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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

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ABSTRACT

This project details transgenic animal technology, applications, ethics and legalities, as an example of technology's impact on society. A transgenic animal has been genetically engineered to incorporate a foreign gene. Transgenic animals can be used as disease models, transpharmers, xenotransplanters, food sources, and other biological models. Although transgenic animals have clearly been documented to benefit society, this technology must weigh these benefits against the potential detriments to the animals or the environment.

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PROJECT OBJECTIVES

The objective of this project was to examine transgenic technology as an example of the effects of technology on society. The first chapter details the various techniques used to create transgenic animals. Chapter two describes the applications of transgenic technology. Chapters three and four examine the ethical and legal issues that stem from this controversial topic. This report aims to provide the information on transgenic animals that is needed for readers to draw their own conclusions on this topic.

CHAPTER-1: TRANSGENIC ANIMAL TECHNOLOGY

Rebecca Cunningham

A transgenic organism is an organism that has been genetically engineered to incorporate a foreign gene. Examples of transgenic organisms include mammals, fish, plants, bacteria and viruses. Transgenic animals include disease models designed to aid our understanding of human diseases, or transpharmers designed to produce life-saving drugs in their milk. Transgenic technology has the potential to save millions of lives by revolutionizing modern medicine and agriculture. Although they have been engineered to benefit society, some types of transgenic animals suffer, so society must weigh the benefits against the detriments to the animals. The purpose of this chapter is to discuss transgenic technology and how such animals are created.

Transgenic animals are created using recombinant deoxyribonucleic acid (rDNA) technology to insert foreign DNA into the animal's genome (Transgenic Mouse, 2005). Pronuclear manipulation and embryonic stem (ES) cell manipulation are the two major techniques used to engineer these animals, but before discussing those techniques, the entire process manipulates the molecule of life, DNA.

Transgenic History

The first transgenic organism was created in 1973 by Stanley N. Cohen and Herbert W. Boyer (Cohen et al., 1973), who were able to construct a new functional plasmid species *in vitro* and insert it into an *E. coli*. Two years later, in February 1975, the Asilomar Conference on recombinant DNA molecules was held in Pacific Grove, California, to assess the risks associated with recombinant DNA research and recommend safety procedures and guidelines (Berg et al., 1975). These procedures also helped prevent and contain biohazards. In the United States, the

National Institutes of Health allowed rDNA research to continue under strict guidelines. In 1974, the world's first transgenic animal was created containing SV40 viral DNA inserted in a mouse genome, although the rDNA was not expressed in this instance (Jaenisch and Mintz, 1974; Transgenic Mouse, 2005). In 1982, the world's first expressing transgenic animal was created, an oversized mouse containing a growth hormone gene under the control of a metallothionein promoter (Palmiter et al., 1982).

DNA

Deoxyribonucleic Acid (DNA) is often called the blueprint of life because it contains the instructions necessary for the construction of cellular components, including ribonucleic acid (RNA) and protein. DNA, RNA and protein are the three main macromolecules essential for all known forms of life (Campbell et al., 1999). Genes are the segments of DNA that contain inherited information. A genome is an organism's unique and complete set of genes (Griffiths et al., 2008).

DNA is a polymer composed of two nucleotide chains (**Figure-1**). Each nucleotide chain has a backbone made from the sugar (deoxyribose) and phosphate. DNA contains four different kinds of nitrogenous bases: adenine (A), thymine (T), guanine (G), and cytosine (C). DNA forms a double helix that is held together by the base pairing of A with T, and G with C. Proteins are synthesized through the transcription of DNA (the synthesis of mRNA) followed by translation (the synthesis of protein) (Griffiths et al., 2008).

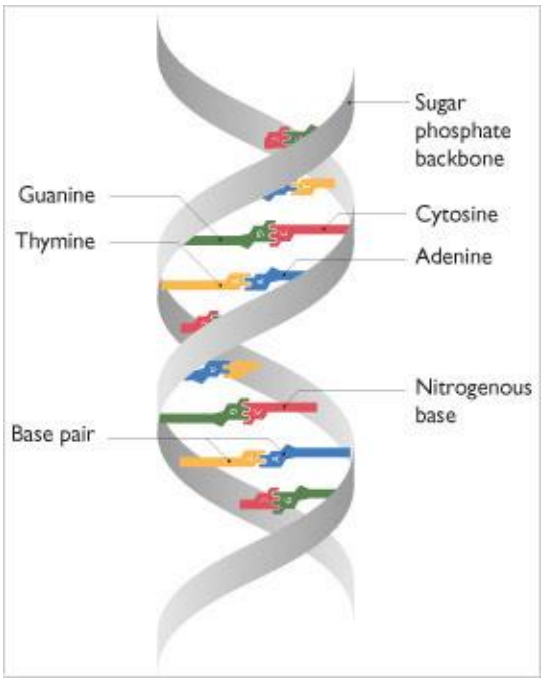

Figure-1: Double Helical Structure of DNA. DNA is a double helix composed of two strands of deoxyribose sugar alternating with phosphate (gray), and four nitrogenous bases (shown in color) (Jones, 2002).

Recombinant DNA Technology Overview

Recombinant DNA (rDNA) represents a new strand of DNA created by combining two or more strands of DNA. rDNA technology is sometimes referred to as cut-and-paste technology because DNA is essentially "cut" using restriction enzymes and then "pasted" and sealed together using DNA ligase (**Figure-2**). rDNA is sometimes referred to as chimeric DNA because it typically contains DNA from two different species. rDNA technology is used to identify, isolate, manipulate, and re-express genes from a given host (Carroll, 1993).

rDNA is usually inserted into a vector system (plasmid, virus, cosmid, or artificial chromosome) to allow the successful propagation of the DNA in a host organism. The most commonly used vector is the plasmid. Plasmids are extra-chromosomal circular DNA found in bacteria (Cohen et al., 1973). The transfer of foreign DNA into a host cell is called

transformation in bacterial cells, and transfection eukaryotic cells. Transduction is the transfer of DNA by a viral vector or cosmid (Carroll, 1993).

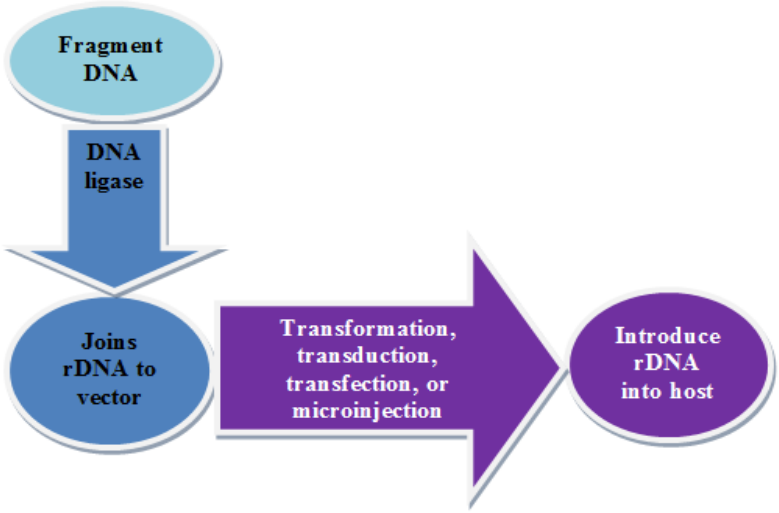

Figure-2: Overview of rDNA Technology. A diagram of the steps used to create a recombinant organism (Carroll, 1993).

Methods for Creating Transgenic Animals

Transgenic animal technologies have come a long way since the creation of the first transgenic mouse in 1974 (Transgenic Mouse, 2005). Many procedures have been developed to increase the efficiency of this generally inefficient process. The three main ways of producing transgenic animals are pronuclear manipulation, embryonic stem (ES) cell manipulation, and nuclear transfer (**Figure-3**).

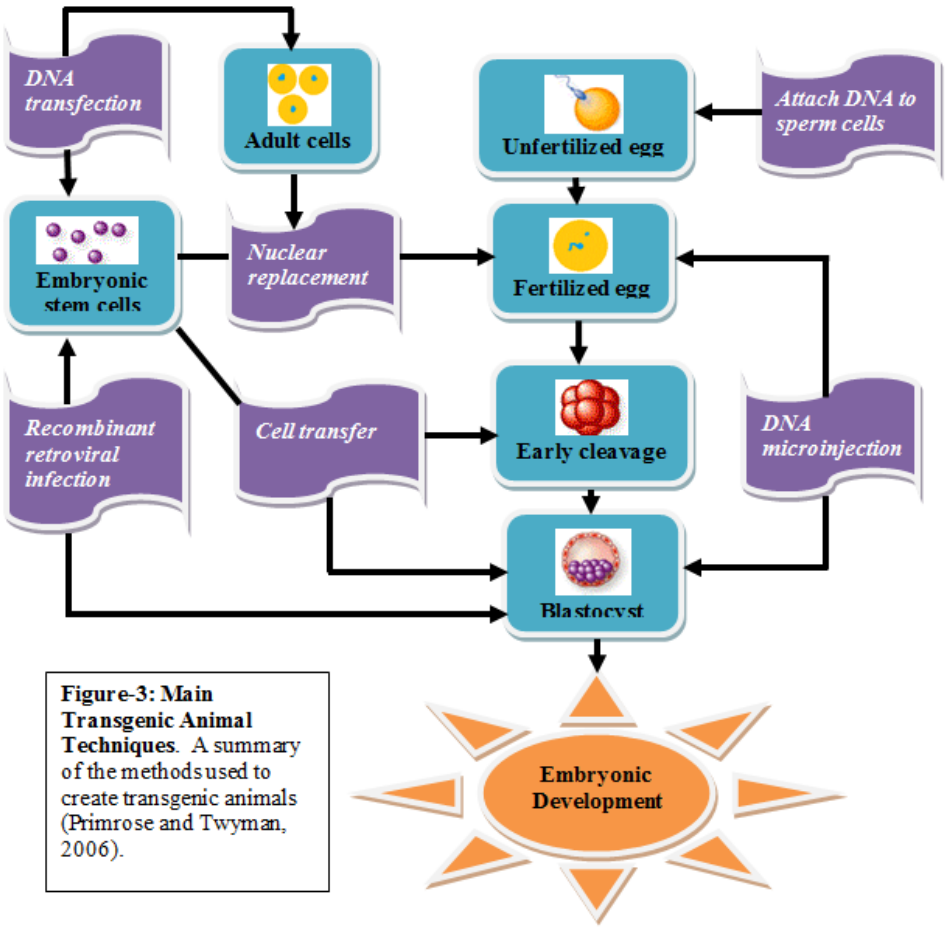

Figure-3: Main Transgenic Animal Techniques. A summary of the methods used to create transgenic animals (Primrose and Twyman, 2006).

Pronuclear Manipulation

The pronuclear microinjection is the most reliable and common method for making a transgenic animal (**Figure-4**). However, the world's first transgenic mice (Jaenisch and Mintz, 1974) were not created by this method. The first transgenic mice created using pronuclear microinjection were created by Gordon and colleagues in 1980 (Gordon et al., 1980). The pronuclear microinjection technique begins by first preparing the rDNA as described previously. Eggs are harvested from super-ovulated animals and are fertilized *in vitro.* The eggs are taken prior to the first cell division while the two pronuclei are still present prior to zygote formation. The male pronucleus is typically selected for microinjection because of its larger size. The rDNA

vector is placed in a syringe and is then microinjected into the pronucleus (Transgenic Animals, 2003).

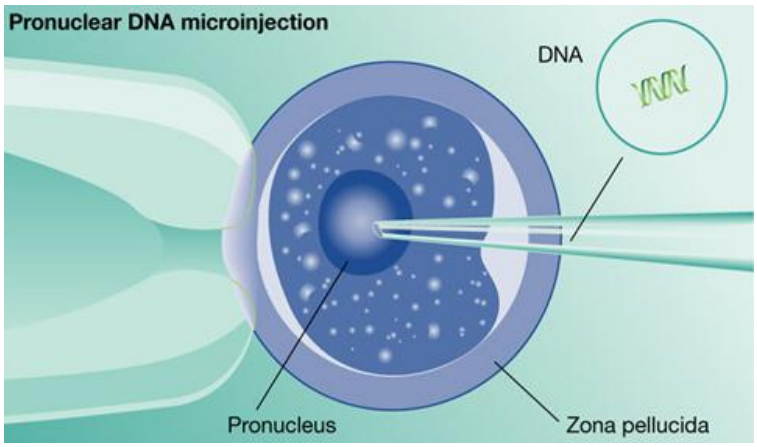

Figure-4: Pronuclear DNA Microinjection. A mild suction pipette (left) is used to hold the egg in place while the glass micropipette (right) microinjects the rDNA into pronucleus (Fässler, 2004).

The microinjected embryo is usually cultured for 5-7 days to increase its vigor. Once the embryo reaches the blastocyst stage *in vitro*, it is implanted into a pseudopregnant female. A pseudo-pregnant female is a female that has been mated with a vasectomized male mouse to prepare the female for pregnancy (Gordon et al., 1980).

Unfortunately, with this technique, the incorporation of the transgene into the genome is a random process, so sometimes the transgene incorporates into an inactive area of the chromosome and is not expressed. However, if the transgene is incorporated into an active region of the host chromosome, then all of the animal's cells will express the transgene. Cells from the pups are screened to confirm the presence of the transgene. DNA pronuclear injection creates pure transgenic animals unlike ES cell manipulation which creates chimeras (Transgenic Animals, 2003).

Other techniques exist for inserting DNA into pronuclei, including electroporation, retroviral infection, and sperm-mediated DNA transfer (Primrose and Twyman, 2006), but pronuclear microinjection remains the most reliable and popular technique.

Embryonic Stem Cell Manipulation

Embryonic stem (ES) cells have the ability to develop into all of the tissues found in the developing embryo. The contribution of these undifferentiated ES cells to the germline was first demonstrated in 1984 (Bradley et al., 1984). ES cells are found in the inner cell mass of the blastocyst. ES cells can be grown into ES cell lines, genetically manipulated, and re-implanted into a blastocyst to create transgenic animals (**Figure-5**). Because some of the blastocyst ES cells represent the original non-transgenic cells, the animals created using this technique are chimeras, not pure transgenic animals, so further breeding of the offspring is required to produce pure transgenic animals (Garvin et al., 1998).

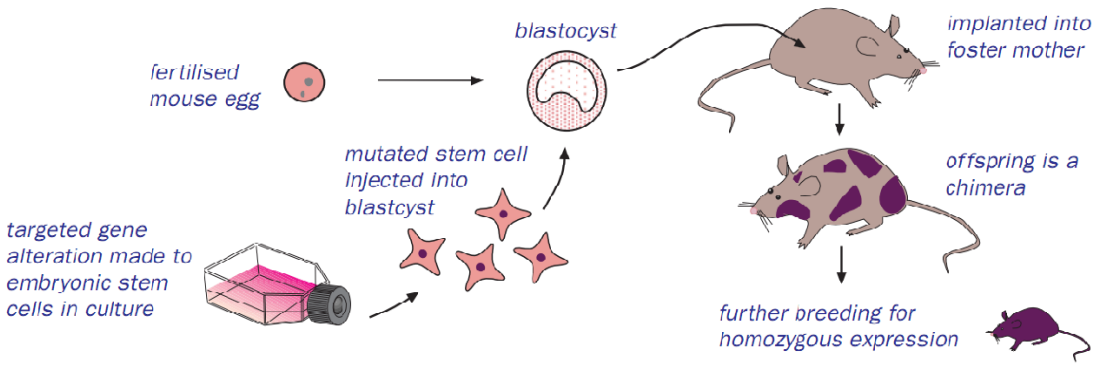

Figure-5: ES Cell Microinjection.
Overview of the steps taken to produce a transgenic mouse by ES cell manipulation (Garvin et al., 1998).

ES cell manipulation begins with the creation of embryo by *in vitro* fertilization (IVF). The embryo is grown for 5-7 days until it reaches blastocyst stage, then ES cells are harvested from the inner cellular mass. The ES cells are cultured, and the transgene is introduced using microinjection, viruses, electroporation, or chemical transfection. The ES cells are then screened to determine which ES cells contain the transgene (Transgenic Animal Science, 1991). The positive ES cells that contain the transgene are inserted into the inner cellular mass of a new blastocyst, and then the manipulated embryo is inserted into the uterus of a pseudo-pregnant host. The offspring are screened to determine which of the offspring are heterozygous for the transgene and then two heterozygous animals are bred to create an animal that is homozygous for the transgene (Transgenic Animals, 2003).

One advantage of ES cell manipulation is that ES cells allow the use of homologous recombination to target where the transgene is inserted in the host's genome. In homologous recombination, regions of host DNA are engineered to flank the transgene. Once the rDNA is inserted into the ES cell, during normal DNA replication and cell division, the homologous DNA regions exchange between the rDNA and the host chromosome, targeting the transgene to the site. So transgenes can be inserted into active areas of the chromosome. The combination of ES cell pluripotency, tolerance of *in vitro* cell manipulation, and capacity for homologous recombination make ES cells an excellent method for creating transgenic animals (Primrose and Twyman, 2006). ES cell manipulation has been successful in creating transgenic mice but has not been used to create other larger mammals (Garvin et al., 1998).

Somatic Cell Nuclear Transfer Technology

In 1996, somatic cell nuclear transfer (SCNT) technology was used to clone Dolly the sheep using six year old cells from a sheep's udder (Campbell et al., 1996). The world's first transgenic lamb, Polly, was also created using SCNT. Polly was born in 1997 and contained the human gene for blood clotting factor IX. Polly's birth proved that creating transgenic animals by SCNT could be successful (Galvin et al., 1998).

SCNT is performed by removing the nucleus of an unfertilized egg cell and replacing it with the nucleus from a somatic donor cell (usually a skin fibroblast cell). To make a transgenic animal, the nucleus is microinjected with transgenic DNA prior to inserting the nucleus in the egg. In order to produce a viable embryo the donor cell genome must be complete, and the egg must be treated with a drug to prevent the extrusion of any DNA in a polar body. An electric current is used to fuse the donor nucleus with the egg cell and to cause the egg cell to divide. The embryos are then implanted into surrogate mothers. The SCNT technique produces transgenic animals in which every cell contains the transgene. One advantage of nuclear transfer is that the gender of the transgenic animal is predetermined, as it will match the sex of the donor of the somatic nucleus (Garvin al., 1998).

Assays for Screening Transgenic Animals

Regardless of which process is used to create transgenic animals, the process in inefficient, and most pups are born non-transgenic. So, the pups must be screened to determine which ones took up the transgene. ES cell manipulation requires that the embryos be screened for the transgene prior to insertion into the blastocyst and then into the foster mother. There are a

variety of screening tests commonly used including: PCR, Southern blotting, Western blotting, and enzyme linked immunosorbant assay (ELISA).

PCR and Southern Blot Tests

Once a potential transgenic animal is born, for mice a short section of tail is taken for analysis. DNA is isolated from the tissue, then screened using the polymerase chain reaction (PCR) technique, or the Southern blotting technique. PCR is used to amplify targeted DNA *in vitro* through a series of polymerization cycles. PCR depends on three temperature dependent steps: template DNA denaturation, primer-template annealing, and DNA synthesis by a thermostable DNA polymerase (Rychlik et al., 1990). If the primers are designed against the transgene, the amplification of a band during PCR indicates the presence of the transgene in the host DNA.

Southern blotting uses restriction enzymes to cut the purified DNA. The DNA fragments are then separated using gel electrophoresis, blotted to a membrane, then exposed to a radiolabeled complement probe of the transgene. X-ray film is used to visualize the radioactive probe. Southern blotting is used to determine the number of copies and even the location of the transgene.

Western Blot Test

Western blotting is very similar to southern blotting, except in this case cellular proteins are separated by electrophoresis. The proteins are blotted to a membrane, and hybridized to an antibody against the transgene. If the transgenic protein is present, the antibody binds the membrane. That antibody can be located using a secondary antibody conjugated to a marker

enzyme that forms a color when reacted with substrate. The difference between Western blotting and Southern blotting is that the former measures expression of the transgene, while the latter measures integration of the transgene (Carroll, 1993).

Enzyme Linked Immunosorbant Assay (ELISA)

ELISA is a biochemical technique used to determine the presence of the transprotein in a fluid like blood or milk. It is a relatively quick and simple method that can be used to quantify the amount of transprotein in a sample. A well in a microtiter dish is coated with antibodies against the transprotein. A test solution (blood or milk) is added to the well, and if the transprotein is present it will be captured by the antibodies and retained in the well during a subsequent wash. Then a secondary antibody is added to detect the captured transprotein. In between each step the well is rinsed with a mild detergent solution to remove all unbound proteins. The remaining protein in the well is used to determine the quantity of transprotein in the original test solution sample (Garvin et al., 1998).

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CHAPTER-2: TRANSGENIC APPLICATIONS

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Transgenic animals have many applications in modern science and medicine. Transgenic animals are divided into five classes based on their purposes. These classes are disease models, transpharmers, xenoplanters, food sources, and biological models. The purpose of this chapter is to describe and provide examples of each class of transgenic animal. This chapter will provide a background on transgenic applications and benefits to society that will serve as an introduction to chapter-3 on transgenic ethics.

Disease Models

Transgenic disease models are animals that have been genetically altered to express some aspect of human disease. Disease models allow scientists to develop potential therapies without the use of human subjects. Once a treatment is successful in animal models, it can be approved for clinical testing in humans. Three successful transgenic disease models that are discussed in this chapter are AIDS mouse, Alzheimer's mouse, and Oncomouse.

AIDS Mouse

Acquired Immune Deficiency Syndrome (AIDS) is the final stages of infection by the retrovirus Human Immunodeficiency Virus (HIV). AIDS is a worldwide pandemic. The CDC estimates that there are more than one million Americans currently living with HIV. In 2009 in Worcester, MA, 42 people were diagnosed with HIV, bringing the total of people living with HIV in our city to 1,375 people (CDC, 2009). There is no known cure or vaccine for AIDS.

Animals are not normally susceptible to HIV infection and AIDS. Monkeys can be infected with the Simian Immunodeficiency Virus (SIV) which is the monkey equivalent to HIV. Monkeys are expensive and cannot be reliably infected with HIV. Transgenic AIDS mice and rats were developed as alternative, less expensive, AIDS models.

The first AIDS mouse was not transgenic; it was a SCID mouse, meaning it lacked an immune system. Human lymph nodes were implanted into the SCID mouse. The implanted human tissue was then infected with HIV (Namikawa et al., 1988). CD4 and CKR5 co-receptors are responsible for the attachment of HIV to human cells. One early transgenic AIDS mouse contained the gene for HIV tat, a viral protein that is needed for HIV infection (Vogel et al., 1988). This early AIDS mouse developed signs of Kaposi's sarcoma, a common condition seen in people infected with AIDS.

Mice are not the only animals used for HIV and AIDS research. Other animals including, monkeys, rats, cats, rabbits, and *Drosophila* have been used as AIDS models. Mice make good models because they are cheap, and much is already known about their genes. Animal models provide important information about the life cycle of HIV and can be used to try and develop treatments and vaccines.

Alzheimer's Mouse

Alzheimer's disease (AD) is an incurable, degenerative, terminal illness. The exact cause of AD is unknown. AD is the most common form of dementia, and is characterized by the development of amyloid plaques in the brain. Amyloid plaques are composed of amyloid β-peptide (Aβ), a fragment of the β-amyloid precursor protein (APP) (Games et al., 1995). Neurofibrillary tangles are twisted strands of tau protein found in dead and dying nerve cells.

Plaques and tangles are found mainly in the cerebral cortex and hippocampus, the parts of the brain responsible for memory and thought. AD usually affects people over 65 years old. Sometimes AD is early-onset affecting people as young as 30 years old (Alzheimer's, 2011).

The world's first Alzheimer's mouse was genetically engineered to incorporate an APP mutation at Worcester Polytechnic Institute in Worcester, Massachusetts, and colleagues at the former Transgenic Sciences Inc. (Games et al., 1995). Under the control of a PDGF-β promoter, the neurotoxic Aβ appeared in the same parts of the Alzheimer's mouse brain as it did in human brains. This first mouse model was used by Elan Pharmaceuticals (South San Francisco, CA) to create an AD vaccine, an antibody against Aβ (Schenk et al., 1999). The AD vaccine was effective in removing amyloid plaques in AD mice. Unfortunately, the first set of human clinical trials were not successful, and were stopped after the vaccine caused swelling of the central nervous system (Herper, 2002), however Elan has initiated a newer clinical trial using a second generation vaccine against a different portion of Aβ that does not appear to cause brain swelling. Phase II trials of Wyeth and Elan's Alzheimer's vaccine ACC-001 were also halted after participants began to develop skin legions (McGuire, 2008).

Oncomouse

Oncomouse (**Figure-1)** was the first patented transgenic animal. Oncomouse was created by Philip Leder and Timothy Stewart of Harvard University in 1984. The term "Oncomouse" actually refers to 13 different strains of mice that were engineered to contain a human oncogene that causes tumor formation (Stewart et al., 1984). This disease model was created to study cancer formation and to screen anti-tumor drugs. The patent for Oncomouse was filed in 1984

(Leder and Stewart, 1984). Four years later Oncomouse became the world's first patented transgenic animal on April 12, 1988.

Figure-1: Oncomouse. Harvard's Oncomouse was created in 1984 by Stewart and Leder (Oncomouse, 2011).

Transpharmers

Transpharmers are genetically engineered to produce a human pharmaceutical in their saliva, milk, urine, or blood. Milk is the most common medium because the pharmaceuticals only have a small amount of contact with the animal's bloodstream, so it has little effect on their physiology. The drug can be collected and purified without harming the animal. Cows, goats, sheep, pigs, rabbits, chicken, and mice have already been used as transpharmers (Genetic, 2011).

Herman the bull the world's first transgenic cow was engineered in September, 1991, by Gen Pharm International of Mountain View, CA. Herman had eight calves in Gen Pharm's European Laboratory in Leiden, The Netherlands (Biotech, 1994). Herman was engineered to carry the human gene for lactoferrin by pronuclear microinjection. Lactoferrin is an iron rich protein that is important for human infant growth. Cow milk and artificial formula do not naturally contain lactoferrin. Herman's female offspring produced lactoferrin in their milk (Brink et al., 2000).

Transpharmers can be created using several approaches. Herman was a lactoferrin founder bull used to create transgenic female offspring. Microinjection can also be directly used to create transgenic females. Nuclear transfer can also be used to create transpharmers. The nuclear transfer approach is popular because gender can be pre-selected, cutting down production time (Brink et al., 2000). **Figure-2** illustrates the three common methods for creating transpharmer cows.

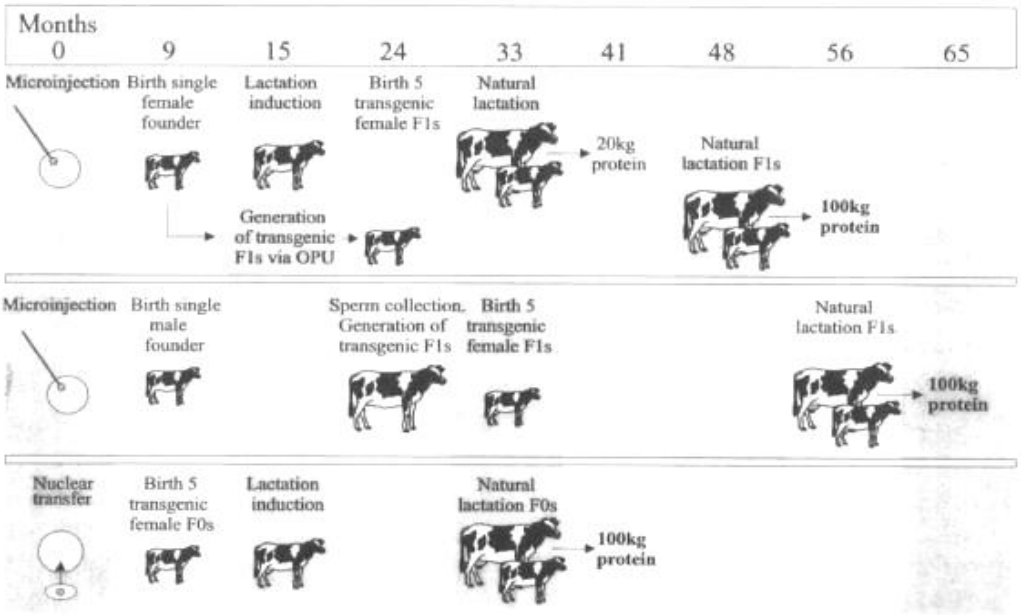

Figure-2: Three Scenarios for Producing Transgenic Cattle Containing Recombinant Protein in their Milk. The figure shows the time lines in months for producing 100 kg of recombinant protein, assuming that a single cow produces 10,000 L of milk in an 8 month lactation period (Brink et al., 2000).

Xenotransplanters

Xenotransplanters are animals that have been genetically modified to have organs that can be transplanted into humans. A xenotransplant is an organ that has been transplanted from a different species. Xenotransplanters are altered so that they do not express key foreign antigens

that cause immuno-rejection. There are currently 111,909 people on the United Network for Organ Sharing (UNOS) waiting list based on OPTN data as of August 23, 2011. The number of transplants performed is limited by a shortage of human organs and tissues. Xenotranplants are a promising alternative to human donors.

The pig is the primary animal used for xenotransplant research. Pigs are used instead of primates because they are widely available and they are not endangered. The size and physiology of pig organs are similar to human organs. Pigs have the sugar α-1,3-galactose (αGal) on the surface of their cells. αGal causes humans to reject pig organs. The enzyme α-1,2-galactosyltransferase (GGAT1) produces αGal (Pearson, 2003). Pigs have been genetically altered to knock out GGAT1, and the genetically modified organs had a much lower instance of rejection in monkeys than the unaltered organs (Pearson, 2003). Porcine heart valves have been successfully transplanted into human hearts for years now, and soon this may extend to livers, kidneys, and lungs. There are concerns about transplanting entire pig organs into humans; including the possibility that porcine viruses may cross the species barrier. But the risks could be minimized by pre-screening the pigs for known viruses.

Food Sources

Transgenic animals have been created in an attempt to produce a new food source. By creating animals that grow larger without much food scientists had hoped to create a more efficient, cost effective, food source. Transgenic animals are not yet produced commercially as food sources because of ethical and safety concerns. They have only been created for research purposes.

Superpig

Superpig was created in an attempt to produce a fast growing, lean animal that consumes less food than normal pigs. The idea was to create a more feed-efficient food source. Superpig was created by microinjecting a gene that expressed either bovine growth hormone or ovine growth hormone (Miller et al., 1989; Pursel et al., 1997). The rate of gain was increased by 15%, feed efficiency by 18%, and carcass fat was reduced by 80% (Rollin, 1996). Unfortunately, Superpig suffered life-shortening pathogenic changes, including kidney and liver problems. Superpig also exhibited lethargy, lameness, uncoordinated gait, bulging eyes, thickening skin, gastric ulcers, severe synovitis, degenerative joint disease, heart disease of various kinds, nephritis, and pneumonia (Rollin, 1996). Scientists decided to put a voluntary moratorium on transgenic experiments with growth hormones in mammals after Superpig suffered such debilitating symptoms.

Superfish

A Superfish does not have the same ethical concerns as Superpig or other mammalian models. AquAdvantage® Salmon (AAS) (**Figure-3**) was developed by AquaBounty Technologies in Waltham, Massachusetts (AquaBounty, 2011). AAS is an Atlantic salmon that contains a gene for Chinook salmon growth hormone. AAS is able to grow to market size in half the time it takes the Atlantic salmon. The Chinook salmon is the largest of the Pacific salmon. AAS is sterile to eliminate the possibility of accidental introduction into the wild (AquaBounty, 2011).

The FDA decided to classify animals with genetically modified traits as veterinary drugs in 2009 (Merris, 2010). The Veterinary Medicine Advisory Committee (VMAC) will hold public

sessions to evaluate the potential benefits, safety concerns and environmental impact of genetically modified salmon. A decision will be made regarding the approval of genetically modified salmon once the VMAC advises FDA's Center for Veterinary Medicine (Merris, 2010).

Figure-3: AquAdvantage® Salmon. An Atlantic salmon that contains the gene for Chinook growth hormone (Marris, 2010).

Transgenic Biological Models

Biological models are created to increase our knowledge about the function of protein by overexpressing the gene encoding that protein, or knocking it out. Transgenic biological models can help us learn more about the fields of biology and genetics. Examples of transgenic animals that have been created for this purpose include ANDi, Smart mouse, and Youth mouse.

ANDi

The world's first transgenic monkey was ANDi. ANDi was one of three rhesus monkeys born that contained the transgene for the green fluorescent protein (GFP) in all tissues. These transgenic monkeys were produced by retroviral gene transfer into mature oocytes (Chan et al., 2001). A fraternal set of twins miscarried at 73 days and three males were born (including ANDi) from the 20 embryo transfers. The fraternal set of twins were confirmed to have carried the transgene by Southern blot analysis (Chan et al., 2001).

Smart Mouse

In 1999 researchers created smart mice that were genetically modified to have superior learning and memory. These mice were named Doogie, and were modified to over-express the NR2B transgene in the cortex and hippocampus, with little expression in the thalamus, brainstem and cerebellum (Tang et al., 1999). Behavioral tests were administered to access learning and memory of the transgenic mice compared to their non-transgenic litter mates. The transgenic mice were found to learn faster and remember objects four to five longer than their normal litter mates (Harmon, 1999).

Youth Mouse

There is a direct correlation between food consumption and life span across multiple species. αMUPA is a line of transgenic mice that eat ~20% less and live ~20% longer than the wild type (Miskin et al., 1999). The αMUPA mice exhibited a young look, even at old ages (**Figure-4)**. Compared to the wild type mice, αMUPA mice had a lower body temperature. The spontaneous decrease in food consumption corresponded to a decrease in body temperature in αMUPA mice at all ages (Miskin et al., 1999). The homeostatic state of delayed aging can be investigated using this transgenic biological model.

Figure-4: αMUPA and Wild Type mice. After 30 months αMUPA mice (right) still appear young compared to the wild type (left).

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Chapter-3: Transgenic Ethics

Andrew Reed

Historically, all types of animals have gone through evolution that has changed their looks and properties. Some change has been natural, while selective breeding has brought about other changes. But with transgenic technology, animal properties can be exchanged between species. Transgenic animals have already provided many benefits to humans including the production of drugs and food, the reduced need to use pesticides or herbicides, and a reduction in the overall number of animals used for scientific testing. But just because we have the ability to create such animals, should we? This chapter will investigate the ethics of transgenic animals, balancing the benefit to society against the detriment to the animals.

Disease Model Ethics

Disease models are animals engineered to mimic specific aspects of human disorders for the purpose of studying disease formation or for testing potential therapies. Examples of animals in this section (discussed in Chapter-1) are Alzheimer's mouse, Oncomouse, and HIV mouse. Disease models are one of the most debated groups of transgenic animals, as they have strong benefits to society while the animals can suffer. So the ethics of this group involves weighing the extent of medical information we can obtain to cure a disease versus how much the animal suffers. The medical benefits are among the highest for any class of transgenic animal, as they give hope to some of the sickest people in the world.

Disease models are needed to help find cures for diseases. When drug companies are testing a new drug, these drugs cannot be tested in humans until their relative safety is first

proven in animals. So scientists use animals to make sure there are no drug side effects. Some of these effects may only happen in a small percentage of the human population, but drug companies are not given permission to test high numbers of patients in phase-I clinical trials for safety, so they need to test fairly large numbers of mice. But mice don't get many human diseases in nature, so scientists must engineer them to mimic these diseases. The animal testing in these situations is crucial if people want to eventually find the cures that they are looking for.

Oncomouse has a huge benefit for medicine because it has taught scientists much about carcinogenesis (cancer formation), and has allowed drugs to be rapidly screened for fighting cancer. But Oncomice can suffer, especially when the tumors are allowed to grow to the advanced stage. As discussed in Chapter-4 on Transgenic Legalities, the probability that Oncomouse would help find a cure for cancer (and provide profit to its creators) was so high that Harvard Medical School and Dupont eventually received a patent on it. The United States Patent Office in 1988 granted patent no. 4,736,866 to Harvard College claiming 'a transgenic non-human mammal whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal…' (Leder and Stewart, 1984). The claim explicitly excluded humans, apparently reflecting the moral and legal concerns about patents on human beings, and about modification of the human genome" (WIPO Magazine 2006). Although Oncomouse was later patented in Europe and Japan, it was denied in Canada, where the Canadian Supreme Court said, "A higher life form is not patentable because it is not a 'manufacture' or 'composition of matter'" (Check, 2002). Following this case, ethical questions arose with respect to the question of suffering caused to the animal? (Check, 2002).

With respect to whether Oncomouse experiments should be continued, the author of this chapter believes that testing should be continued because there is much to be learned from them

about cancer formation. Because the mice suffer with advanced tumor formation, pain killers should be mandated by institutional IACUC committees whenever possible, and the animals should be sacrificed before advanced tumor formation if the experiment allows that.

With respect to Alzheimer's mouse, this mouse also provides a strong societal benefit. The American Alzheimer's Association predicts that over time the 5.4 million people that already have Alzheimer's could grow to over 20 million over the next couple of decades. The original creation of this mouse (Games et al., 1995) provided proof that the formation of neurotoxic Aβ initiates the disease. And the mouse line was used by Elan Pharmaceuticals (South San Francisco, CA) to create the world's first Alzheimer's vaccine, initially tested in mice (Schenk et al., 1999), and later in human clinical trials (Elan, 2011). Thus, this transgenic mouse serves as a good example for how using information gained from animals can be eventually applied to humans. With respect to this line of experimentation, the author of this chapter believes that it should be continued. The mouse line does not appear to suffer by any measurable criteria (there are only small behavioral alterations), yet it has already provided strong societal benefits.

Transgenic Art Ethics

Should we be able to change the genes in animals to make them look "cool" or make it easier to identify the animals? Should we be allowed to genetically engineer animals to aggressively occupy a niche in the ecosystem to destroy another invasive species? There is little scientific knowledge to be made from making such animals, but such experiments are currently being done by private organizations. The one that I found the most interesting is "Alba," the rabbit that glows under UV light, designed by Eduardo Kac. The rabbit is part of Eduardo Kac's plan to create "transgenic art." This plan is a new way of humans interacting with animals and

transforming them solely for the purpose of art. Kac is hoping that artists can create their own species of animals (Kac, 1998). As expected, this line of experimentation came under strong attack because not only was there a plan in place if the animals escaped, but it really had no societal benefit. The author of this chapter believes this is a "cool concept", but since there is no medical benefit it should not be continued.

Transpharmer Ethics

Transpharming is the use of animals to create a life-saving pharmaceutical drug in the milk or blood. Milk is the preferred site of production because the presence of the product has little effect on the animal's physiology, and isolation of the drug does not harm the animal. Transpharmer animals include mice, cows, sheep, and goats. The author of this chapter believes that this line of experimentation should be continued because of the medical benefits and lack of animal suffering.

Xenotransplanter Ethics

While it may sound strange to place an animal's organ into a human, this may eventually turn out to be one of the best transgenic applications yet. A girl in Mexico, after getting newborn pig cells, has not required any insulin or other drugs for her diabetes. This procedure used a new technique which implanted two different types of cells, insulin producing β-cells and sertoli cells, which prevent other cells from attacking the implant. This was the first xenotrasplant that lasted a long time. This idea is not a new one, but has become more popular recently as more patients await organ transplants. Pig hearts are now being used for heart transplants when human donors are not available (Leahy and LePage, 2002). The pig heart valves seem to work

extremely well and have no diseases or medical malfunction.

But some people are concerned with the possibility that pig viruses might jump species into their human host and cause disease. Pigs are well known to carry influenza viruses that can infect humans (swine flu), and they can carry retroviruses. So the author of this chapter agrees with scientists who argue that any pigs used for organ donation should be screened in advance for pig viruses that are known to cause diseases in humans.

Transgenic Food Source Ethics

Chapter-1 discussed examples of transgenic animals engineered for consumption. In this category of transgenic animals, the ethics focuses on the benefit to mankind of helping alleviate hunger, versus the death of the animal to provide man's food. This debate is not specific to transgenic animals, but could be applied to any farm animal currently sacrificed for our food.

One successful example of a transgenic food source is Aquabounty's Superfish that grows to a larger size on less food. This fish was designed for the aquaculture industry, and is near to receiving FDA approval as the world's first transgenic animal for consumption (Marris, 2010). This fish will enable the aquaculture industry to raise larger fish cheaper. The ethical question here is should we be changing the size of animals for our benefit. In fact, mankind has been increasing the size and other desirable characteristics of livestock for generations by traditional breeding, so this debate is not new. I feel the type of experimentation should continue, as it will eventually provide a crucial food source to third world countries that do not have access to much meat.

Another example of a transgenic food source discussed in Chapter-1 was Superpig, who also grew to a large size faster than his non-transgenic littermates. Although this animal is

similar to Superfish in that they both grow to large sizes, Superpig developed severe health problems, including severe arthritis and large tumors (Rollin, 1996). The health problems became so bad the animal had to be euthanized, and scientists put in place a voluntary moratorium banning all growth hormone experiments in mammals.

Scientific Model Ethics

Transgenic scientific models are created to test the function of specific proteins *in vivo* by over-expressing them or under-expressing them, then assaying the changes to the animal. These animals are a great help to the human community when trying to learn about the effects of specific proteins on the body, and are a huge help expanding what we know about genetics and biology. For most of these models, the animals do not suffer, but some do not survive the altered gene expression patterns.

One example discussed in Chapter-1 was Doogie the Smartmouse. This mouse was created by a Princeton scientist (Tang et al., 1999), who over-expressed the NR2B subunit of the glutamate receptor. This subunit predominates when mammals are young and can learn more easily, so the hypothesis was that by over-expressing NR2B, the mice would learn faster, which they did. This mouse was a breakthrough in memory research and revealed a common biochemical mechanism involving the glutamate receptor at the root of nearly all learning. It shows that the brain uses the same basic mechanism even though parts of the brain deal with diverse types of information, such as sights, sounds, and touch (Harmon, 1999). So it is now plausible to think that we could change the genetics of an animal to make them smarter. So with respect to ethics, should we be thinking about engineering humans to make them smarter? This is too large a jump for society to even consider now, but the author of this chapter believes that

Smartmouse provides an excellent example of how such animals can provide valuable scientific information without harming the animal.

Chapter-3 Conclusions

It is the author's conclusion that all categories of transgenic animals being tested are helpful to society. The social benefit in my mind far outweighs animal sacrifice to save lives for cancer, Alzheimer's disease, HIV, hunger, organ transplants, etc. Millions of people dying every year because of these diseases, so further transgenic testing should be continued. Having family members that have had cancer, I believe we should especially continue all cancer transgenic experiments, even if that includes the death of some of the animals. When saving human lives, the benefits greatly outweigh the death of the mice.

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Chapter-4: Transgenic Legalities

Andrew Reed

The United States' laws for allowing patents are challenging, but when trying to patent a life form these laws become even more challenging. The purpose of this chapter is to discuss the patenting of transgenic animals, and whether such patents facilitate medical research or hinder it.

In the US, the definition of a patent is as follows, "any new and useful art, machine, manufacture or composition of matter, and any new and useful improvement on any art, machine, manufacture or composition of matter" (A Brief History, 2003). The invention must be novel (not created before), and not obvious to most people in that field. With respect to patenting life, the courts debated the phrase "composition of matter" and whether it applied to animals. Before the first transgenic animal patent was awarded, the courts first had to approve a patent for a microbe in *Diamond v Chakrabarty*.

Diamond v. Chakrabarty

Dr. Chakrabarty was a microbiologist and one forefathers in the field of engineering microbes. He engineered a *Pseudomonas* bacterium to be able to break down crude oil. In 1972, he filed his patent with 36 claims related to "a bacterium from the genus *Pseudomonas* containing therein at least two stable energy-generating plasmids, each of said plasmids providing a separate hydrocarbon degradative pathway" (*Diamond v Chakrabarty*, 1980). "Chakrabarty's patent claims were of three types: 1) process claims for the method of producing the bacteria (447 USC 306), 2) claims for an inoculum comprised of a carrier material floating on water, such as straw, containing the new bacteria, and 3) claims to the bacteria themselves (206 USC 196). The patent examiner initially allowed the claims from the first two categories,

but rejected the claim for the bacteria. His decision rested on two grounds: (1) that micro-organisms are "products of nature," not composition of matter, and (2) that as living things they are not patentable subject matter under 35 USC §101 (*Diamond v Chakrabarty*, 1980).

When Chakrabarty realized one of his claims was not going to be allowed, he filed an appeal. He first relied on the legislative history of the 1930 Plant Patent Act, in which congress allowed patent protection to asexually produced plants. But the Appeals Board said that US §101 was not envisioned to cover living microorganisms, so the Plant Patent Act was ruled outdated for this purpose. If Chakrabarty wanted to get the appeal, he would have to find another case as precedent.

Almost two years later, on May 20, 1976, the three-man Appeals Board conceded that Chakrabarty's bacteria did not occur naturally, so were not products of nature, but it still upheld the rejection of the third claim on a new explicit ground -- that the bacteria were not patentable because they were living organisms (Kevles, 2002). But after waiting five years from the start of the case, and being in several lawsuits to try to get the patent passed, MaLossi argued Chakrabarty's case on December 5, 1977, and on March 2, 1978 the Court ruled three-to-two in Chakrabarty's favor. Judge Rich, speaking for the majority, saw only one issue --- the patentability of living organisms, and agreed to allow patents for microbes.

This landmark case was cited considerably in the subsequent Oncomouse case for patenting animals, and is the reason that animals can now be patented.

Animal Patents

Following the Chakrabarty court case allowing the patenting of a living microbe, patents were filed in 1984 by Harvard and Dupont for Oncomouse (Leder and Stewart, 1984). This

filing initiated fierce debate in the courts as to whether multicellular organisms including animals could be patented. Finally, on April 21, 1987, about ten years after the case of *Diamond v. Chakrabarty,* the US Patent and Trade Office (PTO) released this statement: "The Patent and Trademark Office now considers non-naturally occurring non-human multicellular living organisms, including animals, to be patentable subject matter within the scope of 35 U.S.C. 101" (Patent and Trademark Office Notice, 1987). The PTO statement opened the door for the Oncomouse case; the award given in 1988 was the first animal patented, and was the first patent allowed under the 1987 PTO ruling that animals could now be protected under the new patent law. This case in the history of United States cases was one of the most complex and included several appeals, but was eventually passed and is now viewed by all people as a breakthrough case.

Since the landmark Oncomouse court case, over 800 animals have been patented including birds, sheep, pigs, fish, horses, and chimps. Not only are the animals themselves patented, but in some cases the award extends to include the process used to make the animal. The number of patents grew as the technology advanced (Environment News Service, 2000). In 2009, the US FDA published new simpler guidelines for patenting transgenic animals (FDA.gov, 2009), and this makes it even easier to patent these animals today. The new guidelines should make the filing and approval of animal patents much more routine, and make it easier for biotechnology companies to raise money if investors feel that patents likely will be awarded.

Oncomouse In Canada

While the Oncomouse patent was awarded in the US, Europe and Japan, that was not the case in Canada. In Canada the courts argue that life cannot be patented (Bird and MacOdrum,

2008). "In their ruling that a patent should not be granted for the mouse, the Canadian Court rejected the argument that the genetically engineered mouse constitutes either a "manufacture" or a "composition of matter" within the statutory definition of "invention" in the Canadian *Patent Act.* Interestingly, the majority of the Supreme Court members accepted that the genetically altered egg obtained by injecting the oncogene into a fertilized egg satisfies the definition of "composition of matter", but they still rejected the patent (Check, 2002; Barrigar 2008). Canada remains the only country where Dupont applied for the Oncomouse patent where it was not awarded.

Erythropoietin and the World-Wide Patent War

Erythropoietin (EPO) is a hormone that controls red blood cell production. EPO patents are considered the most wanted patents in the world because of the billions in annual sales. EPO patents are litigated in over 30 countries worldwide using powerful legal teams.

> "EPO, a glycoprotein, acts as a hormone that stimulates the production of red blood cells and is mainly produced in the kidney. It is well known to make the headlines as doping in sports. Its main use and also value, however, is the treatment of anaemia associated with kidney insufficiency and other chronic anaemias, e.g. resulting from chemotherapies, bone marrow transplantations or HIV infections. The volume of the global market for EPO has been estimated to nearly USD 10 billion per year, and patent protection plays a key role for access to this market." (Schutt, 2004)

Agmen, a pharmaceutical company, first cloned the EPO gene allowing its mass production in genetically engineered cells. They eventually received a patent on the process. Then another company, TKT, created a system that allowed the EPO-synthesizing cells to divide twice as fast. Agmen sued TKT arguing patent infringement, which was eventually upheld. Blockbuster drugs with billions of dollars at stake tell interesting stories about initial patent

protection for scientists and companies who initially created the new drugs, but then most everyone else, including the patients, are happy once the patent expires and the drug can go generic, saving patients much money.

Benefits of Patenting Transgenic Animals

Designing a new transgenic animal is not easy. For example, when making a new disease model, years in the lab must be spent understanding enough about the disease in humans to be able to mimic its formation in mice by implanting a few carefully chosen genes. And when making a new transpharmer, years must be spent deciding which human protein is worth manufacturing in large quantities, and in proving it can be produced in mammary tissue at full bioactivity. This requires large amounts of investment cash, and the investment can be risky depending on how far along the research has progressed. "It is obvious that biotechnological advances have many uses that will benefit public health and wellness, but the research required to create these advances is very expensive to undertake" (Walter, 1998). If the work is performed as part of a start-up company, as the project costs increase beyond the ability of the small company to pay, it will often seek larger pharmaceutical company as a partner to help shoulder the costs. Once the transgenic animal has been successfully created, most investors want to protect the invention with a patent to ensure the invention generates some revenue. So in this sense, patents can sometimes help facilitate the advancement of medical science by helping ensure more money comes back to the company to allow more advances.

> "Patents stimulate the growth of industry, and the biotechnology industry welcomes any patent protection it receives. Due to the controversial nature of patenting "life," or products intimately associated with life, it is necessary to pursue patent protection with solid grounding in patent law that is adequately suited to biological advances." (Walter, 1998)

Negatives of Patenting Transgenic Animals

In some cases, scientists have argued that patenting a transgenic animal has actually *hindered* scientific research. This was the case in the early years following the patenting of Oncomouse when Harvard and Dupont charged a large licensing fee for any lab to work with the animal (Marshall, 2002). This allowed large relatively wealthy labs to experiment with Oncomouse, but left out smaller labs that could not easily afford the fee. This seemed unfair for the smaller labs because they had the same technology. This helped smaller labs because they were then able to do the research for the price that was right for them. As time went on these fees were lowered which allowed some of the smaller labs to be able to afford the price.

In other cases, people are concerned with the total cost of producing the animal versus how else that money could have been spent. For example, should the several million dollars it took to create ANDi the world's first transgenic primate who contained a jellyfish gene have been better spent on providing vaccines to the poor? This question is hard to answer until the subsequent benefits of ANDi are more fully realized with the production of other transgenic primates that directly provide a benefit. There are no guarantees in this industry, so in the end, for some cases it could use millions of dollars to create an animal that in the end provides no benefit to society. However, this gets to the heart of the debate about long term research dollars versus direct medical care, and if research dollars are discontinued, some diseases will not be cured.

In other cases, the public is worried about the escape of transgenic animals into the wild. For example, with AquaBounty's Superfish some are worried they may escape their fenced in cage to interbreed with other wild type Salmon, passing on their genetic modification. However, in this case, AquaBounty made the Superfish sterile, so they are incapable of breeding, but the main point of an environmental escape is always worth our planning for. A Purdue professor

Bill Muir did a study on transgenic Medaka (Japanese fish) and put them in a tank with Atlantic salmon and the study showed that when these transgenic fish are partnered with the normal fish things could be catastrophic. These professors say that other fish could become extinct because of the larger size of the transgenic fish and also confer an advantage in attracting mates. (Purdue.edu 2000) Based on this information it is key that these animals are kept with their own breeds until there is further information and studies showing that this would not happen to the natural animals

Some religious leaders have a problem with patenting life because it places a price tag on life that should not come with a price. This topic was covered in Chapter-3, and in the end some of these individuals may never be convinced regardless of the human lives saved. Other people are concerned that now that animal patents are allowed, will transgenic human patents be next? Will we allow genetic modifications to be made to humans to make them more disease resistant? We currently allow genes to be inserted to treat specific genetic disorders in gene therapy, but genetically altering a normal human is not currently allowed.

In any case, due to ethical problems associated with some categories of transgenic animals and their patenting, the industry would be better served by a more open patenting process with better visibility to the public. With better visibility and information, the public would be better equipped to enter into discussions of transgenic ethics in full view of their medical benefits.

FDA Guidelines

The new patenting guidelines for transgenic animals provided by the FDA in 2009 should help in this process by making the process simpler and more open (FDA.gov, 2009). "The

FFDCA defines "articles (other than food) intended to affect the structure or any function of the body of man or other animals" as drugs. An rDNA construct that is in a GE animal and is intended to affect the animal's structure or function meets the definition of an animal drug, whether the animal is intended for food, or used to produce another substance. Developers of these animals must demonstrate that the construct and any new products expressed from the inserted construct are safe for the health of the GE animal and, if they are food animals, for food consumption." (FDA.gov, 2009) This new definition provided by the FDA will greatly help people understand the transgender legalities. The FDA outlined the animals that can be copied as generics and can be seen in the FDA Green Book. (FDA.gov, 2009)

> "The GADPTRA provides for a period of 3 years of marketing exclusivity for a new use of an animal drug (a use that required reports of new clinical or field investigations for its approval), during which time no abbreviated application for a generic copy may be approved for the new use. The law provides for a period of 5 years of marketing exclusivity for an animal drug that has not been previously approved in any new animal drug application. During this period, no abbreviated application may be submitted. (Exception: An abbreviated application may be submitted after 4 years if the generic applicant claims non-infringement of a listed patent that is claimed for the approved product or its use.) The law also provides for another form of marketing exclusivity, known as patent term restoration. This type of exclusivity extends the period of protection by U.S. patent for an animal drug, or its method of use, that was approved after November 16, 1988, to compensate for the time that was required for investigation and regulatory review of the animal drug prior to its approval. Patent term restoration is not related to the exclusivity periods described above and may overlap those exclusivity periods." (FDA.gov, 2009)

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PROJECT CONCLUSIONS

A transgenic animal has been engineered to have a foreign gene inserted in its genome. The foreign gene causes the animal to express characteristics that are not normally found in nature. Potential applications include the ability of the animal to produce a human pharmaceutical in its milk, or the ability to serve as a human disease model. Transgenic animals can be created using pronuclear manipulation, embryonic stem cell manipulation, or nuclear transfer. In pronuclear manipulation and embryonic stem cell manipulation, the transgene of interest is usually isolated using restriction nucleases or it can be amplified by polymerase chain reaction (PCR), and then inserted into an embryo using a vector system. Vector systems include viruses, cosmids, and plasmids. The plasmid is the most commonly used vector. Transgenic technology is not efficient; most of the offspring will not contain the transgene or may be a chimera depending on the method used. Thus, potential transgenic pups are typically screened by PCR or by Southern blot analysis to detect the presence of the transgene in the animal's DNA.

Transgenic animals can be divided into five main categories: disease models (that mimic specific aspects of a human disorder), transpharmers (that produce human pharmaceuticals), xenotransplanters (that produce organs for human transplants), food sources (for consumption), and biological models (that study the effects of specific proteins *in vivo*). Each category was examined, weighing the benefits to society with the detriments to animals or the environment. Disease models, transpharmers, and xenotransplanters, have strong medical benefits, however some disease models are especially prone to pain and suffering (i.e. Oncomouse). To minimize this suffering strong regulations are necessary. Painkillers and euthanasia should be used to prevent animal suffering. Disease models, transpharmers, and

xenotransplanters offer potential cures to cancer, new drug delivery methods to newborns via milk, and a potential solution to the human organ shortage. Research in these categories should be continued since the applications have the potential to save millions of lives.

Transgenic animal food sources are one of the most controversial categories of transgenic research because as they would be consumed by humans. "Super animals" have been created to grow faster with less food. Unfortunately, Superpig developed extremely serious side effects and had to be euthanized. The authors believe the creation of "Super mammals" should only be revisited if absolutely necessary for human survival, because of the severe side effects exhibited in the case of Superpig. However, Superfish have been very successful with no observed negative side effects. AquaBounty's salmon and trout had no observable deleterious effects and soon will be approved by the FDA for human consumption. To ensure that AquaBounty's Superfish do not breed they are sterilized; this helps ensure that delicate ecosystems are not affected in the event of an escape.

The patenting of life is a controversial topic in transgenic research. The world's first patented *lifeform* was Chakrabarty's bacteria, engineered to consume oil slicks. This was a very difficult patent to obtain, but its lead the way for the world's first *animal* patent, for Oncomouse. Canada does not allow the patenting of animals. Patenting allows a company to protect its profits which can increase their funds for other medical research. However, patents can also have a negative effect on research if the fees are so high they discourage smaller labs from performing research.

Based on the findings of this project, the authors of this project believe that all five major classes of transgenic animals should be continued with extreme caution. In all cases, every effort should be made to minimize any animal suffering. Containment procedures should be

implemented to prevent the introduction of a foreign species into the environment. The authors also believe that transgenic fish should be approved by the FDA to help fight world hunger, but agree that any "Super mammals" (such as Superpig) should be disallowed. In all cases, strong legislative oversights should be followed to help ensure that any experiments gone wrong are contained and terminated.