they believe that those involved with the research will be entitled to bad karmic consequences (Ethics, 2009). Hinduism is especially respectful of animals, and cows are especially important and considered sacred. But Hindus also identify the significance of animal research for humans (Ethics, 2009).

There are some religious concerns of "playing God." Religions, like those that stem from Christianity, believe that God creates everything in a certain way because it is His will, and He has reasons behind the way things are. These individuals believe there are ethical boundaries to putting a foreign gene into an animal, as it alters God's will. Playing God continues to be one of the greatest religious concerns with genetic research. Each religion has its own views on animal research and transgenesis, yet each individual person is responsible for the magnitude to which they wish to follow the beliefs that is recognized by their faith.

Transpharmer Ethics

In comparison to other transgenic animal categories, these genetically modified animals have less risk for pain, suffering, and complications. Most transpharmer animals are perfectly healthy; they look and act the same, with the added benefit of producing proteins in their milk. However, the scientific world is not always exact, and there is always the risk when creatig a new strain of a gene being expressed in the wrong part of the body, or being over-expressed to create unwanted complications (Gillespie, 2010). Because newer and better methods are

constantly being tested, there is no way of knowing in advance with certainty that a newly developed transgene will not also be expressed in the wrong area which could have harmful results.

Xenotransplantation Ethics

As mentioned in the previous chapter, there are hundreds of thousands of people waiting in the US for an organ (US Transplantation Data, 2010). Xenotransplantation, which uses animal organs for transplantation in place of human organs, is one way to overcome this overwhelming demand for human organs. If the animal organ can survive long enough, it eliminates the need for one human to die in order to save another. But ethically, one major fear is the risk of disease transmission from the donor animal (Carnell, 2000). Around the globe it is often seen that some diseases easily pass from animal to human. In an attempt to prevent disease passage with xenotransplantation, or with any animal research, the US Public Health Service has very strict requirements regarding animal health surveillance (Carnell, 2000). Raising animals under disease free conditions would help mimimize disease spread during xenotransplantation, as would performing tests on known pathogens just prior to transplant. So this problem is real, but the risk is manageable.

Another fear associated with xenotransplantation is the immunosuppressive drugs that must be administered to prevent organ rejection, as these drugs can create opportunistic infections. But these concerns also apply to standard organ donation. As described in the previous chapter, some animals have already been genetically modified to increase their organ compatibility with humans, so this is a step in the right direction (Carnell, 2000).

Animal welfare is a major concern in this category, as the pigs would be sacrificed for their organs. This criticism also applies to all categories except transpharmers that live their full lives. Some religions believe animal sacrifice can be justified because a human life can be saved. The Vatican, for instance, addresses guidelines for xenotransplantation (Correa, 2001). These guidelines were set forth to protect both the animal that will be used for the organ donation, and the patient that will be receiving the xenograft. In addition to guidelines regarding the assessment and reduction of any sort of pain, when animals are used for xenografts, the organ removal must occur by surgical operation (Correa, 2001).

Superpig and Superfish Ethics

Superpig, Superfish, and other animals that have had foreign growth hormones implemented in their genomes were designed to grow faster and contain less fat content for human agriculture and food sources. These specific transgenic animals in which the costs far outweigh the benefits. With the exception of Superfish, any mammalian model of growth hormone addition has caused outcomes that *severely* impact the welfare of the animals. The original Superpig experiment, performed in the 1980s, was successful in decreasing the amount of animal fat, but each pig that expressed the transgene developed many problems regarding their physical health, including lethargy, arthritis, diarrhea, dementia, skin problems, and even mammary development in males ("Uniqueness," 2002). In this original Beltsville Superpig experiment, seventeen out of the nineteen transgenic pigs died within a year due to health problems from pneumonia and peptic ulcers ("Uniqueness," 2002).

Transgenic salmonids either showed no disease symptoms or less severe symptoms. Some of these transgenic fish had growth abnormalities in the head and the jaw, which made it

difficult for the fish to eat (Eenennaam, 2006). With further experiments, most of these genetic problems were overcome in salmon, and these transgenic aquafarmed fish are only a few years away from becoming FDA approved for human consumption (Aquabounty, 2009). Although Superpig and Superfish may initially seem like a good idea for agricultural benefits, the health problems that are developed by some these "super-animals" seem too costly. Since many of the health problems associated with Superfish were oversome, perhaps less-sufferable Superpigs and other super animals are on their way.

Chapter-3 Conclusion

The ethical debate that surrounds transgenic animal research will, most likely, always exist. There will always be groups that want to protect animals from manipulation and experimentation, and others that favor human welfare over the welfare of animals. This chapter showed that some transgenic experiments, such as transpharming, can be done with minimal risk to the animal. This low-risk genetic modification can save human lives. On the other end, some transgenic research has been proven to cause pain and extreme complications, such as Superpig. In this latter case, perhaps it is best to find alternative ways to increase our food sources.

Chapter-3 Resources

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CHAPTER-4: TRANSGENIC LEGALITIES

The first three chapters covered how transgenic animals are made, the ways in which transgenic animals can be used, and the ethical debate that surrounds these genetically modified organisms. When reviewing both sides of the ethical debate, both human and animal welfares were considered. But when discussing transgenic ethics, questions pertaining to patents on these transgenic animals begin to rise – should life be allowed to be patented? And, if so, where do we draw the line, should humans be patented?

The first living *organism* patented in the United States was a microbe genetically modified to break down crude oil, which could be especially significant in the clean-up of oil spills (Diamond v Chakrabarty, 1980). This opened the doors for further patents on life; however, the ethical debate roared between researchers who wanted to own the rights of their transgenic "inventions", and activists who wanted to protect animals from any sort of genetic modification and prevent the legal possession and potential income that could result from those animals.

The world's first patented *animal* was a transgenic mouse that contained human oncogenes that was developed and patented by researchers at Harvard University. The Oncomouse was the first complex organism to be patented, and this caused a boom in patent applications on other genetically modified organisms and other life. The scientists at Harvard were eventually able to obtain patents for their Oncomouse in not only the United States, but in Europe, Australia, Japan, and numerous other countries. The only country to remain obstinate is Canada; Canadian law continues to deny any patents on Oncomouse or any other complex life form. The Harvard Oncomouse debate raised many ethical issues regarding patents of transgenic

animals. One issue questioned whether patents should be granted to all types of animals, regardless of whether they meet patentability criteria. Another issue raised was the moral implications addressed in specific cases, such as for suffering transgenic animals (Bioethics, 2006).

Patented Microbe

In 1972, Ananda Chakrabarty, a microbiologist working at General Electric, filed a patent application for his genetically engineered bacterium (Diamond v Chakrabarty, 1980; Lumelsky, 2004). His bacterium was developed to break down crude oil, and was created by transferring four plasmids into a *Pseudomonas* bacterium, each one with the capability of breaking down different components of the oil (Lumelsky, 2004). Prior to this patent application, microorganisms and any other form of life were not allowed to be patented (Diamond, 1980). Initially, only two of the three claims that Chakrabarty filed were allowed: claims for the *method* of producing the bacteria, and claims for the transgenic DNA itself (Diamond, 1980). The claims for the *bacteria* themselves were initially rejected, as they are living products of nature and not patentable by law.

The federal statute that defines patentable subject matter is as follows: "Whoever invests or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title" (Lumelsky, 2004). The question became whether or not Chakrabary's transgenic bacterium was considered a "manufacture" or a "composition of matter." It also had to be determined whether it was a product of nature. Chakrabarty appealed this initial rejection to the Patent Office Board of Appeals, and the Board determined that these genetically modified

bacteria were not technically products of nature because they contained energy-generating plasmids that do not naturally occur in nature (Diamond, 1980). In June of 1980, the Supreme Court ruled with a 5-4 majority that living organisms could be patented, and the first patent on a living organism was granted to Ananda Chakrabarty for his oil-eating bacterium (Lumelsky, 2004).

Harvard Oncomouse

The Oncomouse, also known as the Harvard Mouse, was developed by Harvard professor Philip Leder in the early 1980s, just after the Chakrabarty's bacterium patent was approved. The transgenic mouse was engineered to express the mouse mammary tumor virus promoter and *myc* oncogene. This allowed the genetically modified mice to develop a variety of different human-occurring tumors (Blaug, 2004). In 1981, Du Pont contributed six million dollars to the research done on Oncomouse at Harvard (Blaug, 2004). By 1983, their *myc*-expressing mice were created and they began the patent application process.

The patent filed by Leder and Stewart had twelve claims (Fuller, 2008). These claims included any non-human transgenic animal that contains an activated oncogene via transgenic methods. The patent also includes claims for the chromosome within the transgenic animal, an oncogene sequence that is inserted into the animal genome at a different location than what would otherwise be its natural occurrence, and a non-naturally occurring promoter placement to regulate the oncogene sequence (Fuller, 2008).

The Oncomouse was granted the first US patent on a higher-level animal. Once this initial patent was granted, Leder and his colleagues were initially granted rights to all genetically modified NON-HUMAN mammals and the methods used to produce those animals. This meant

that the patent not only claimed the *myc*-expressing mice, but any transgenic animal that that expressed oncogenes.

Due to their initial financial investment, DuPont was allowed an exclusive license to these transgenic mice. Initially, licensing fees for the use of the Oncomouse were small and had few limitations, but as the technology and research in this area began to boom, and DuPont began working with larger commercial companies, fees rose tremendously and restrictions – particularly those pertaining to university research – became stricter (Blaug, 2004). Additionally, DuPont initially required any results obtained from research with these transgenic animals to be approved prior to being shared with any other laboratory or research company (Blaug, 2004).

Eventually, the National Institutes of Health (NIH) and DuPont negotiated an agreement on the cost and restrictions for using Oncomouse (Blaug, 2004). The NIH stressed the importance of these animals for research to benefit human welfare, and that they should not be so restricted. In 1999, DuPont and NIH signed an agreement that would allow free-of-charge research access for scientists and researchers dedicated to public health service, as long as the research was not being done for commercial or for-profit companies (Blaug, 2004).

Although this agreement helped those involved with the NIH, research that was being done in labs at universities were still severely restricted, which was seemingly outrageous since the Oncomouse patent was held by the prestigious Harvard University. Only universities that were granted funding by the NIH were allowed free-of-charge accessibility to the oncomice (Blaug, 2004).

The Harvard Oncomouse case was the first complex animal life to be patented. This not only opened the doors for future patents on animals, but also began more debates on whether life

should be allowed to be patented. A major concern included the worry that patenting an animal could potentially lead to patenting human life, which was and still is a concern in Canada, the only developed country currently without an Oncomouse patent.

Canadian Oncomouse

In 2002, the Canadian Supreme Court turned down Harvard's patent application for their genetically modified Oncomouse. They applied for the methods pertaining to the development of the oncomouse and other genetically modified cancer-expressing nonhuman mammals in the United States, Canada, Europe, and Japan (Check, 2002). The US, Europe, and Japan, and Australia all granted patents for the transgenic mice without too much debate, as the mice were not considered to be products of nature and were thus patentable. These patents give Harvard University and DuPont exclusive rights to the creation of the mice, as well as charging licensing fees for their use (Ching, 2003).

After years of trying to gain patent rights for the Oncomouse in Canada, Harvard's appeal for their Oncomouse patent was initially approved in Canada on August 2000 by the Federal Court of Appeal (Barrigar, 2002). The Court of Appeal decided that the law would allow these non-natural mice to be patented. The Canadian Patent Act of 1869 defines an invention as a new "manufacture" or "composition of matter."

But the case was appealed to the Supreme court, and in a 5-4 majority decision, the Supreme Court of Canada ruled that the Oncomouse would *not* be allowed to be patented under Canadian law, and that the earlier Federal Court of Appeal for patents had been wrong and misleading (Barrigar, 2002). In its ruling, the Court stated that higher life cannot be patented because it is not a "manufactured" entity. The majority of the vote agreed that higher animal life

forms are not allowed to be considered a part of the definition of invention, while the minority believed that the term "invention" is supposed to cover new and emerging technologies such as the Oncomouse, and that the Patent Act should have a wider definition as technology becomes more advanced (Barrigar, 2002). It was decided that any form of higher life could not be patented until the emerging field of science was reviewed by the Canadian Parliament, and new laws regarding genetically modified animals were passed (Ching, 2003). One of the major concerns with granting patents for animal lives in Canada included the worry of future allowance for human patenting. Justice Ian Binnie, who was involved with the Canadian Oncomouse debate, expressed his concern that by patenting Oncomouse, doors would be opened that would allow humans to be patented and eventually allow legal possession of human life (Ching, 2003).

Although the patent for Oncomouse was denied, the claims for the *methodology* in the production of the mice were not immediately ruled out (Check, 2002). The lives of the mice themselves were considered impermissible for patenting. Just a year later, in 2003, Canada allowed *single-cell organisms* to be patented, as well as genetically modified *plants*. But the Canadian Supreme Court continued to rule that higher, more complex life forms, such as the Oncomouse, were not permissible. Canada continues to disallow any patents on complex life forms today.

Transgenic FDA Approval and Guidelines

In September of 2008, the FDA drafted guidelines proposing that genetically altered animals should be regulated as drugs. The argument for this was that the foreign recombinant DNA is a type of drug. When viewed as drugs, transgenic animals can be investigated thoroughly as any drug is, including safety factors and environmental impacts (FDA to Regulate, 2009). In 2009,

the FDA implemented a policy that would administer the approval and use of transgenic animals. After over ten years of creating the policy's guidelines, genetically modified animals would follow similar guidelines as those pertaining to any new drug.

In early 2009, the FDA approved their first-ever transgenic animal product, ATryn. ATryn is an anti-clotting drug produced by transpharmer goats (as described in Chapters 2 and 3), as a safe drug to be used in humans. This is the first step towards the allowance of marketing and sales for the drug (FDA to Regulate, 2009). ATryn is an anticoagulant used for preventing blood clots in patients with hereditary antithrombin deficiency, a rare inheritable disease that causes harmful blood clots. ATryn comes from a protein that is obtained from the milk of transgenic goats that have been engineered to secrete the protein into the mammary glands and be produced in the milk (FDA.gov, 2009).

The FDA regulates transgenic animals under the Federal Food, Drug, and Cosmetic Act (FFDCA), and the FDA's regulations for new animal drugs. The animals, and any products that come from these animals, are regulated the same way in which any new animal drug is regulated. Transgenic animals are treated as drugs because, according to the FFDCA, anything other than food that intentionally affects the structure or function of the body (either human or animal) is considered a drug. Foreign DNA that is inserted into the genome of an animal – a transgene – is essentially changing the structure and function of what the normal, wild-type animal would be.

Chapter-4 Conclusions

There is much controversy over whether animals should be able to be patented. The Oncomouse patent opened up doors for all kinds of patents on higher life, especially those pertaining to newly engineered transgenic animals. The author of this project believes it is in the

best interest for society, and for research and development, to continue granting patents on life because patenting provides both motivation and incentive for the continuation of finding cures and drugs, and using these animals to better human lives. Although there is serious concern regarding the possible future of cloning humans, it is doubtful and highly unlikely that a any government will ever allow this. Patents encourage a competitive spirit among scientists, and it is this competition and incentive that often results in new ideas and faster progress.

Chapter-4 References

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PROJECT CONCLUSIONS

Transgenic animals have been used in a variety of ways for human benefit. Disease models have provided new knowledge about diseases like HIV/AIDS and Alzheimer's. Some animals have been genetically modified so their organs can be successfully transplanted into humans. Some have been engineered to contain growth hormones for providing better sources of food. Other transgenic animals have been made to produce pharmaceutical proteins in their milk that can be used to treat deadly human diseases. Each one of these applications has had successes and failures, however even each failure can be viewed as a success in the biological world as it still provides new information that was once unknown.

The ethical debate that surrounds transgenic animal research will, most likely, always exist. There will always be groups that want to protect animals from manipulation and experimentation, and others that favor human welfare over the welfare of animals. It is the opinion of this author that transgenic research should continue, as the benefits to society are tremendous. Treatments and drugs for some of the worst diseases could be discovered with transgenic animal research.

There is also much controversy over whether higher life forms such as animals should be able to be patented, but this author believes that patents encourage a competitive spirit among scientists, and it is this competition and incentive that often results in new ideas and faster progress. It is because of this that patents on animals should be granted. The competitive spirit will one day find a cure for AIDS or cancer, or will one day find methods for creating transgenic animals with no animal suffering at all.