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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

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of the

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Degree of Bachelor of Science

By

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Abstract

The goal of this project was to show how genetic modification, used to create transgenic animals, affects society. Each section is defined to provide background on the methods used for creation, examples of transgenic animals that have been created, a summary of the legal and ethical issues encountered, and to express project team members' feelings and reactions to the information presented. There are great societal advantages associated with creating transgenic animals, so long as ethical issues are considered.

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Executive Summary

Modern medicinal science, in its endless effort to free humanity from its frailties, has created incredible tools to battle our most severe ailments. Perhaps the most notable and ingenious development of the 20th century has been the ability of doctors and scientists to alter the DNA of a living organism. These techniques have been used on all forms of life, from single celled bacteria, to plants to animals. While genetically modified bacteria are broadly useful for generating proteins and even cleaning oil spills, genetically modified animals have opened up huge possibilities for the research of human disease causes and cures. The ability to generate these Transgenic Animals has allowed doctors to imitate and model normal human functions in ways never before possible.

Ordinary lab mice can now be modified to be susceptible to specific human diseases, providing doctors with an invaluable model for testing prevention and treatment. Doctors can now use otherwise ordinary genetically modified farm animals to produce human proteins in their milk or eggs. Progress has even been made towards growing animals that are suitable xenotransplantation donors (xenotransplantation is the process of replacing a human organ with an animal organ).

The advent of a new technology eventually creates a need for regulation. The ability to create new and non-natural animals has raised issues at all levels of government. The most notable of these questions has been patent protection for living organisms. Although the PTO originally refused patent protection for organisms that had

been genetically altered, the Supreme Court overturned that refusal based on the wording of the patent statutes in the United States Code. Patent protection was thus allowed and has since been granted to animals as well. The PTO has been definite in its refusal to patent anything human in nature, but has still granted patents on methods involved with human embryonic stem cell research. With the expansion of human ES cell research caused by the recent allowance of federal funding for some of these experiments, these patents have generated significant controversy and are currently the object of legal action.

As is to be expected with any concept so novel and powerful, the creation of transgenic animals has generated its share of moral controversy as well. Fundamental questions such as whether humans are within their right to "play god" with the natural expression of life are important, and have received their share of debate. With any scientific experimentation the subjects' lives are altered to some extent. Some suffer no worse than any captive animal and in fact live very comfortable, disease-free lives. Some suffer a great deal and many are sacrificed. Many people whose beliefs include the sacredness of animals are appalled at animal experimentation. When that experimentation crosses the line from beast to human, such as it has for human embryonic stem cell research, many more people protest. If you discuss the rights of laboratory mice to a cancer patient, however, be prepared to hear a different perspective.

As with any new technology there is potential for disaster. Contamination of the natural wild species, dangerous mutations, and new diseases are all possibilities that need

to be addressed. Although the eventual good seems destined to outweigh the possible bad, these potential harms need consideration.

Transgenic technology is remarkable. It is changing the face of medicine, science, agriculture and industry right now. With any powerful new technology there are drawbacks as well as a chance for disaster, but there is no stopping progress. Scientists are going to keep doing what they do best, commerce will always try to commercialize, and everyday people will constantly be drawn by the words, "cheaper", "better", and "healthy". Should we be careful? Always. Should the welfare of the animals be considered? Of course, quantitatively and qualitatively, who's lives are more important, the mice or ours'? Some questions aren't answered so easily. For now we need to be open minded and forward thinking to make our newfound knowledge work for our planet and us.

Project Objective

The purpose of this Interactive Qualifying Project was to research the topic of transgenic animals, determining the effect of this new technology on society, and presenting the information in layman's terms. In order to fully understand the topic, we began by investigating the various methods used for creating transgenic animals. Within this document you will find several examples of transgenic animals that have been created, as well as the purpose of their creation. There is a summary of ethical considerations that a scientist must evaluate before creating a transgenic animal. In addition, there is a section describing the legal issues of transgenic technology. In conclusion we present a summary of the writers' view on the information presented.

Chapter 1: Introduction to Transgenic Animals

What is a transgenic animal?

The term transgenic animal refers to an animal that has been created by deliberate modification of the animal's genes (Buy, 1997b). A gene is a unit that contains a sequence of deoxyribonucleic acid (DNA) that is responsible for creating specific characteristics of an organism (Lexico LLC, 2001).

Why are they created?

Transgenic animals are being produced in order to increase resistance to disease, food safety, animal productivity, and to create medicines for humans (AviGenics, 2000). In addition, transgenic animals may be used for creating organs used in xenotransplantation procedures (Altweb, 2001). Xenotransplantation is a process involving the transfer of an organ from one species to another (Winston, 2000). The main advantages of creating transgenic animals for medical purposes are the low-costs and simplicity of production scale-up compared to mammalian cell culturing (Yangene, 2000). To illustrate the differences of cell culturing versus transgenic methods, a study completed by the Wall Street Journal and AviGenics, a transgenic chicken company, has provided estimated costs of monoclonal antibody production from mammalian cell cultures versus transgenic chicken and goat protein collections, seen in Table 1.1 below.

	Method of Production		
	Cell Culture	**Chickens**	**Goats**
Expression Level (g/egg or g/liter)	0.1-10	1	20-25
Total Raw Material Volume	170,000	250	21,000
Reactor Capacity	8,500	400 Hens	35 goats
Cost per Animal	$100 Million	$1,000 per	$10,000-$50,000
Annual Maintenance or Keeping Cost per Animal, $	$100,000	$10	$2,500
Unit Cost of Protein, $/g	$100	$0.10-0.25	$2-20

Table 1.1. Relative Costs for 100kg of raw material per year of monoclonal antibody (Mab) Production Source: Wall Street Journal & Avigenics Estimates, 2000.

How are transgenic animals created?

In transgenic animal creation, germ cells, such as sperm and egg cells, are altered so specific animal traits are over- or under-expressed (Industry Canada, 2000). In preparation, scientists must identify and isolate the genes that produce proteins responsible for expression of the desired animal traits. The isolated genes are then inserted into a fertilized egg, scientifically called an embryo, using one of the several transgenic techniques: DNA microinjection, Embryonic Stem (ES) cell-mediated gene transfer, retrovirus-mediated transgenesis, or nuclear transfer (Trangenic Animals, 2000). Of the various methods, DNA microinjection and ES cell-mediated gene transfer are the most commonly used techniques and are detailed in subsequent sections.

DNA Microinjection

The process of DNA microinjection is an injection of a specific or a combination of isolated genes into the male nucleus of a fertilized egg cell. J.W. Gordon and F.H. Ruddle performed the first successful DNA microinjection in 1981 (Redway, 2001).

The process is performed at the single-cell stage to ensure the desired qualities will exist in all of the cells making up that animal. Ideally, the injected DNA will enter the offspring's germ cells so that the modified characteristics will also be passed on and expressed in the offspring.

Upon successfully targeting and isolating the desired DNA sequence, fertilized eggs are harvested. The injection process involves several tools: an inverted microscope, micromanipulation equipment, and injection and holding devices. An inverted microscope is used to make targeting of injection into the male nucleus of the fertilized egg quickly and easily. A suction tool, such as Eppendorf's CellTram Air equipped with a vacuum tip, is used to hold the egg in place for the injection (Brinkmann, 2001). On the opposite side of the egg a hollow fine-tipped glass tube controlled by micromanipulation equipment is used to introduce the foreign DNA into the pronucleus of the male cell, as seen in Figure 1.1 below. Scientists can verify a successful injection by the expansion of the pronucleus size. The modified egg is then reintroduced into the ovum of a psudopregnant female where it will produce an animal with an over- or under-expression of certain genes or will create a new trait to the species. The offspring are tested for presence of the transgene(s), and bred based on case study or protein needs. An overview of the DNA microinjection method can be seen in Figure 1.2.

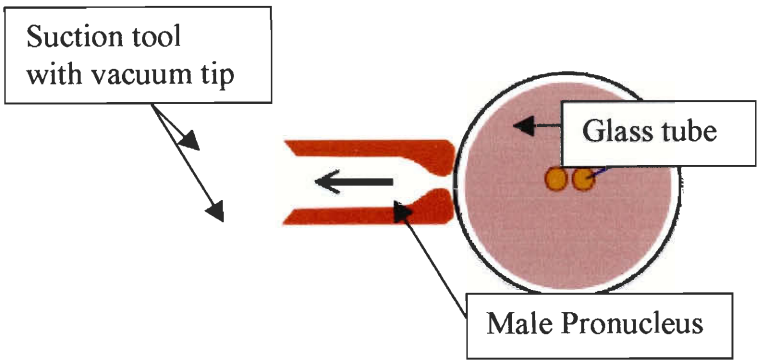

Figure 1.1. DNA Microinjection Source: Charles Jetzer adapted from Brinkmann, 2001.

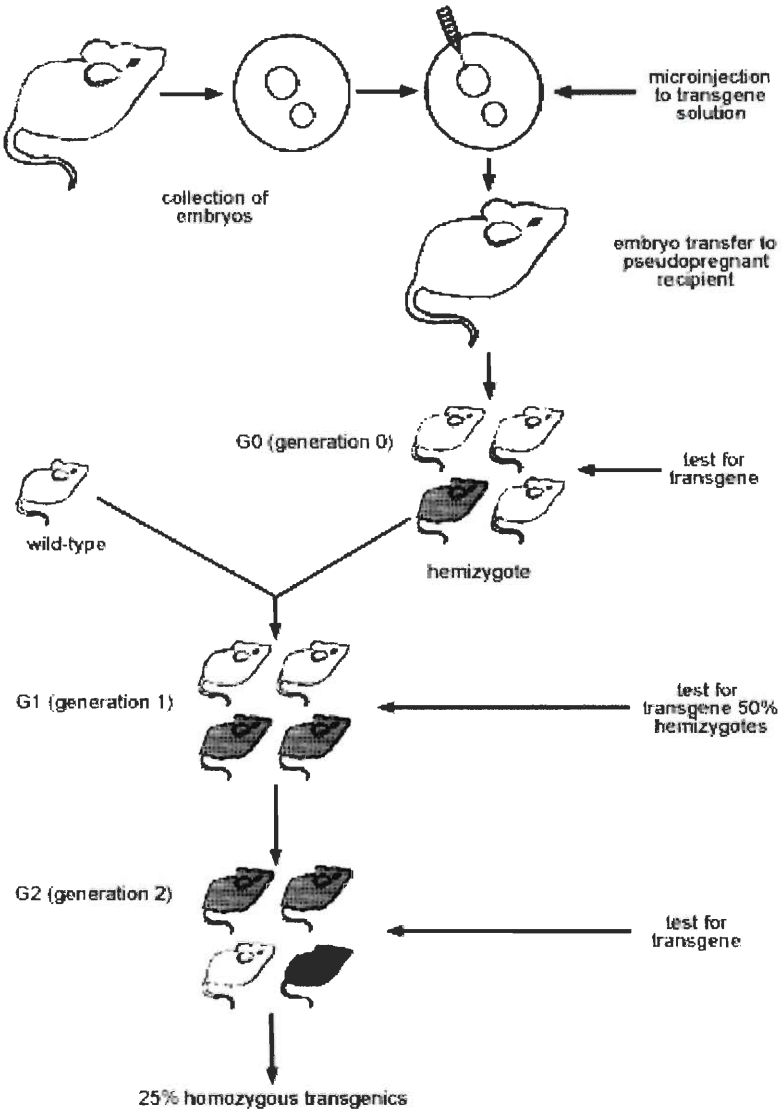

Figure 1.2. Creation of a Transgenic Mouse Using Microinjection Source: Altweb, 2001.

DNA microinjection disadvantages

Unfortunately, incorporation of DNA using microinjection is a random process and there is a high probability that the intended transgene will not combine with the original DNA. In some cases, if the DNA incorporation does not occur before the first cell division, there is a possibility that the genome characteristics will be present in the transgenic animal, but it will not be present in all of the animal's cells. In other cases, the host DNA may also accept the foreign DNA, but the animal will not express the desired gene traits. This problem occurs because the transgene integrated into an inactive area of the host's chromosome.

Microinjection is about twenty five percent successful using mice. Unfortunately, the success rate decreases rapidly in larger animals to ten percent in pigs and to one percent in cattle (Buy, 1997a).

DNA Microinjection Advantages

The advantage of this process is that it can be performed on a large number of different species and has proven to be the most successful method of transgenic animal creation (Transgenic Animal Technology, 2000). The DNA microinjection process was one of the first transgenic methods found to be effective on mammals (Buy, 1997a).

Embryonic Stem (ES) cell-mediated gene transfer

ES cells are recent descendants of a fertilized egg cell. Stem cells are pluripotent, meaning that when subjected to different conditions they can become one of a number of different cell types (Glick et al., 1998). Stem cells can be grown in the lab, thus a stem

cell that survives the microinjection of donor DNA and accepts the gene can then be grown to create transgenic stem cells that can be injected into multiple blastocysts. A blastocyst is an embryo in which the cells have formed a sphere with a mass of cells attached to the inside (Thomas, 1981). These animals are chimeric, i.e., they have cells of both genotypes, the normal and the modified (Glick et al., 1998).

The creation of chimeric mice from ES cell-mediated gene transfer begins with the same steps as the DNA Microinjection method. The female mouse is caused to super-ovulate in the same manner and then mated. The eggs are harvested and injected with DNA. Once the stem cells are injected with the new DNA, however, they are cultured rather than placed directly in a foster mother (Figure 1.3). The first successful ES cell gene transfer occurred in 1980 by the Evans team (Transgenic Animal Technology, 2000).

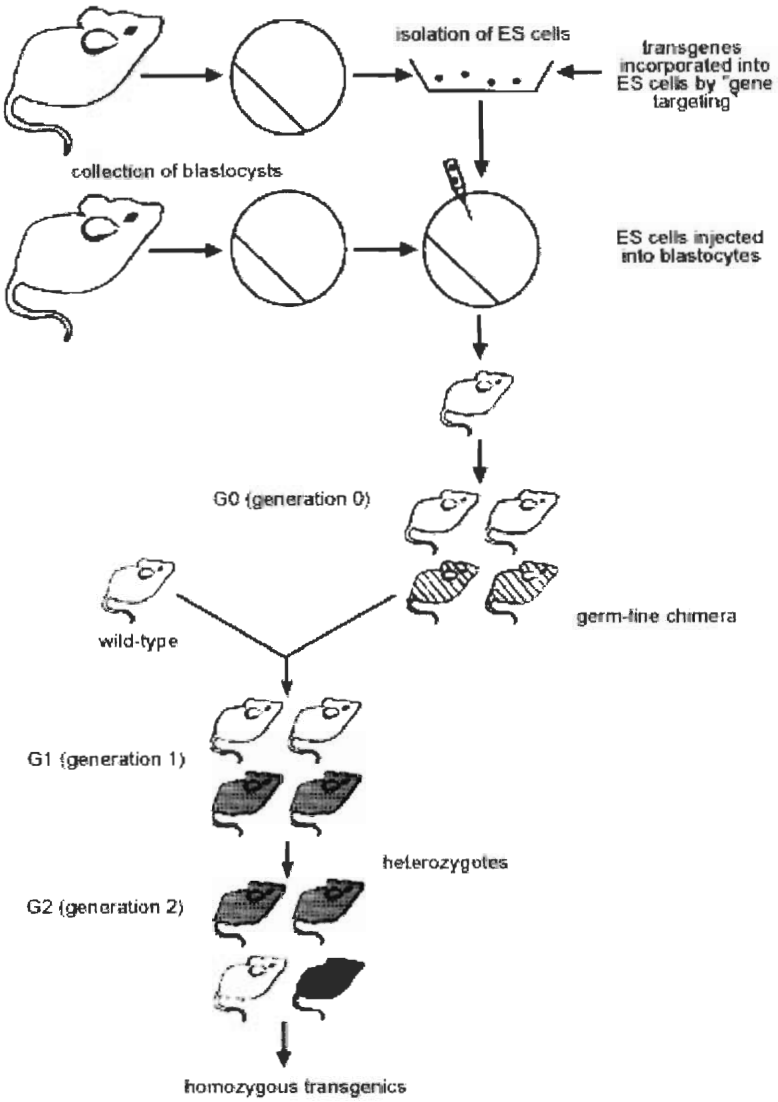

Figure 1.3. Embryonic Stem Cell Method Source: Altweb, 2001.

To ensure a high production yield of transgenic stem cells, a method of positive-negative selection has been developed (Glick et al., 1998). The selection method has two mechanisms for ensuring the donated DNA is transcribed and has been transcribed correctly. The donated DNA consists of two genes, the transgene that we wish the future animal to express, and another gene that will give the cell a resistance to a specific agent, such as an antibiotic. If the new genes are assimilated then they will have a higher rate of

survival when the culture is exposed to the agent than cells where the transgenes are not absorbed. This is the positive selection.

Unfortunately, the transgenes do not always align themselves properly within the genome. To avoid nonspecific integration two genes are added, one on either side of the two transgenes that are homologous to two genes on the target site (CCAC, 1997). The genes are then flanked by two other genes, tk1 and tk2, from the herpes simplex virus (Figure 1.4). The homologous genes will tend to attach themselves where their naturally occurring counterparts had previously existed on the DNA chain. If this happens the genes from the herpes simplex virus will be excluded from the recombination and will not be contained in the new DNA strand. If the new DNA transcribes itself at spurious sites the herpes simplex virus strands will be included in the recombination. These herpes simplex genes will eventually cause the destruction of the host cell containing randomly inserted DNA. This is the negative selection.

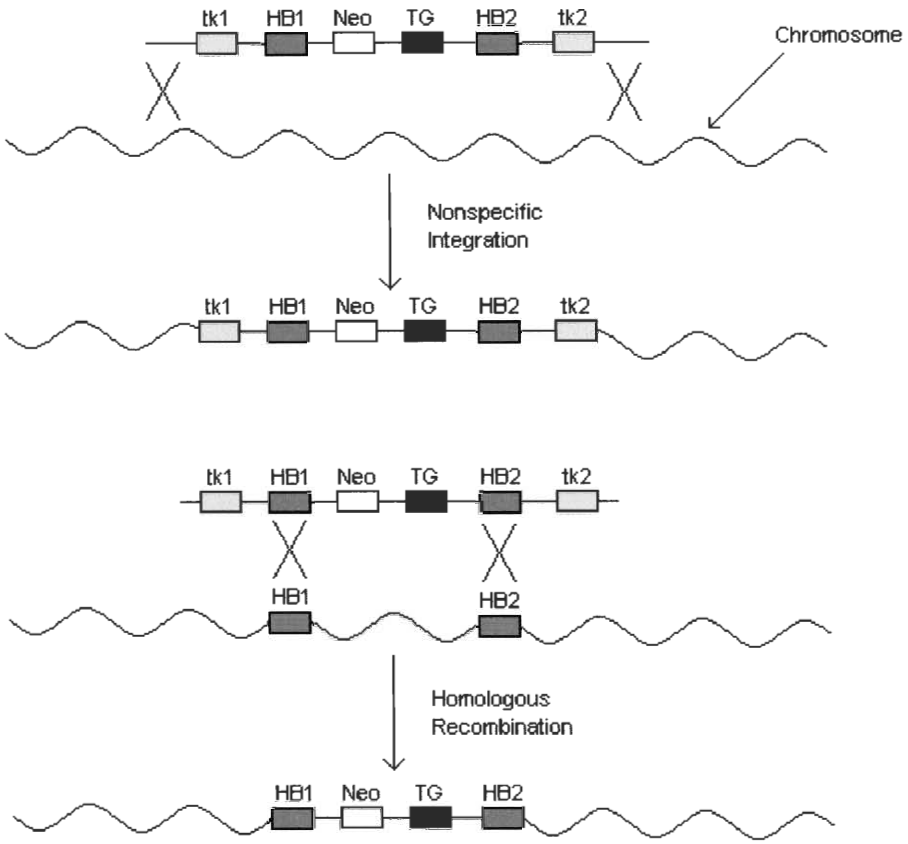

Figure 1.4. ES Cell Method: Positive-Negative Selection Source: Glick, 1998.

In this manner ES cells are modified, and the concentration of the modified cells in a culture is maximized. ES cells can then be harvested from the culture and injected into blastocysts to create transgenic embryos for implantation into host mothers.

ES Cell Method Disadvantages

The first generation of transgenic animals created in this manner is chimeric. Once the stem cells start to differentiate, the germ cells created may or may not be of the new genotype. If they are not then the offspring will not carry the transgene.

The ES Cell method has been proven to work well in mice. As of 1998, however, it has not been proven successful in any of the other species commonly used for genetic manipulation such as cattle, sheep, pigs or chickens (Glick, 1998).

ES Stem Cell Method Advantages

The ES method allows for very specific placement of the donor gene. If it is desirable to inactivate a particular host gene, it can be replaced with a functionless gene. The effect is essentially a knock out; it will no longer be expressed in future generations of that cell. Therefore the ES method can be used to express a non-natural gene, exaggerate the expression of a natural gene, or to suppress the expression of a natural gene.

The other advantage of the ES method is that single transgenic stem cells can be reproduced and injected into many blastocyst stage embryos. Thus a single modified stem cell can be cultured and eventually used to create many transgenic animals.

Chapter 2: Transgenic Examples

Transgenic animals are normally categorized by the reason for creation. Transgenic animals are created for one or more of the following reasons: disease models, food sources, xenotransplantation, transpharming, and scientific or developmental models. In the following sections, each category will be explained, and transgenic examples will be given.

The dominant animal selection for transgenics is mice due to their small size, low housing costs compared to larger vertebrates, short generation time, and their genetics have been well defined (Buy, 1997b).

With the wide variety of animals that could be used as a transgenic platform, selection is based on several characteristics including housing costs, milk yield, animal size, generation time and animal genetics. A comparison in milk yield per animal can be seen in Table 2.1.

Animal	Generation Time	Milk Yield (/year/animal)
Mice	3 months	1 milliliter
Chickens	7 months	6 liters egg white
Rabbits	8 months	4 liters
Goats	18 months	800 liters
Cows	3 years	8000 liters

Table 2.1. Animal Characteristic Comparisons Source: Avigenics, 2000.

Disease Models

Scientists consider transgenic animals such as OncoMouse™ and Alzheimer's Mouse disease models because they have been designed to express traits mimicking aspects of human diseases.

OncoMouse

First engineered in 1988, it was used to develop breast and lymph cancer tumors all over its body (Leder et al., 1990). These mice are used worldwide to test drugs and therapies against these cancers (Hardin, 1994). The patent was subsequently upheld, and will be discussed in Chapter 4. OncoMouse™ was created by Dr. Philip Leder at the Harvard Medical School at Harvard University in Cambridge, Massachusetts (Taconic, 1998). OncoMouse™ is scientifically known with the prefix v-Ha-ras(TG.AC). The mouse was named because it carries the v-Ha-ras oncogene (Leder et al., 1990). The oncogene was introduced using DNA microinjection. The United States allowed DuPont, the major provider of the project's funding, to patent OncoMouse™ on April 12, 1989, but their patent application was declined by the European Patent Office due to a clause that refuses the patenting of anything offensive to the public's sense of morality (Woessner et al., 1999; NOAH, 1992). OncoMouse™ has been the most famous transgenic mouse in history due to the controversial patenting and the milestone for cancer treatment developments (Woessner et al., 1992). This strain of mice successfully passes on their oncogene to their offspring due to the total infection of the animal's cells.

Alzheimer's Mouse

After years of development of an Alzheimer's mouse turned sour in 1992 when a group of scientists were unable to reproduce data, the hopes of many scientists fell (King, 1995). Three years later, David Adams, a professor at Worcester Polytechnic Institute, in Worcester, Massachusetts, helped develop a mouse carrying the Alzheimer's disease

gene. Inserting the human gene for amyloid protein in embryonic cells was the method used for creating the Alzheimer's mouse ("WPI professor's", 1995). Alzheimer's is a disease that affects mental capabilities, reducing memory and judgment; it's estimated that nearly four million Americans suffer from this disease ("WPI professor's", 1995). The creation of the mouse is a major milestone in disease modeling, it has allowed scientists to better understand the causes of the disease, determine how to slow it down, or cure it (King, 1995). David Adams worked with TSI Corp. to develop Alzheimer's mouse, the company was later renamed Exemplar Corp. and eventually sold to Athena ("WPI professor's", 1995).

Transgenic Mouse Disease Model Limitations

Human diseases that have been introduced into transgenic mice have encountered several limitations. While the disease may express similarities to the human forms, there are many differences between a human and a mouse, and some of the diseases are not suitable for the mice. A good example of this problem is with cystic fibrosis. In humans, severe respiratory problems commonly develop, but in transgenic mice, this problem has not been present even after the cystic fibrosis gene was given to the mice. After-birth deaths also occurred more often in these model mice than with humans (Hardin, 1994).

Food Sources

Transgenic animals that are created with increased growth hormones and disease resistance fall into the food sources category. Animals such as A/F Protein's Superfish and AviGenics' Salmonella and Campylobacter resistant chickens are just two examples of transgenic creations with a purpose of being consumed by humans.

Several transgenic fish, Atlantic and coho salmon, channel catfish, striped bass, rainbow trout, etc, have been engineered to express increased growth hormones or similar growth factors. Their growth rates have varied and average between a 30 to 60 percent increase in size (Hallerman, 1996).

Similarly, coldwater fish have been genetically modified by adding an "antifreeze protein" which allows the fish to survive in subzero waters. The genome modifications prevent the formation of ice crystals in the blood, ultimately preventing freezing (Hallerman, 1996).

Superfish

Superfish are Atlantic salmon engineered by A/F Protein to grow six times faster and twice as large as the usual farmed salmon, and to consume 25 percent less food. To create the transgenic salmon, human growth hormone (hGH) was combined with DNA from a winter flounder (Cousteau, 2000). The fish were accidentally discovered when attempting to combine the antifreeze gene with the genes of a salmon. The discovery formed the company A/F Protein (antifreeze protein) in Waltham, MA. A downside to the increased growth is the decreased survival rate of superfish eggs. In addition, the fish have less muscle structure and do not swim as well as normal salmon as a result (Lewis, 2000). The fish have increased growth rates because of the addition of the hGH and because the antifreeze gene does not permit their metabolism to slow during the winter (Stoll, 1999). It only takes the salmon fourteen months to reach food market size, normally 28 months (Stoll, 1999). The area of Superfish development has lead to great

controversy among the public due to fear of fish escape and accidental spawning with unmodified salmon. A possible future enhancement of the fish is mass sterilization to eliminate undesirable breeding outside the pharming facilities (Golden, 2000).

Figure 2.1. Superfish: Salmon transgenic fish on right side of picture Source: AQUA Bounty Farms, 2000.

AviGenics Chickens

AviGenics, an Athens, Georgia based company, has produced a transgenic chicken in the laboratory of cofounder Dr. Robert Ivarie, with financial sponsoring by the University of Georgia Research Foundation. The transgenic chickens (Figure 2.2) have been engineered to produce increased therapeutic proteins in egg whites and to increase the productivity of the chickens through disease resistance and increased egg production. AviGenics has successfully introduced several types of glycosylated proteins; glycosylation is the process of adding sugars to certain proteins, and is necessary for protein activity or human acceptance of the proteins. The proteins have a high level of expression within the oviduct of the chicken and contain sugars that attach at specific sites within the mature protein molecule (AviGenics, 2001). AviGenics uses their retrovirus-mediated transfection of blastodermal cells technique to create human drugs

(Equicom, 2001). The company plans to increase the safety of egg consumption by humans in the future, by making the chickens Salmonella and Campylobacter resistant as well as engineering them to become resistant to bird diseases such as Coccidiosis and Marek's Disease (AviGenics, 2001).

Figure 2.2. AviGenics Chicken Source: AviGenics, 2001.

Xenotransplanters

Companies have been working to create transgenic animals that are used as organ donors. The animals are genetically altered so that the human body will not reject them upon transplantation. An example of this technology is the production of organs in pigs for xenotransplantation. The advantage of this type of transgenic animal is an increased amount of donors for the long lists of organ recipients with very specific human requirements such as blood type. There has been proof that animal organs can be transplanted into humans, pig heart halves have been used to replace human heart halves when they have worn out ("Xenotransplantation: Animal", 1997). There is a high demand for organs and without willing donors nearly 4000 people die each year (Klug, 1998).

The major problem that has occurred in xenotransplantation is the human body's immune system rejecting the organs. The key to developing transgenic animals for use as organ donors is to make the animal's parts as close to human as possible. The first steps to achieving organ acceptance occurred in 1996 when Jeffrey Platt of Duke University successfully introduced three proteins in a pig, that act as shields when organs are

xenotransplanted: delay accelerating factor (DAF), CD46, and CD59 (Platt, 1998). Work done by John Atkinson at the Washington University, in St. Louis, assisted Platt in creating his xenotransplantation pigs. In the 1980s, Atkinson and coworkers discovered that membrane cofactor protein (MCP) and DAF reduced rejection of transplants while working with mice and hamsters (Allen, 1995). Both Platt's and Atkinson's genes, for insertion, prevent the human immune system from recognizing the organs as foreign and destroying them. The success of Platt's transgenic pigs was temporary. Unfortunately, the transplanted organ only survived thirty hours ("Xenotransplantation: Animal", 1997). A source of the problem was found to be a complex sugar molecule produced in the pig cells that are found in all mammals except monkeys, apes, and humans ("Xenotransplantation: Animal", 1997). The sugar molecule makes the organ identifiable to the human body and is the signal for the immune system to reject and destroy it.

PPL Therapeutics transgenic cloned xenotransplanter pigs

PPL Therapeutics has created five cloned piglets that contain a "marker gene" introduced into their DNA (PPL Therapeutics, 2001). The alpha 1-3 gal transferase gene has been knocked out (Figure 2.3) of the pig genome; the gene is normally responsible for indicating to the human immune system that an organ is foreign to the body. Scientifically this is called called hyperacute rejection ("PPL Produces", 2001). The PPL strategy builds on the works of Atkinson and Platt to eliminate the complex sugar molecule from the pig genome. The company also plans to include an additional gene from the human immune system, in order to increase the acceptability of the organs.

Hyperacute rejection is only the first of three problems to overcome, before organ transplants will not require suppressants.

The second hurdle is a combination of two interrelated elements caused by delayed xenograft rejection (DXR). DXR is a result of anti-coagulation factors lost in transplantation, and increased amounts of the vascular cell adhesion molecule (VCAM) (PPL Therapeutics, 2001). Normally, the anti-coagulation factors stop blood clotting, and the VCAM increase indicates to the immune system that there is an infection or inflammation and white blood cells are rushed to the site. PPL plans to introduce a gene to replace the anti-coagulation factors (Figure 2.4) to be expressed only when they are required. The increase of white blood cells eventually kills the organ, thus scientists are working to also add a gene to stop VCAM from getting to the surface of cells (Figure 2.5).

The last problem is long term rejection related to an attack by T cells. The attack will be overcome by a transfusion of cells from the donating pig before the xenotransplantation takes place (PPL Therapeutics, 2001). The transfusion will be done to deactivate the host T cells responsible for the attack on the foreign organ (Figure 2.6). After all the T cells responsible for attacking the foreign organ have been inactivated, the xenotransplantation can take place without any problems.

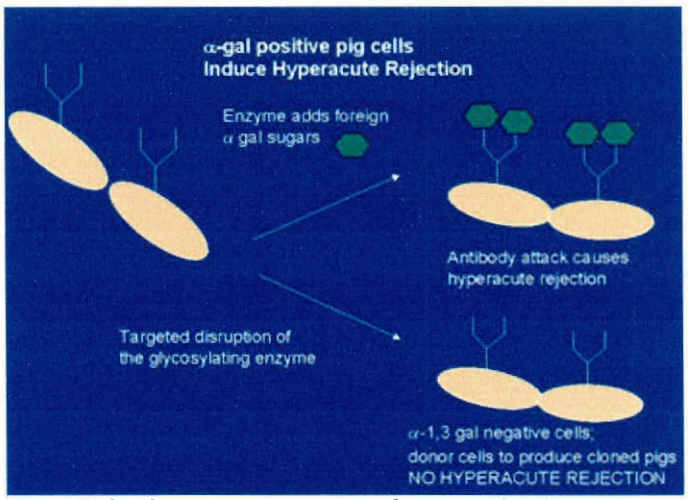

Figure 2.3. Hyperaute Rejection due to presence of alpha gal 1-3 sugar molecule Source: PPL Therapeutics, 2001.

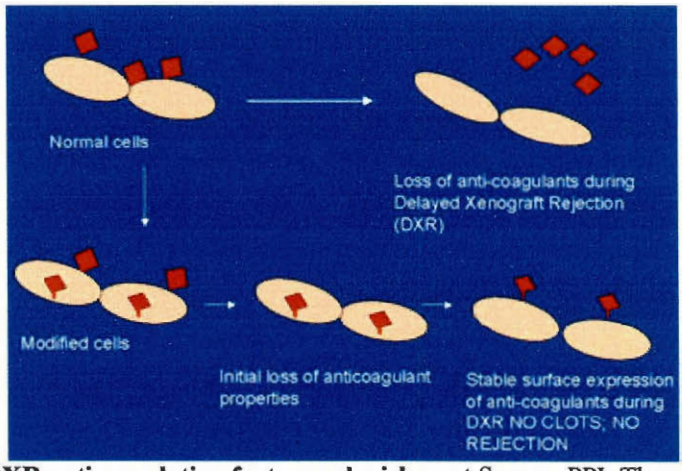

Figure 2.4. DXR anticoagulation factor replenishment Source: PPL Therapeutics, 2001.

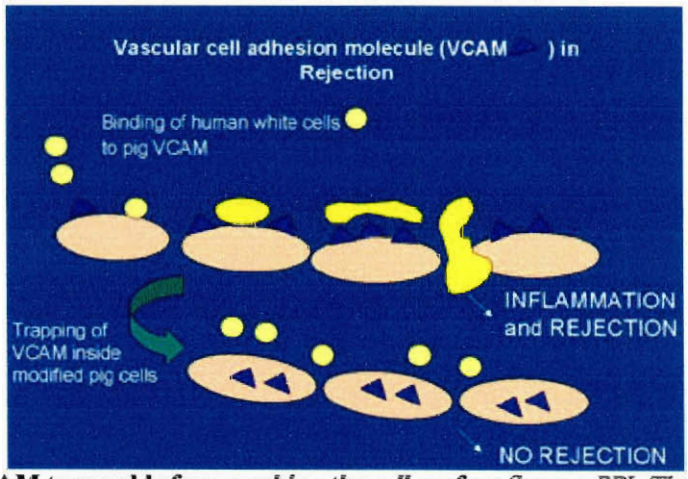

Figure 2.5. VCAM trapped before reaching the cell surface Source: PPL Therapeutics, 2001.

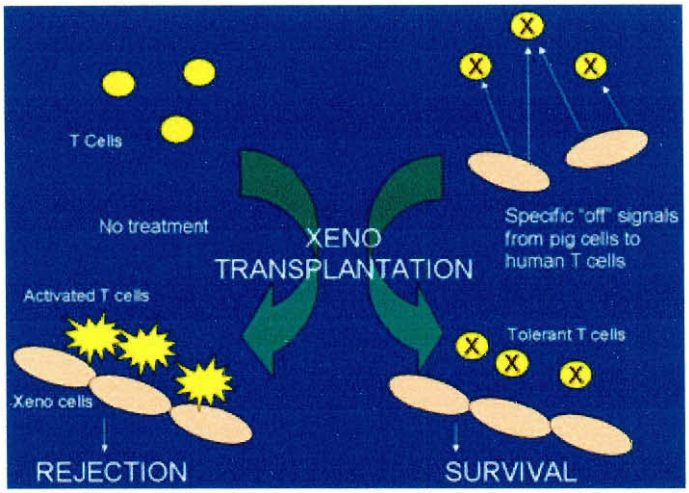

Figure 2.6. T Cell Inactivation Transfusion Source: PPL Therapeutics, 2001.

Transpharming

When animals are engineered to create medicines, the animals fit into the transpharming category. In this category, pharmaceuticals are harvested from the animal's milk. There are many examples of this technology, including Nexia Biotechnologies' BioSteel® and BELE® goats, AviGenic's chickens, the Pharming Group's Herman the Bull, Rosilin Institute and PPL Therapeutics' Polly, and Genzyme's TPA and rhAT goats.

Nexia Biotechnologies BELE® Goats

Goats have been developed by Nexia Biotechnologies to produce an anti-clotting protein called human antithrombin III (ATIII), which can be given to patients during a method of open-heart surgery, coronary artery bypass grafting (CABG). BELE® (Breed Early, Lactate Early) goats have been successfully produced by Nexia Biotechnologies, a Canadian transgenic company (Figure 2.7). Nexia's first transgenic goat, named Willow,

was successfully born on August 31, 1998. The company uses DNA microinjection to create transgenic animals that contain human genes producing human tissue plasminogen activator (htPA) proteins in the goat milk. The target of the htPA proteins is for use in treating heart attacks and isschemic strokes (Turner, 2000). The BELE® program was instated to increase the "speed and efficiency of transgenic goat production" (Nexia Biotechnologies, 2001). Success in their transgenic goats has been seen with males becoming sexually active three months after birth, and females becoming fertile after three to six months (Nexia Biotechnologies, 2001). The transgenic goats are Nigerian dwarfs that breed year round, unlike other goats that cannot breed during the summer. Due to the small size of the goats, overall housing and food consumption costs are decreased compared to standard goats.

Figure 2.7. Nexia Biotechnologies BELE® goat Source: Nexia Biotechnologies, 2001.

A downside to the use of goats as a transgenic platform is the low success rate (5 to 10 %) of creating a new animal using transgenic techniques (Summers, 2001). To avoid the success problems, once a BELE goat is successfully produced it is then used for breeding. After a transgenic goat has matured, the 305-day lactation period begins and the goat produces approximately one liter of milk per day containing the desired recombinant proteins.

Nexia Biotechnologies "BioSteel® Goats"

As an extension to their BELE® goats, Nexia has created two transgenic goats capable of producing un-spun spider web silk protein, called BioSilx™, within their milk. The goats, Webster and Peter (Figure 2.8) were born on January 12, 2000.

The DNA microinjection method was used in creating the transgenic animals. Spider web silk has a very high tensile strength of 400,000 pounds per square inch, is very flexible and lightweight (Equicom Group Inc, 2001). BioSilx™ protein can be extracted from the goat milk and spun to create fibers (BioSteel® fibers) for applications in cosmetics, aerospace products and medical devices such as wound closure systems (Nexia Biotechnologies, 2001).

Figure 2.8. Nexia Biotechnologies Webster and Peter BioSteel® Goats Source: www.cosmiverse.com

AviGenics Chickens

AviGenics has also been able to produce monoclonal antibodies (MAb's) within their chicken egg whites. Antibodies are among the most complex of the recombinant proteins that have been expressed using transgenic technology. AviGenics was permitted to attain a patent on their transgenic methods, called "Windowing Technology", in the

United States (August, 1997) and in Europe (March, 2000) (USPTO, 2001; Espacenet, 2001). The "Windowing Technology" name was derived from a hole created in an egg's shell where they introduce foreign DNA. Antibody formation requires the combination of two different genes that are synthesized in a cell simultaneously to ensure equal amounts of each gene are expressed. Unfortunately, the precision required for creating the antibodies does not guarantee production. The cells must also assemble the subunits correctly into four identical complex proteins. Fully human monoclonal antibodies have been successfully produced for the purpose of providing an alternate solution to human antibodies due to the increasing annual human treatment demands (AviGenics, 2001). AviGenics has also successfully produced human alpha interferon in their chickens, used in treating Hepatitis C and some cancers (The Dealflow Staff, 2000). Currently, the company has created chickens that produce between 250 to 330 eggs per year and produce up to four grams of medicine per egg ("AviGenics announces", 2000). Ordinary chickens produce between 208 to 260 eggs per year (University of Tennessee, 2000).

Rosilin Institute and PPL Therapeutics' Polly

On July 24th, 1997 the Roslin Institute and PPL Therapeutics announced that a genetically altered lamb was born, Polly (Figure 2.9). The lamb has been engineered to include the human gene coding for blood-clotting Factor IX. By experimentation, it was found that the genes were being expressed in every cell of its body (Buy, 1997b). The Factor IX protein is present in Polly's milk and is used to treat hemophiliacs who lack the normal blood-clotting factor (Schnieke et al., 1997). Scientists created Polly by using the nuclear transfer method where cells containing the desired genes were transferred to a sheep's eggs where the DNA was removed (Mc Keen,

1997). The disadvantage to Polly's discovery is that the company was not sure whether there was an infection of scrapie, a sheep disease possibly related to BSE, or mad-cow disease (Henahan, 1997).

Figure 2.9. Roslin Institute and PPL Therapeutics' Polly Source: Henahan, 1997.

PPL has also engineered sheep to produce alpha-1-antitrypsin (AAT) in their milk, this is the protein used for treating cystic fibrosis patients ("Now A Cow", 1998).

Herman the Bull

The success of Roslin Institute and PPL Therapeutics' Dolly and Polly helped lead to the production of the first transgenic cow, Herman, in 1990 (Figure 2.10). Gen Pharm International, genetically engineered the bull at their Mountain View, California laboratories, to produce the human gene for lactoferrin, an iron-containing protein vital for infant growth. Herman was created using the microinjection of the gene coding for human lactoferrin. Cow's milk does not normally contain lactoferrin, thus infants must receive it from another source such as formula or mother's milk ("Herman the Bull", 1994). The protein is produced in Herman's offspring's milk and provides a natural immunity to diseases in infants. Herman became father to at least eight calves in 1994, at Gen Pharm's Leiden, Netherlands laboratory, and each of them received the lactoferrin gene (U.S. Dept. of Agriculture, 1994). The development of lactoferrin in cow's milk provides protection from bacterial infections in the gastro-intestinal tract and may

provide increased immune response in cancer and AIDS patients (Ag-West Biotech Inc, 1994). Lactoferrin may also be used to neutralize heparin in open-heart surgeries and possibly for older patients on chemo- or radiotherapy (Pharming Group, 2001; Kreeger, 1997). Testing of lactoferrin for medicinal purposes has been in clinical trial at the Netherlands Academic Medical Center since 1999 (Acid Maltase Deficiency Association, 1999).

Figure 2.10. Herman the Bull Source: Kreeger, 1997.

Genzyme Transgenics TPA and rhAT Transgenic Animals

In April, 1999 Genzyme Transgenics Corporation of Framingham, Massachusetts created the first recombinant human antithrombin III (rhAT) goat. The human protein rhAT is normally found in the blood as a clotting regulator, but is deficient in some patients. Genzyme has also been working on transgenic animals that will express fifty different human proteins in their milk. Included in the proteins are tissue Plasminogen Activator (tPA) and antibodies. TPA is the human protein that allows blood to circulate through our bodies. The TPA protein was inserted into a sheep embryo, and the milk

produced by the sheep contained the protein expressed from that human gene providing an anti-clotting agent for heart attack patients (Figure 2.11).

Figure 2.11. Genzyme's TPA Sheep Source: NBCi member web page, 2001.

Scientific or Developmental Models

The final classification of transgenic animals includes genetically altered animals that are used as proof of concepts for future work and scientific studies. These animals do not fit into any of the other categories because of their unique genetic alterations. Examples of these animals include zebrafish used to study fast paced human development and Joe Tsien's SuperMice or "Doogies" developed to have enhanced memory capabilities.

Transgenic Zebrafish

Transgenic zebrafish have been engineered in order to study how genes are activated in embryo development. These animals have provided insight on human development, due to similarities between human and fish development. The zebrafish was chosen because of its increased similarities to human gene sequences and functions (Ekker, 1998). The study of growth effects in zebrafish is much easier than in most

animals because they are transparent. Zebrafish have been studied for neurological development as well as cellular growth using time-lapse photography (Kanki, 2000). Modifications to zebrafish are accomplished using a laser microbeam, to activate or deactivate specific genes. One type of transgenic zebrafish that has been created for modeling is the addition of green florescent protein (GFP) from jellyfish by Kaethner and Stuermer in 1992 (Figure 2.12) (Amsterdam et al., 1998).

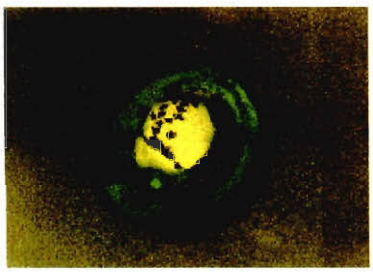

Figure 2.12. GFP transgene expression in zebrafish embryo Source: Amsterdam, A. et al., 1998.

Joe Tsien's SmartMice

Doogies, named after the TV character Doogie Howser, M.D. are a new strain of mice genetically engineered to be smarter than the normal mouse (Figure 2.13). Joe Tsien, a neurobiologist at Priceton University, created these mice around September in 1999 by injecting fertilized mouse eggs with an extra copy of the NR2B gene (Tang et al, 1999). The NR2B gene is part of a brain cell receptor, called the NMDA receptor, which receives chemical signals from neurons. The NMDA receptor receives specific chemical signals training the brain cells to fire in repeated patterns; the patterns are what we know as memories (Guynup, 2000).

To prove the increased mental capabilities, a rodent SAT was created by Guosong Liu that evaluated the amount and response of NMDA receptors. The test showed that the mice performed tasks about 40 percent faster than normal mice (Leutwyler, 1999).

Figure 2.13. Smart Mouse Source: Princeton University, 1999.

Chapter 3: Transgenic Ethics

For many years, animal experimentation has been a hotly debated topic. On one side, the animal rights activists protesting that animals have the same rights as humans, and on the other side the researchers who are trying to cure the deadly diseases that haunt our society. With the increase of biotechnology comes a new issue-the transgenic animal. Because of the makeup of the transgenic animal there are more ethical issues than just animal research alone. The idea of taking a set of genes from one animal and putting them into another has sparked a hot debate among our society, scientists and layman alike.

For as long as man and animals have been around there have been disagreements on whether or not to use the animals for our own purposes. Everyone has a different point of view of animals and their place in our society. Their beliefs stem from three major viewpoints. The first is that humans are "number one" in the animal kingdom. We have been created superior to other animals despite the similarities between us and other species. Humans are unique and supremely valuable as compared to other species. Another view is that humans "own" animals. Animals other than our self are considered inferior, and they basically only have value because we can use them. The third view is that humans are one of many animals and we have no grounds to be superior to anything else on the planet. With people basically believing one of these principles, there is a lot of room for disagreement in how animals should be used in research if at all. People in each of these categories basically have the same belief on animal testing. The people that believe we are superior will usually be in support of animal testing, and the people who

believe that we are just one of the many species on the planet are usually against. That is not to say that one's mind cannot be changed given other evidence.

On one side are the researchers; the people that create the animal and the people that use them for testing. Most scientists today feel as though there are too many benefits to this transgenic technique to abandon it. The work done so far using transgenic animals has had far more success than regular animal testing alone. One example would be the Alzheimer's mouse (Gamer et al., 1995). This mouse model would not have been possible without the insertion of an outside gene to form the debilitating plaques in the animal's brain. The only animal that naturally gets Alzheimer's other than humans, is the orangutan and it would take 60 years to develop the plaques that are evident in humans with this disease. This Alzheimer's mouse model went on to be used by Elan Pharmaceuticals to make the worlds first vaccine for the disease that could help hundreds of people. Anyone who knows someone with this disease can vouch for the suffering that the patient endures. If transgenic animal research was abandoned then this vaccine might not have been developed. Studies on this mouse show that it was in minimal or no pain throughout the study. If the animal isn't suffering and can be used as a model to treat this severe illness that affects many Americans, then there isn't much justification for stopping the experiment.

Another successful animal experiment is the use of sheep to produce human alpha-1-antitrypsin. This is a drug that is used to treat patients with fatal liver disease. This is done using a new technique called transpharming, which is a way of using farm animals. Using an animal that has foreign genes inserted into its genome, the animal can then be milked for the drug. The farm animal will produce the drug in its milk and then

milk is then purified and the drug can be used. The animal can live a normal life, and some scientists argue that this technique when used with cloning could treat hundreds of patients with the disease. Up and coming techniques will have the drug of choice produced in the urine which could ironically make the drug purer and easier to collect.

These are a couple of examples of how transgenic animals can help many people. Most people do realize that transgenic animals can help treat a wide range of diseases, however some do still feel it is not right no matter how many people could be treated. Transgenic animals are not always in minimal or no pain. There have been many experiments where the animals were in serious pain. Even though we have no direct measurement of animal pain, it can be obvious to the investigator that the animal is suffering. For example, many mice have gross disfigurements that occur through the onset of the disease being studied. In these cases the experiment should be reevaluated and a decision should be made whether to continue the transgenic line.

Because human genes are being introduced into the animal then they will sometimes have more serious suffering than they would have had if non-transgenic animals had just been used for regular animal testing. An example of an experiment that went slightly wrong in the eyes of many people would be the animal known as Superpig. Scientists inserted human growth hormone into a pig so that it would grow larger and have more meat to feed hungry humans. This genetically modified animal became known to everyone as Superpig. Unfortunately because of its size, Superpig developed bad arthritis and was crippled. This pig ended up being in a lot of pain that could have been prevented had the human growth hormone not been introduced. In this case the animal is not being used for medical benefit and the experiment is perhaps not as justified

as if the animal was being used to help cure a fatal disease. Also, instead of the transgenic animal being created, more pigs could have been bred. This also opens up a whole new door of what is acceptable in transgenics and what is not. Had this pig not had arthritis and been able to live a pain free lifestyle, he would have been able to feed many more people-especially in underdeveloped countries. This would be especially helpful in combating world hunger in many nations.

In animal research it is speculated that the more advanced an animal is being used, the higher the amount of pain is felt. Therefore, if used, transgenic animals should be kept to a less complex animal when possible. After the experiment is done on a less complex animal and the results are interpreted, then a more complex animal can be used. In every case the amount of suffering should be kept to an absolute minimum and at no time should the animal be in serious pain. Even if there is a small chance of pain in the animal, scientists still do not know how the other animals, including humans, will react when the genome is tried in a more complex species. This is not to say that we shouldn't perform experiments in more complex animals though. Strict regulations and monitoring should be in place.

There are many gray areas when working with transgenic animals. Sometimes there is suffering speculated when there isn't, and vice versa. Or other times the animal may have some suffering, but the animal model is just too useful for scientists to abandon it completely. A well-known example of this type of a transgenic animal is the OncoMouse™. OncoMouse™ is used for cancer research due to its ability to grow tumors that have been xenografted into the animal. It is obvious to any onlooker that the animal is in discomfort based on the fact that there are large tumors on its body. There

has been no evidence to support this theory, but previous research points in this direction. Even though there is probably a large chance of suffering, the mouse will most likely help in the eventual cure. With the number of people being diagnosed with cancer increasing all the time, it is a major pressing problem in our society. This little mouse can be used to test out so many treatments and also further our understanding of the disease.

People opposed to the use of genetically modified animals sometimes have a religious influence. They argue that we would be playing God by creating a new species even if it was a transfer of just one gene. Through playing God, the research is going to get thrown back in our face and could harm us. Other people argue that the basic integrity of the animal is not preserved in the use of transgenic animals. They believe that a pig may lose its "pig-ness" if outside genes are inserted into the animal's own genome. But who's to say whether the pig could actually lose its "pig-ness" and if it could even affect the animal.

Other religious issues surrounding transgenic animals are the ideas that certain animals are sacred in certain religions. For example the Hindu's believe that the cow is a sacred animal and therefore no harm should come to this animal. In this case a transgenic cow would definitely not be accepted in their culture. Because the Hindu's feel that the cow should not be used in research, we as a society have a decision to make about this specific species. If the cow is used in research we are offending a large group of people. Even though majorities of the Hindu's do not live in our country, we would still be offending the few that do. Hindu's are just one type of religion. In most every religion, certain animals are sacred and if they were all banned from research there would be a

limited group of animals to pick from. This is a tough decision to make because people are going to end up getting offended no matter what happens.

Along with the loss of the animal's basic integrity comes the danger that we are setting ourselves up for catastrophe. By changing the structure of all these animals we could be compromising ourselves in the long run. An example would be if we accidentally created an organism that could severely harm us, whether animal or bacteria. This, of course, would not be done on purpose, but with all the techniques being used, accidents in the lab could happen. The expressed gene could have very different consequences in the animal as opposed to a human. A fictional example of this was used in Robin Cook's novel Chromosome 6. In this book scientists created monkeys that were identical genetically to their human counterpart. What the scientists didn't realize when they were manipulating the genome was that there was a gene on the chromosome number 6 that controlled behavior in humans. The altered monkeys ended up being extremely violent and harming other monkeys through advanced humanlike behaviors. This is an example of a technique that ended up backfiring for the humans in the long run. Even though this hasn't happened in humans yet and may never, the potential for this type of experimentation is there and this could be extremely harmful to us as a species.

Another view on the modification of organisms is that species evolve over time and by transferring genes from one organism to another we are helping the pattern of evolution. Even though this may be a far cry from natural evolution, this could be a result of all the technology that humans have been creating, and could be a new type of evolution. With an increase of technology comes new and innovative ways to do things

to alter our own environment. We could have created a new way to evolve species by way of gene transfer. In natural evolution, genes are swapped between species, which strengthens the gene pool and allows the strongest to survive. Therefore this could be a more technical evolution. Humans have evolved throughout centuries becoming smarter and this could be the animal's way of evolving and becoming smarter through our own interference.

A big issue that surrounds the making of transgenic animals is the actual creation of the embryo. Because genes are transferred from one nucleus to the other, certain techniques are used. Certain drugs induce super ovulation, and then a timed mating is performed. For the collection of eggs in mice, the animal is sacrificed, and in larger animals a laparotomy is performed. After the eggs have been collected they are then placed into a surrogate mother. This surrogate mother is obtained by mating a female with a sterile male so that a pseudopregnancy is performed. The male therefore needs to have a vasectomy, which can be painful if the right anesthetic is not administered. In order to obtain a transgenic animal the parents go through a lot of stress, especially if the technique is employed when the animals are younger (Hubrecht, 1994). Another problem with this technique is that a lot of animals are sacrificed, and some argue that this is done unnecessarily. In the making of the embryo there will inevitably be some sacrificing whether intentional or not, and then on top of that the mothers are sacrificed. Some people believe that this is an abundance of killing and is not necessary. In one study carried out 1360 out of 1585 embryos actually survived microinjection, and out of the implanted 29% survived until weaning (Wight et al., 1994). This means that just over a quarter of the offspring were successfully manipulated. This is a low number and is hard

to justify through ethics and cost. The present survival rate is even lower for farm animals. Even though techniques have improved since this study, there will still be many embryos that are sacrificed for the sake of a transgenic animal.

In introducing a new gene to the animal, the welfare of that animal is often jeopardized. Genes introduced can have serious side effects that the researcher could not predict. Certain physiological changes can occur when the gene is introduced. Such cases include mice born with deformed limbs or kidney malfunction through no fault of the investigator (Moore et al.,1995; Mepham, 1998). The embryonic stem cell manipulation can target genes better than before, but there is still room for error. Scientists are still learning how all the genes interact, and whether the removal or addition of several can have consequences on the animal.

One area that could benefit highly from the creation of transgenic animals is the farming industry. For many years farmers have come under a shower of abuse for the way the animals are treated, and the conditions that they live in. Disease almost always strikes a farm, and whether one animal or one hundred animals are affected the disease is still there. People in opposition of animal research are always quick to point out that animals being used in research are only being used selfishly by humans, but that is not necessarily the case. An example of this is if pigs were resistant to foot and mouth disease, they would benefit enormously. The pig wouldn't have to endure the suffering that usually accompanies this disease. This isn't just true in farming but also everyday animals. The recent outbreak of foot and mouth in England had devastating results for the economy and for animals everywhere. This could all be prevented if there were a way that pigs were immune to this deadly disease.

Another way that farming could benefit is by modifying the animal to produce more meat. By engineering the animal this way, there would be fewer of these animals running around. With fewer of these animals comes less waste, and with less waste comes less pollution. With farming each year producing 14 billion pounds of manure and 28 billion gallons of wastewater, we cannot afford to do nothing. With the creation of a genetically modified animal comes a cleaner environment. On the flip side of this is the human end. Are people going to want to eat a genetically modified chicken? With all the modification comes many viruses' and the animals are not as healthy as their non-transgenic counterparts. No matter how safe scientists claim their techniques are, people are always going to be skeptical of the fact that their food has been tampered with.

Ever since the creation of the transgenic animal, studies have shown that the number of animals used in research has increased dramatically. From 1995 to 1997 the number of procedures on genetically modified animals has risen by 64% (Home office, 1998). This rise could be because there are more experiments being done in general so therefore the number of animals increases because of the increase in experimentation. Even though the number is currently rising, the validity of the animal is increased so therefore in time scientists anticipate that the number of animal experiments will be reduced. This will be because the results will be obtained more quickly and therefore fewer animals will be used. Even though researchers anticipate that the number of animals used could be reduced, there is also a probability that the number could increase even more. With over 100,000 genes in the human genome, there is a huge potential for new studies. With increasing studies comes increasing usage of animals. This is

something that only time will tell us. We cannot predict how many animals will be used, nor can we predict how many experiments will be done in the future.

A concern that few people would think of is the safety of these experiments. From a distance no one would think that there was a possibility of danger in using transgenic animals in research, but there is a potential risk to humans and the environment. A concern of some is that the genetically modified animal could escape and breed with other animals. Even though this is somewhat of a long shot, it is a possibility, and care should be taken to make sure that this doesn't happen. With the breeding between the animals there is concern that the retroviruses' used in creating a transgenic animal could infect the other organism. A more realistic concern is that risk consuming a genetically modified animal poses to the human population. If the farm animal has a disease-resistant gene implanted into its genome, this could inadvertedly be transferred to a human. This could also be true if there is some drug-resistance in use for the farm animal. These pose serious concerns to those who oppose transgenic animals and any type of research involving them.

Another concern with transgenic animals is the money surrounding them. Many millions of dollars are put into creating the animals then many more are invested in trying to keep them disease free. A typical transgenic animal housing room has to be kept completely sterile. This requires the people taking care of them to use time putting on the necessary protective gear, the tweezers used for placing one mouse into another cage kept sterile, fume hoods to prevent contamination between the species and many more special procedures. Not only is there an increased labor cost, but an increased equipment cost

also. If disease breaks out in the room it can be devastating with thousands of hours and dollars down the drain.

With all the embryos that are lost in the creation of a transgenic animal there is also a huge monetary loss. The people in opposition to this research could argue that the money spent in creating these animals could be used to benefit other research. But as in any research results are not guaranteed, and if there is a possibility that this research could benefit many people then it is worth a try.

History has shown that humans do not have a great record with the treatment of animals. Native Americans and religious groups have many different rituals that involve the torturing and killing of animals. Technically there is no difference between taking an animal and torturing it through that ritual, and taking an animal and implanting foreign genes into it and sacrificing the animal in the end. If anything the transgenic animal can help humans cure disease whether the sacrificing is used for a set of beliefs by a certain culture. Even non-scientists were never kind in their treatment of animals. In the past housewives cut off the feet of geese in order to make the meat more tender, and London poulterers kept thousands of birds in the attic. This is far crueler to the animal than inserting foreign genes but it was accepted in society without much of a protest.

Another argument is that humans have been given the ability to create and use all this technology so why should we sit back and not use our own abilities. If a team of scientists is intelligent enough to come up with an animal model unlike any others that can treat disease, then why shouldn't we use it? Many people feel that humans have worked hard to bring together many different techniques throughout the years and shouldn't be stopped because some bioethicists believe that it is wrong.

This is a tough debate and can go around and around without getting any answers and not pleasing anyone. If we eliminate transgenic animal research then the people suffering from these deadly diseases and their loved ones are upset, and if we keep transgenic animals in research the animal rights activists among other people are also upset. As long as humans keep developing new techniques to keep people alive they will have to answer to the ethical questions that keep being brought up. Once we decide who's more important the animals or the humans then we can become a lot closer to finding an answer to whether we should use transgenic animals or not. Such an answer will probably never be found as long as there are different people with different points of view. Some people argue that maybe diseases that don't have any cures to them are put on to this planet to keep the human population in check. Others wonder whether humans are outsmarting themselves? Or maybe someday these technologies will all backfire and when we find a cure for every disease then something else will then crop up in our environment. Technology has a history of doing that to us. For example with the invention of guns came the killing of people. Should we abandon guns because some people aren't responsible? With everything in life comes ethical and moral questions, and they cannot be avoided.

Some of the research that is being done is somewhat uncalled for and needs to be kept in check, but there is also a lot of research done with the transgenic animals that could benefit many people. By stopping the use of OncoMouse™, progress in cancer research will be slowed incredibly, and many more people will end up suffering unnecessarily. Unfortunately there are some experiments that are done just because scientists are able to do them, and those are the ones that need to be stopped. Also to be

stopped are the experiments that severely harm the animal with no medical benefits such as in the case of Superpig. When an animal gets a severely debilitating disease and is in obvious pain there is a responsibility of the investigators and the people involved to stop the experiment. There should definitely be a strict governing body over the use of transgenic animals and their welfare, but the use of transgenic animals should not be discontinued in today's research. Too many new discoveries have been made about the use of genes in today's diseases and treatments to abandon this type of testing. The animals that are used for medical research are the only ones that should be created.

As with any technology we will have to wait to see how it affects us down the line. If we weren't meant to use transgenic animals then something will happen and it will blow up in our faces. Who knows, maybe we'll create a species by accident that will come back and kill us all off. One of these days if technology keeps progressing at the rate that it is, we will all outsmart ourselves and there will be tragic consequences. Until then we will just have to wait and see.

Chapter 4: Legal Issues of Transgenic Technology

Introduction

The Constitution of the United States of America was drafted over two hundred years ago. It was impossible to foresee the direction of change of society and technology that has occurred in the years since. Fully aware of the fact that times change, the framers of the United States Constitution were careful to add enough flexibility so that federal laws could adapt to changing times and opinions. The Constitution is the backbone of all laws in this country. It establishes the three branches of government, details how their members are elected, and defines a system of checks and balances to prevent any single branch from having too much power. The Constitution also sets clear and strict guidelines as to what laws may and may not be passed, and how laws are passed and repealed. The United States Code is the blueprint that defines the agencies of government and the power they have. It establishes and regulates the Food and Drug Administration, the Census Bureau, and the National Guard to name a few. The United States Code is divided into 50 Titles, each defining a specific area of federal law.

Acts of congress, the Legislative branch of the government, are responsible for changes to the United States Code. These acts may be vetoed by the Executive branch, headed by the President, but that veto may then be overturned by a 2/3-majority vote in both houses of congress, the House of Representatives and the Senate. The J0udicial Branch, headed by the Supreme Court, decides the constitutionality of legislation passed by the federal and state governments. Again, however, Congress has the ability to amend

the Constitution or the U.S. Code if they believe the interpretation of it differs from what they believe was the intent of the drafters, or if they feel the change will bring about a greater good.

Changes in society and technology bring about controversies that often lead to amendments to the Constitution and acts of Congress. Over the years, the Constitution has been amended to limit the number of terms a president may serve in office, to forbid the passing of laws intended to segregate on the basis of race or religion, and to broaden the right to vote to include all citizens above the age of eighteen (Article V, U.S. Constitution).

New developments in technology have spurred controversy leading to changes in the United States Code. For example, with the invention of the telegraph, Title 47 of the United States Code was created to regulate this new invention. As wireless radio technology emerged in the 20th century, Chapters 3 (Radiotelegraphs) and 4 (The Radio Act of 1927), were added (47 U.S.C. Sec 40-150). Later, as the business of wireless communication grew, those chapters were repealed to make way for the Federal Communication Commission, created by the addition of Chapter 5 (47 U.S.C. Sec 151-615).

The advent of gene-altering technology has also had an effect on federal law. This IQP chapter discusses how the federal government has addressed the issues raised by the origination and growth of transgenic technology. Controversies have arisen over

whether or not living organisms should have federal patent protection, whether federal funding is acceptable for such experimentation, and what laws must be placed to prevent harm from such technologies.

The legislative, judicial and executive branches of the government primarily act to enforce the U.S. Code and seek to change it only when necessary. Therefore the federal government is reluctant to act on the fundamental ethical issue of whether or not genetic experimentation should be allowed at all. This is not a decision that should be made prematurely, especially in the light of the enormous potential for greater good. The U.S. Patent and Trademark Office (PTO) and the Supreme Court have had the most influence over the progression of Transgenic Technology, and, conversely, are the areas of government most affected by it. This chapter deals primarily with the role of the PTO and the decisions of the Supreme Court regarding transgenic technology and the ownership of life.

A brief history of non-human life in the law before transgenic technology

The origin of laws in this country pertaining to the ownership of discoveries and inventions dates back to the framing of the Constitution. Article 1 Section 8 empowers the federal government, "To promote the Progress of Science and useful Arts, by securing for limited times to Authors and Inventors the exclusive Right to their respective writings and discoveries" (N.A.R.A., 1998). The first patent laws were enacted in 1790 and were finalized into their present form with the Patent Act of 1952 (Perpich, 1986).

These laws allow the ownership of intellectual property, discoveries and inventions and the right to profit from them.

With the discovery or creation of a new form of life, whether it be a new breed of plant or a genetically altered mouse, comes the double question of is it ownable and if so, who owns it? The federal government first addressed the ownerships of newly discovered or created living organisms in 1930 with the Plant Protection Act (35 U.S.C. Sec 161). This act allowed certain asexually reproduced plants the same patent protection allowed to inventions. The incorporation of this act into Title 35 of the U.S.C. allowed for new varieties of plants that had been "invented" or "discovered" to be patented. This protection extended to the use and sale of future asexually produced progeny of this plant from anyone but the original patentee (35 U.S.C. Sec 163).

The purpose of the Plant Protection Act was to allow breeders and horticulturalists to profit from their inventions/discoveries. The act gives incentive to ingenuity and discovery in the spirit of the original patent act.

The Plant Variety Protection Act of 1970 (P.V.P.A.) created a body for the protection of plant varieties separate from the Patent and Trademark Office. The Department of Agriculture became the Plant Variety Protection Office (7 U.S.C. 2321). Rather than offering patent protection for new plant varieties, The Plant Variety Protection Office was given the power to grant and enforce certificates of variety

protection (7 U.S.C. Sec 2481-3 and 2561-70). The scope of protection was wider than the 1930 act as it included seed-grown plants.

Although Congress wished to provide protection for progress in horticulture, it did allow for exemptions for the sake of agriculture or research. Under this exemption a farmer is allowed to store and sell seeds obtained, or those descended from seeds obtained, by authority of the owner of the variety for purposes other than reproduction, and to sell harvests from such seeds (7 U.S.C. Sec 2543). The farmers' exemption was added to protect small family farms from unfair competition from larger farms under license to grow superior plant species (Edwards, 2001). Use and reproduction of the protected variety for research purposes is also allowed and does not constitute infringement under the P.V.P.A. (7 U.S.C. Sec 2544).

Patent protection for plant varieties was tested in 2000 in the case Pioneer Hi-Bred International v. J.E.M. Ag Supply. Pioneer HI-Bred International sued J.E.M. Ag Supply and some of their colleagues for patent infringement. The defendants, who were selling protected varieties of seed without permission, were found to be in infringement of Pioneer's patent rights. They appealed the decision and brought the case to the Federal Circuit Court ("Pioneer Hi-bred", 2000).

The defendant pointed out that at the time of the P.V.P.A. Congress believed that seed-grown plants were not included in the patent statute (35 U.S.C. 161), and that Title 35, Sec 101 can not now be interpreted as available to seed-grown plants when Congress

believed otherwise. They also objected that Pioneer held patents under Title 35 as well as certificates under the P.V.P.A., and that these statutes are in conflict.

The court cited the *Ex Parte* Hibberd decision (227 USPQ 443, 444; 1985) where the court authorized the PTO to grant patents to transgenic plants. The court observed that the PTO had been granting patents on new and unobvious varieties of seed-grown plants for fifteen years. To the second argument regarding statute conflict the court cited Radzanower v. Touche Ross & Co. (426 US 148, 155; 1976) "when two statutes are capable of co-existence, it is the duty of the courts... to regard each as effective" and concluded that a person who develops a new plant variety may have recourse either to patenting under Title 35 or to registration under the P.V.P.A., or, as in this case, both.

Patenting of plants after the transgenic revolution

In 1985, the PTO extended patent protection to transgenic plants (*Ex Parte* Hibberd, 1985). This does not become a landmark decision because the protection available under the Plant Variety Protection Act is usually adequate while the patent process is more costly ("Who Owns", 2001). This statement, however, allows dual protection for plant varieties under both patent law and the Plant Variety Protection Act.

Agracetus, a biotechnology company from Wisconsin was awarded patent number 5,159,135 in 1992 for genetically engineered cotton plants or seeds. In 1994 the PTO cancelled this patent as being too broad in scope ("Who Owns", 2001). This effectively

narrowed the scope of transgenic plant patents to specific lines, whereas the original patent in effect gave protection to any modified cotton plant or seed.

This patent was originally awarded in the spirit of the General Electric, Chakrabarty patent (discussed in the next section). While the PTO refused to grant patent protection to Chakrabarty's specific genetically altered bacteria, they awarded a patent to the method of genetically engineering the *B. cepacia* bacteria. By that reasoning any attempt to alter the same species of bacteria could be considered infringement of the patent. Thus Chakrabarty and GE could be said to have a patent on all genetically modified strains of *B. cepacia*. Agracetus similarly owned such a monopoly on cotton before the patent was cancelled.

Diamond v. Chakrabarty: Patenting a non-natural living organism

The first transgenic organisms were single celled bacteria. Although the issue of patent protection for a single celled organism would have seemed simple based on the simplicity of the organism in question and its impersonal nature, it was anything but. The Plant Patent Act of 1930 made no mention of single celled organisms, and the late Plant Variety Protection Act of 1970 specifically excluded bacteria and fungi. This left a void of indecision before the PTO when first presented with a patent application for a novel, human made, non-naturally occurring, gene-altered bacterium.

Shortly after the Plant Variety Act of 1970 a micro-biologist working for General Electric applied for a patent for a new strain of *B. cepacia* bacteria who's DNA he had

modified. Dr. Ananda Chakrabarty had genetically modified *B. cepacia* adding the ability to metabolize large amounts of crude oil into substances suitable for consumption by other marine life forms (Diamond, 1979). The bacterium was created as a tool to clean up crude oil spills. The patent application was for three patents: the process used to genetically alter the bacteria; the inoculum made up of the bacteria and a floating carrier material; and the bacteria themselves. Patents were granted for the first two. The third was denied on the grounds that the subject in question was a product of nature and that living things are not patentable (Diamond, 1979). The decision was appealed to the Patent Office Board of appeals who upheld the decision that living things are not patentable subject matter.

Eight years later, Diamond vs. Chakrabarty (Diamond was the commissioner of the PTO in 1972) came before the Supreme Court (Diamond, 1979). The Supreme Court held 5 to 4 that genetically altered bacteria were patentable subject matter. The court felt that because of the broad wording of the patent laws, "...any new and useful process, machine, manufacture, or composition of matter..." (35 U.S.C Sec 101), congress intended for patent laws to have broad scope. They felt, "composition of matter" could not be interpreted to exclude living organisms. They observed that the Plant Act of 1930 extended the scope of patent law to include living things, and although the Plant Variety Protection Act of 1970 specifically excluded bacteria, this did not mean that congress meant for bacteria to be excluded from patent law.

The Supreme Court decision left an opening for Congress to decide whether microorganisms, and living organisms in general, should be patentable. Part C of the decision states that, "Arguments against patentability under [35 U.S.C.] section 101, based on potential hazards that may be generated by genetic research, should be addressed to the Congress and the Executive, not to the Judiciary" (Diamond, 1979). This section of the decision also states that despite the fact that genetic technology was unforeseen when Congress enacted Title 35 of the U.S.C., as long as the invention fulfills the requirements of "novelty" and "non-obviousness", which Chakrabarty's bacteria did fulfill, it is not the place of the PTO or of the Supreme Court itself to deny patent protection.

The dissenting opinion of the Supreme Court in the Diamond vs. Chakrabarty case did, however, raise some good points. It was stated by Justice Brennan for the dissenting justices that the Plant act of 1930 protects plants specifically, leading court to believe that creations unforeseen by congress need congress's expressed protection. The Plant act of 1970, while extending the protection of unique varieties of life, specifically excluded bacteria, leading the court to believe that congress had reserved the right to specifically allow or disallow patent protections to other forms of life (Diamond, 1979).

The dissenting opinion had no objections to the patents awarded for the process of creating the transgenic bacteria, or it's method of delivery. In this the Supreme Court agreed with the PTO. The irony of this decision is that the process for altering the *B. cepacia* bacteria to create the new strain could be very useful. The same process could be

use to generate many different strains of bacteria that could metabolize and neutralize other pollutants. Had the position of the PTO and the dissenting opinion of the court been upheld, future research into bacteria that could clean up waste would have been stifled by a perceived lack of profit potential. The bacteria itself, which could easily be collected and cultured from sites of usage, would not be protected, and therefore the inventor would not be able to profit from the invention itself. For this reason the Supreme Court showed good foresight and upheld the original purpose of the patent act.

This was a landmark decision in that it was the first concrete act of the federal government concerning ownership rights for new, non-plant species of life.

The unfortunate outcome of the Chakrabarty case was that the application of the bacteria was never developed. Chakrabarty created his bacteria while employed at General Electric Company, thus the patent was awarded to GE in 1980 following the Supreme Court's decision. GE had no aspirations of cleaning up oil spills, however, and chose not to develop Chakrabarty's invention (Helfferich, 1989). Nor has GE found anyone else to license the patent to develop the crude eating system.

In this case the fact that patent protection was awarded to the invention actually discouraged the actual, potentially useful, application of it. The decision, however, has encouraged research in the area of genetics, which has led to many new and useful tools for biological and biotechnological research. A spokesman for Genetech, a company

specializing in genetics and transgenic manipulation said the Supreme Court's decision has, "...assured this country's technology future" (Chakrabarty, 2000).

Patenting of Animals

In 1987 an application for patent protection to an animal was brought before the PTO. The invention was a polyploid oyster, an oyster with more than the normal number of chromosomes in its nucleus (Thomas, 1981). The case became known as *Ex Parte* Allen, and although patent protection was refused on the grounds of obviousness (*Ex Parte* Allen, 1985), the PTO stated in their Official Gazette, "The Patent and Trademark Office now considers non-naturally occurring non-human multicellular organisms, including animals, to be patentable subject matter within the scope of 35 U.S.C. 101." ("Patent and", 1987). This opened the floodgates to numerous applications for patent protection for animals.

In issuing this statement, the PTO was careful to exclude human and humanoid animals from patent protection. No doubt this was to avoid the incredible controversy that would surround such a patent attempt. Despite the PTO's clear and public refusal to award patent protection to any transgenic humanoid, in 1997 Jeremy Rifkin and Stuart Newman applied for patent on the creation and implantation into a surrogate mother of transgenic human-animal chimera. Six months later the patent was denied on the basis that, "the claimed invention embraces a human being." ("Who Owns", 2001)

The first animal to be awarded a patent in the United States was the now famed Harvard OncoMouse™ (US Pat. 4,736,866, 1984). OncoMouse™ was created by DNA microinjection. OncoMouse™ has since become and invaluable medical research tool. A human gene known to cause cancer was introduced into the mouse's DNA. The result was mouse that is extremely prone to breast cancer. One benefit of this is the reduced number of mice needed to study cancer treatments. Whereas huge numbers of normal mice would be needed to yield a reasonable number of individuals with cancer, far fewer oncomice would be necessary to yield the same number with cancer. This can drastically reduce the cost and labor associated with such and experiment because of the reduced amount of care, repeated observation and diagnosis of the test population.

In an interesting side note to the Harvard OncoMouse™, the gentlemen who unknowingly donated spleen cells to the project later sued. In 1984, the same year as the Harvard OncoMouse™ patent application, John Moore sued his doctor, the University of California, and two biotechnology firms for the use of his cells without his expressed permission. *John Moore v. The Regents of the University of California et. al.* went before the Supreme Court of California in 1990. Moore's basis for the suit was, "that his physician failed to disclose preexisting research and economic interests in the cells before obtaining consent to the medical procedures by which they were extracted." ("Moore v.", 1990). Interestingly, the court found that Moore had no property rights to the cells that were taken from him.

At this point it is important to note the specification requirement of a patent application as described in section 112 of Title 35 of the US Code. In short, this section requires the applicant to document the invention and the manner of process for making it in enough detail, "as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same". For example, the description of the creation, analysis, production and testing of the OncoMouse™ is in excess of 4000 words. While this description won Harvard its OncoMouse™ patent, it is a complete recipe for anyone wishing to recreate the invention.

While in academia patents are prized, and publishing one's work is customary, in commerce the purpose is to capitalize. Patent protection is intended to allow inventors to profit from their efforts but there are some drawbacks. Full disclosure of the invention is one drawback. Another is the limited term of the patent, which is the greater of either 20 years from the date of application, or 17 years from the date of grant (35 U.S.C. 154). Often for-profit companies opt for complete trade secrecy instead of patent protection. Many inventions would be easily replicated. The microscopic and subtle nature of DNA makes reverse engineering as difficult as starting from scratch, making secrecy a viable option. Athena Neurosciences of San Francisco, CA chose to maintain their trade secret rather than patenting and publishing their findings.

In 1995 Athena Neurosciences announced that it had created the first workable Alzheimer's mouse model (Nohlgren, 2001). The mouse was originally created by Professor Adams and colleagues at TSI in Worcester, MA (Games et al., 1995). It

developed amyloid plaques in the brain similar to human Alzheimer's sufferers. Athena and Elan Pharmaceuticals, who later acquired them, never published in detail. They intended to use the mice to perform their own experiments.

Their decision backfired, however, as other researchers developed similar Alzheimer's mouse models. One in particular not only developed a mouse with the characteristic amyloid deposits, but also behavioral problems as well (Finn, 1997). University of Minnesota researcher Karen Hsiao's mice showed weakened memories after developing the amyloid plaques (Hsiao, 1996). The Mayo Foundation for Medical Education and Research was licensed by Hsiao and the University of Minnesota to sell breeder mice. The mice were reportedly sold for as much as $800,000 to commercial drug manufacturers. (Non-profit researchers and Universities were given mice for free under the agreement that they give the Mayo Foundation a chance at buying the rights to any commercial products derived from experiments on the mouse.)

Had Athena Neuroscience patented their original Alzheimer's mouse they would have had a precedent for being able to secure royalties from the creators of later Alzheimer's mouse models. *Graver Mf. Co.* v. *Linde Co.* (Graver Mfg., 1950) declared that two different inventions that, "...do the same work in substantially the same way, and accomplish substantially the same result, they are the same, even though they differ in name, form or shape." Athena Neuroscience would have a case to claim infringement by the University of Minnesota and other developers of Alzheimer's mouse models.

Current and Future Concerns

The latest news in the realm of transgenic research is the recent decision of the president to allow federal funding for research of Embryonic Stem Cells. ES cells represent the earliest stage of human life. Being such, many religious conservatives are strongly opposed to research on ES cells, claiming that it is a destruction of human life. Congress has imposed a ban on federal funding for research, although privately funded companies have been continuing their research.

This month (August 2001) the president agreed to allow federal funding for ES cell research on the existing ES cell lines. The catch being that the funding may not be used to experiment with new embryos, only the ES cell lines already identified. A worldwide inventory counts 60 different ES cell lines (Stolberg, 2001b). The exception was to allow academic and other non-profit research to progress in an area of medicine that is very promising. Previously, institutions receiving federal funding for other, non-related experiments were apprehensive about experimenting on stem cells with private money for fear of loosing their federal funds. Several scientists emigrated to countries like the U.K. who imposes less stringent restrictions on that type of research.

Now that the government has opened the door to academic and non-profit research into ES cells, one legal issue in particular has concerned scientists. The Wisconsin Alumni Research Foundation (WARF), holds a patent covering the isolation of ES cells, the isolated cells themselves and the conversion into liver, muscle, nerve, pancreas, blood and bone stem cells (US Pat. 6,200,806, 2001). WARF has been

providing its ES cells to research institutions around the world for a flat fee of $5000 with no restrictions other than those federally mandated (Wade, 2001).

The problem is that exclusive rights to converting ES cells into the six tissue types listed above is owned by Geron Corporation of California who financed the research of the ES cells that led to the patent. Despite the outwardly generous nature of WARF's distribution of their line of ES cells, under the patent and the licensing agreement Geron still has a claim to all ES cell derivatives of the six types listed in the patent. Dr. Douglas Melton of Harvard University, frustrated with Geron's monopoly, objected that, "Those conditions would mean that I am the ideal employee of Geron, they don't pay my salary, they don't pay my benefits, but anything I discover they own" (Stolberg, 2001a). WARF is also discontent with Geron's control over the ES cell line on which WARF holds the patent. On Wednesday, August 15th they filed suit against Geron.

Clearly there is still much to be resolved in the issue of stem cell research. What research should be allowed, and who owns the knowledge and products of that research are still being decided. For the time being, however, scientists are content with having access to healthy ES cell cultures and the funds to experiment with them.

Conclusion

This chapter has addressed the relationship between transgenic technology and the federal law: How the law has effected the development of transgenic technology and in return, how transgenic technology has shaped the law. All three branches of the federal

government have their share of influence over this science. Congress has passed laws in the form of federal statues concerning patent protection, the Supreme Court has interpreted these laws to help smooth their implementation and the executive branch has determined where federal funds may be allowed.

Advancements in science and technology originate from two separate camps, for-profit and non-profit institutions, with shared and individual concerns. Patent protection has been the area of most debate, and is a concern for both camps, but the government's role in funding non-profit research is an issue as well. Few laws have been passed at the federal level that seeks to restrict the type of research performed, or how it may be implemented. There are exceptions, though, one of which being the governments ban on experimenting with human embryos, a practice that many people view as being too close to experimenting on live humans.

Patent law has been affected by the advent of genetic engineering (35 U.S.C. 103, 271). More notable, however, is the interpretations of the patent law by both the PTO and the Supreme Court. At first the PTO refused to award patent protection to living organisms, but allowed patents for the process of manipulation. Later the Supreme Court decided that congress had worded the patent statutes in a way that made patenting of genetically altered living organisms acceptable. The PTO later refused a patent on the process of genetically engineering an organism because it was too broad, in effect granting a monopoly on all genetic variations. Thus their original position was completely reversed.

Based on conservative views, mainly religious, congress did impose a ban on experimentation with human embryos. In August of 2001 the president defied this statute somewhat by allowing federal funding for research on ES cells, which are directly obtained from human embryos, but a restriction was made that only ES cell cultures currently in existence are usable and that no new ones may be obtained from human embryos. This decision by the executive branch came very close to being in breech of congressional legislation. At best it was exploitation of a loophole, but that is the executive's right, and they hope it will lead to the greater good.

It is in this manner that the relationship between technology and the law changes as each evolves. A big change in either, such as the advent of genetic engineering or the ban on cloning, causes the other to adapt. The genetic revolution caused patent law to encompass living organisms that were made unique because of man's ingenuity. The extension of patent law to include the living thus spawned heightened research in the area of transgenic organisms, which has lead to numerous advances in medicine and biotechnology. The transition has been smooth. Laws didn't need to be rewritten so much as reinterpreted. Similarly, research was never halted, rather in a few cases it was redirected. This mutualism is a tribute to the solid reasoning behind the legal system, and the resiliency and determination of science.

Conclusion

This project has been a detailed account of Transgenic Technology. The ability to alter the genetic code of living organisms has been hailed, along with splitting the atom, escaping earth's gravity, and the computer revolution, as one of the four major scientific revolutions of this century (Perpich, 1986). Discussed in the chapters of this project have been the theories behind the technology, examples of application, ethical issues, and legal issues.

A transgenic animal is created by modifying its DNA at an early stage of development, either at the single-cell stage or shortly after. The two predominant methods of manipulation are DNA Microinjection and Embryonic Stem Cell Mediated Gene Transfer. DNA microinjection is the most common method and has been effective on several different species. The ES cell method's main advantage is the precision with which genes can be placed or removed. This method works well on mice, but as of yet has been unsuccessful on other species.

Transgenic animals have been used for a variety of different purposes. OncoMouse and the Alzheimer's mouse have provided scientists with invaluable models for human diseases. Transgenic technology has also been used to create animals that can be used as organ donors for humans. The process of implanting an animal's organ into a human is called xenotransplantation. Successful implantation of half of a pig's heart into a human has been accomplished. Tranpharming is the use of genetically modified animals as producers of proteins and other chemicals they would not ordinarily produce. Herman the bull, the first transgenic cattle, was implanted with a gene for the production of human lactoferrin, a chemical that strengthens the immune system of infants.

Herman's offspring generate lactoferrin in their milk. Transgenic animals have also been used as food sources such as A/F Protein's Superfish. Superfish grow twice as large as normal salmon in 1/6 the time. More remarkably, they consume 25% less food.

There is no doubt that all of these are great accomplishments that serve the greater good of humanity. There have been more frivolous 'inventions' such as a rabbit that was implanted with a jellyfish gene. The rabbit glows green under a black light.

When dealing with a technology as powerful as genetic engineering, at some point one needs to look at the bigger picture. We are toying with life for our own purposes. We are creating new species of animals at will with little thought as to whether we should be. We must ask ourselves, after four billion years of planetary evolution who are we to decide the course evolution of species on this planet? Can we justify experimenting on helpless animals for our own good? The facts remain: animals have been the object of human experimentation for centuries; humans have been creating new species by selectively breeding and domesticating animals for millennia; and we have been harvesting their products and lives for our own needs since before history began. The fact that we've recently gained the ability to expand and accelerate these processes does not change the original, moral question facing us: What right do we have? This is an important question and anyone would agree that science needs to use some discretion when life is involved.

Another critical question regarding Transgenic Technology is who owns a life? Now that man has the ability to create new species of animals and plants almost at will there is the very important legal question of protection for the intellectual and monetary investment involved. The Patent and Trademark Office, with the encouragement of the

Supreme Court, has decided that yes, a man-made, non-natural invention is patentable, despite the fact that it may be a living organism. To an extent it is proper that scientists be able to profit from their work. That is simple enough for non-human organisms but when the line is crossed between beasts and human, the subject becomes touchy. Experimenting on human embryos, as in the case of stem cell research, and patents in that area have created some controversy. This area of law is still awaiting resolution.

In the end Transgenic Technology will show great advancement in medicine, agriculture, science and industry. As is so often the case, however, there is a downside. We've found yet another way to exploit Mother Nature for our own needs. Our short-term goal is the improvement in our lives and health. Transgenic Technology shows a lot of potential towards those ends. To mature as a species, however, we need to learn to coexist with all other species. Our long-term goad needs to be the improvement in the lives and health of the earth as a whole.

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