

TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

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ABSTRACT

The goal of this project was to research the controversial topic of transgenic animals, and to look at both pros and cons of this ever-broadening technology. The goal was accomplished by describing the process by which these animals are created, the ways in which they have been used, and the ways in which they can be beneficial to the society. The ethical and moral implications of existing transgenic technologies, as well as the legal issues of patenting and owning life and genomes are discussed, presenting both sides of the arguments. Transgenic technology has a massive potential to benefit society, so long as the effect on the animals is minimal.

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PROJECT OBJECTIVES

The goal of this project was to examine the topic of transgenic animals, and to discuss the effect of this controversial new technology on society. First, the technology was investigated, by examining how transgenic animals are created and screened, and how they are used. Then their effects on society are examined by an investigation of their ethics and legalities. Examples of ethically justified and non-justified experiments are presented, and the laws governing the creation and use of transgenic animals are analyzed. Finally, the authors make a conclusion based on their research.

Chapter 1: Transgenic Technology

Gregory Richmond

A transgenic animal has had its genome intentionally altered to allow that animal to better serve man. Such animals include disease models to aid our understanding of diseases, transpharmers to produce pharmaceuticals in milk, xenotransplanters to produce organs for transplant, food sources, and scientific models to aid our understanding of biology. Such amazing technological feats are performed through the use of recombinant DNA technology (rDNA), which involves splicing and cutting DNA (including between species) for the purpose of inserting or removing pieces from an animal's genome. For this technology to be successful the inserted foreign gene must be successfully incorporated into the DNA of the host and be expressed correctly to give the animal its new trait. The purpose of this chapter is to describe the way such animals are created, as a background for subsequent discussions on their effects on society.

Microinjection of DNA Into a Pronucleus

Transgenic animals are created through two main methods: 1) microinjection into the male pronucleus of a newly fertilized egg, or 2) microinjection, transfection, or viral infection of embryonic stem (ES) cells ("Transgenic Animals", 2003). The most common way of developing a transgenic animal is through microinjection of a gene into a male pronucleus. A male pronucleus is the nucleus of the sperm inside a newly fertilized egg after they have have fertilized but before the two nuclei fuse. The male pronucleus is used because it is slightly larger than the female pronucleus. The transgene injection is performed by holding the fertilized egg in

place by a pipette with a small amount of suction, while another micropipette injects over 200 copies of the desired gene into the pronucleus (**Figure-1**).

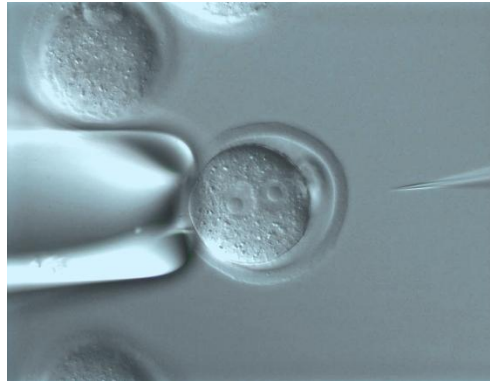


Figure 1: Photograph of Microinjection into the Male Pronucleus.

The photo shows a large pipette (left side) holding the fertilized egg in place with gentle suction. The micropipette for injecting DNA is shown on the right. The male pronucleus is the sphere at the center left (Oregon Health, 2009).

After microinjection with DNA, the fertilized egg is grown *in vitro* for about 5 days to the blastocyst stage to improve its vigor, then it is implanted into the uterus of a foster animal that has been prepared with hormone injections to mimic pregnancy (**Figure-2**). If the technique works, the injected gene will be incorporated into the genome and passed on to the founder animal's offspring. The technique is reliable, but one problem is that the injected DNA can be incorporated anywhere in the genome, producing wildly different results among different founders even when injected with the same transgene (Walinski, 2004). So a subsequent screening of the transgenics made using this technique is important to map the site of insertion to ensure the transgene did not insert into an inactive region of the chromosome, and that no essential animal genes have become inactivated.

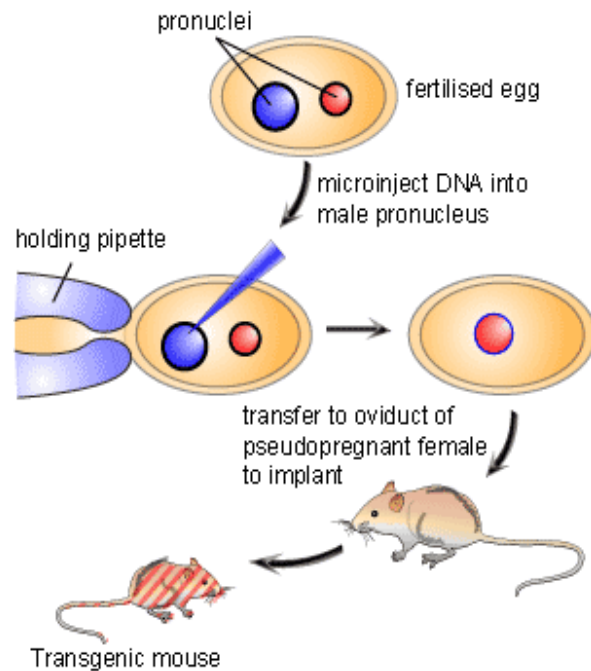


Figure-2: Diagram of the Egg Microinjection Process for Making a Transgenic Animal. The male pronucleus is shown in blue and the female pronucleus in red. The embryo is held in place with gentle suction (right side), and the male pronucleus is injected with DNA (blue needle). The nuclei merge following mitosis (diagram right), and the embryo is implanted into the uterus of a recipient (diagram lower). (<http://www.scq.ubc.ca/studying-gene-function-creating-knockout-mice/>)

Creating Transgenics Using Embryonic Stem Cells

The second main technique for creating a transgenic animal is manipulation of embryonic stem (ES) cells. In this process, an embryo is created by *in vitro* fertilization. The embryo is grown about 5 days to the blastocyst stage, and its ES cells are isolated from the inner cell mass. The ES cells are then manipulated to take up foreign DNA using microinjection, transfection, or viral infection. The manipulated ES cells are then injected back into a blastocyst (**Figure-3**), and the embryo implanted into the uterus of a recipient (**Figure-4**).



Figure-3: Photograph of ES Cell Microinjection into a Mouse Blastocyst. The mouse blastocyst is being held in place by suction with a pipette on the left and on the right are the genetically modified ES Stem Cells being injected inside (Oregon Health and Science University, 2009).

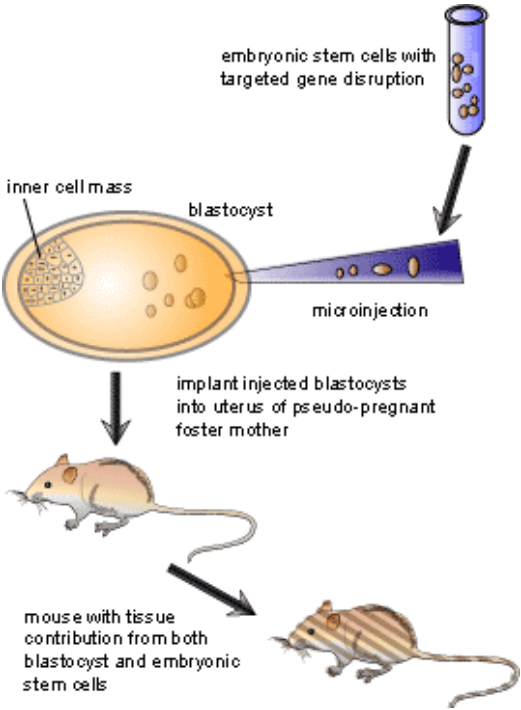


Figure-4: Diagram of the ES Method for Making a Transgenic Animal. The diagram shows the microinjection of manipulated embryonic stem (ES) cells (diagram upper right) into a blastocyst (upper left). The reconstituted embryo is then implanted into the uterus of a recipient (diagram center) to eventually create a transgenic pup (lower right) (Walinski, 2004).

The main advantage of the ES technique is it allows the use of the natural biological process of homologous recombination to target the foreign gene to a specific site in the host chromosome to ensure the transgene is not inserted in an inactive region of the chromosome, and to ensure no essential host gene is inadvertently inactivated. Homologous recombination is a natural biological process that takes place during mitosis where two pairs of sister chromatids align side by side (**Figure-5**). At some points along the aligned chromosomes they connect at a point called the chiasma (panel B) and exchange DNA segments (panel C) (Shorn et al., 2003). When making a transgenic animal, this naturally occurring recombinant process is used to exchange genetic information from the cloning vector we create to the identical region of the host genome we wish to target (Davidson College, 2002). The cloning vector contains the foreign transgene plus two regulatory genes to insure proper expression of the gene. The regulatory genes (or their flanking sequences) are also complementary to specific sites in the host genome. After manipulation of the ES cells to insert the vector DNA, the complementary sequences in the vector bind to the complementary sites in the host chromosome to pair, insuring predictable site integration.

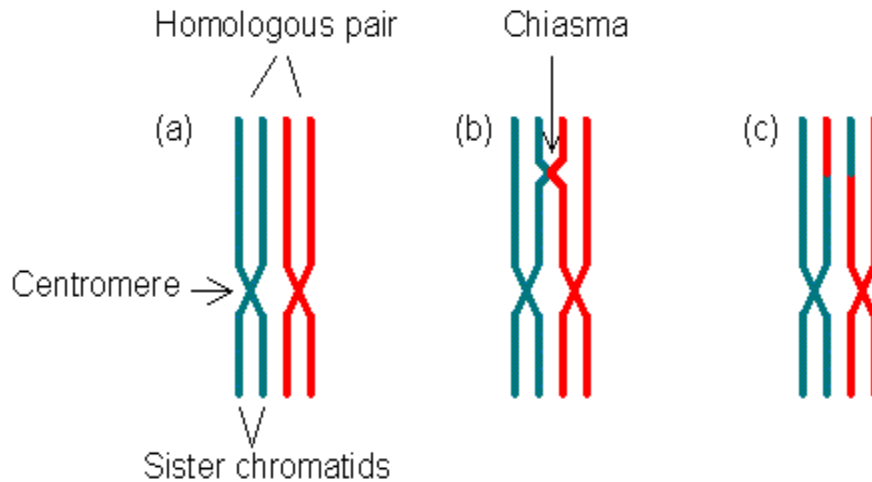


Figure-5: Diagram of Homologous Recombination. During the natural biological process of mitosis, homologous chromosomes pair (panel A). During the pairing, DNA sometimes exchanges between complementary regions of chromatid strands (panel B) to create chromatin exchange (panel C). The enzymes that perform this natural exchange process can be used to target transgene DNA to specific sites in the host animal's chromosome. (<http://www.web-books.com/MoBio/Free/Ch8D1.htm>)

Another popular method of integration of transgene DNA into an animal genome uses viral delivery. In nature, viruses efficiently infect their host cells with their own genetic material, so viruses engineered to contain a transgene can be an efficient delivery method. The most commonly used viruses used for gene delivery in transgenesis are retroviruses because they integrate viral DNA directly into a chromosome (**Figure-6**). However, with this technique there are safety issues with respect to viral replication. Although the virus used for gene delivery is usually engineered to lack key replicative genes, there is a concern that the engineered viruses could recombine with wild type viruses to create an infectious virus. Retrovirus delivery usually yields a 5 to 10% transfection efficiency (Lasic, 1997).

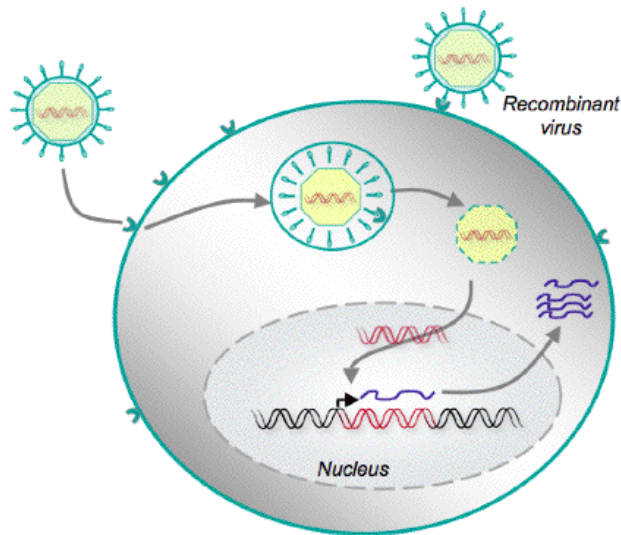


Figure 6: Diagram of Gene Insertion by Retroviruses. A mature retrovirus (diagram upper left) attaches to receptors present on the surface of a host cell. The virus enters the cell (diagram upper center), and the capsid disassembles (upper right) exposing the viral genome. The RNA genome is converted to DNA by reverse transcriptase (diagram center) and the viral DNA integrates into the host genome (diagram lower center). This natural infection process has been used to deliver foreign DNA into animal genomes for transgenesis (Lund University, 2007).

Screening Transgenic Animals

In order to determine the success of creating a transgenic animal, the DNA of the transgenic founder animal or its offspring is tested for the presence of the transgene and its expression. The assays usually used first test for the presence of the transgene in the DNA of the host animal. Usually Southern blots or PCR are used for this purpose. A Southern blot detects specific DNA sequences (i.e. a transgene) in the presence of a complex mixture of DNA (i.e. a host animal's genomic DNA) (Khalsa, 2000). The host DNA is broken up with restriction

enzymes which cut DNA at certain sequences. The DNA fragments are then separated by size on an agarose gel using an electric current. DNA is negatively charged so it moves towards the anode, the smaller fragments move further. The DNA is then split into single strands, called denaturing, to allow it to hybridize to a probe for the transgene. The denatured DNA is then blotted to a nitrocellulose membrane and treated with UV light linking the DNA to the membrane. For accuracy you can then add nonspecific single stranded DNA to limit the binding of our probe to unwanted sequences (pre-hybridization), after which is followed by hybridization to the transgene probe. After hybridization the probe will be visually apparent, whether by radioactive labeling, bioluminescence, or of colorimetric methods (**Figure 7**).

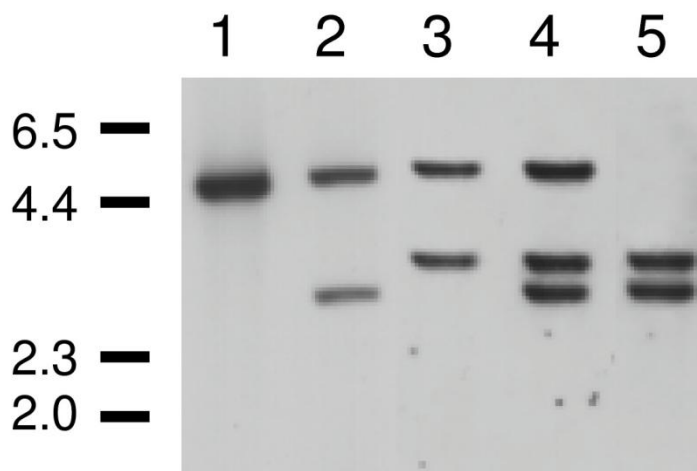


Figure 7: Southern Blot Analysis. The dark black bands denote specific DNA fragments that have hybridized to the DNA probe indicating their presence in the cut DNA fragments present on the gel. This technique can be used to determine whether a specific transgene is present in a host animal's DNA (Wang et al., 2005).

To determine whether a transgene is being expressed into protein in the transgenic animal, usually Western blots or ELISAs are used. These techniques are well suited for verifying the presence of a specific protein (i.e. trans-protein) in a complex mixture of proteins

(i.e. cellular proteins of the host animal). In the Western blot (immunoblot), cellular proteins are separated by size using SDS-polyacrylamide gel electrophoresis (Khalsa, 2000). The proteins on the gel are then blotted to a nitrocellulose membrane, and the membrane is incubated with a primary antibody that specifically attaches to the protein of interest (**Figure-8**) to confirm whether it is present or absent, and if present at which levels (Khalsa, 2000).

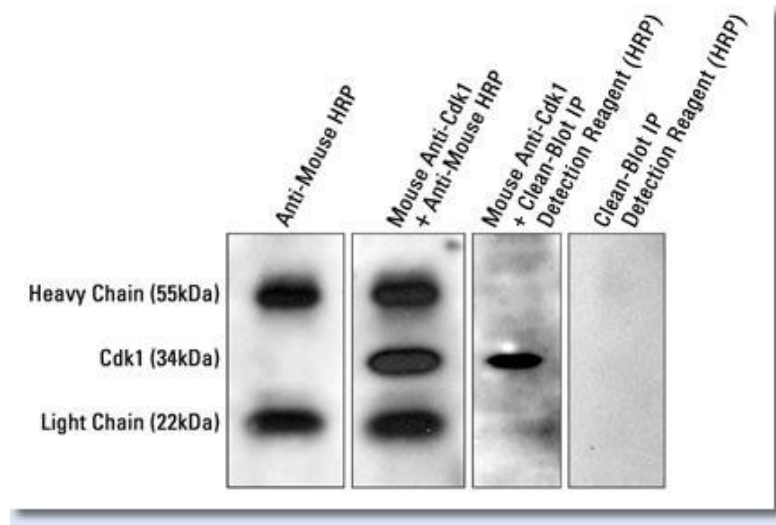


Figure 8: Sample Western Blot. The photo shows the use of three different antibodies (first three lanes) to detect three different proteins in a mixture of proteins. This technique can be used to determine whether a transgene is correctly expressed (ThermoScientific, 2009).

The second assay commonly used to determine transgene expression is the enzyme-linked immunosorbent assay (ELISA). Plastic wells are coated with an antibody to be used to capture the protein of interest (i.e. trans-protein) (**Figure-9**). The sample is then added (i.e. a blood sample from a potential transgenic animal). If the trans-protein is present in the blood sample, it binds to the antibody coating the well. Unbound proteins are removed by washing, then a second antibody is used to detect the presence of the captured transprotein. The greater

the level of attachment of secondary antibody to the well, the greater the presence of transprotein in the well, and the stronger the color formation (University of Arizona, 1998).

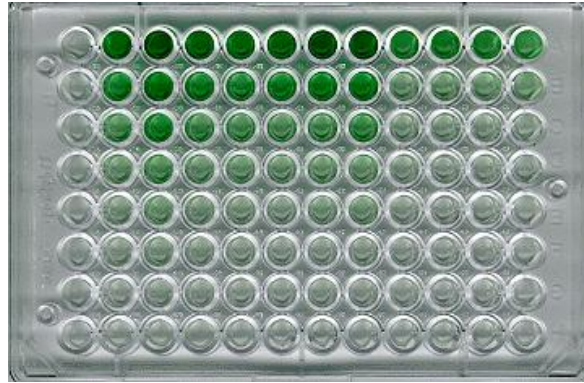


Figure 9: Photograph of an ELISA. Shown is a 96-well microtiter plate treated as described in the text. The darker the green color, the higher the concentration of specific protein in the well. This technique can be used to quantitate the level of transgenic protein present in a transgenic animal (University of Arizona, 1998).

Using the techniques described in this chapter, scientists are able to manipulate an animal's genome to insert a foreign gene and screen whether they were successful in their experiment. Subsequent chapters will focus on the types of animals that have been created, and their effects on society.

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Chapter 2: Types of Transgenic Animals

Ryan Clinton

Now that we have discussed the main *technology* for making and screening transgenic animals, we turn our attention to *which* animals have been produced so far. The purpose of this chapter is to categorize and document the main types of transgenic animals created to date, as a basis for discussion in the next chapter as to whether such animals *should* be made. There are five main categories of transgenic animals, each with a very specific and important use: disease models, transpharmers, xenotransplanters, food sources, and scientific models.

Disease Models

This particular branch of transgenics is one of the most medically beneficial branches because of the immediate impact they have on our knowledge of human disease initiation and cures. They offer vast numbers of subjects for testing potential medicines for human diseases, without the risk of human test subject casualties. These offer hope for people with specific diseases which currently have no cure, such as cancer, AIDS, Alzheimer's disease, and Parkinson's disease.

Oncomouse

Cancer is an uncontrolled replication of cells in the body. This disease can be caused by damage to DNA, especially tumor suppressor genes that normally work to hinder tumor formation, or by activation of oncogenes that lead to uncontrolled cell growth. Oncomouse was first created at Harvard University by Philip Leder and Timothy Stewart in the mid 1980's

(Leder and Stewart, 1984). The animal was created by inserting the human oncogene *ras* into the mouse genome, so the mouse showed a predisposition to tumors. This would allow an investigation into tumorigenesis and the screening of potential therapeutics. In their patent application, Leder and Stewart mentioned the potential use of this transgenic as a test to see if a specific material is carcinogenic (Leder and Stewart, 1984). Another important potential use of these transgenics is the development of anti-cancer or anti-tumor drugs. Since its creation, Oncomouse has been used for many research venues, including adding to our knowledge of various chemopreventive agents which prevent uncontrollable cell growth (Alexander, 2000).

AIDS Mouse

AIDS is an immunodeficiency disease caused by infection with HIV. The virus predominately infects macrophages and helper T-cells, critical components of the immune system. Unfortunately there currently is no cure for AIDS. Progress has been hindered due the the lack of an affordable animal model other than primates in which to study infection and potential cures. Initially, scientists studied the process of immuno-suppression in the SCID (severe combined immunodeficiency disease) mouse which lacks an immune system. When homozygous for this mutation, the animal has diminished B-cell, T-cell, and Natural Killer cell populations and functions (Senpuke et al, 2003).

But the SCID mouse does not support HIV replication since it lacks appropriate HIV receptors and key host proteins required for viral replication, so other transgenic rodents have recently been created as HIV models containing these key proteins (Reid et al., 2001; Ambrose, 2007). Robert Gallo's lab created an HIV rat by inserting the human gene for cyclin-T, a host cell protein required for HIV replication (Reid et al., 2001). Other mice have been created that

express human CD4 and CCR5 HIV co-receptors required for HIV entry. Thus research scientists now have better models than expensive primates to study this disease (Ambrose, 2007).

Alzheimer's Mouse

Alzheimer's disease is caused by the buildup of neurotoxic β -amyloid (a waxy protein fragment) in various areas of the brain. The interaction of β -amyloid with receptors on the neuronal cell surface causes the hyperphosphorylation of tau protein, which destabilizes microtubules to form neurofibrillary tangles, eventually resulting in cell death. The β -amyloid builds up both outside and inside of neurons, when it is outside of the neurons, it eventually aggregates to form senile plaques. When it builds up inside a neuron, it typically finds its way into the mitochondria, which further disrupts cell function, and can also trigger apoptosis.

The first Alzheimer's Mouse disease model was created by Prof. Dave Adams and colleagues (Games et al., 1995) by inserting the gene for human amyloid precursor protein (APP) into mice. The APP inserted was a particular version first identified in a family in Indiana that develops an early onset version of the disease (the Indiana mutation). The human APP protein gets cleaved to β -amyloid to initiate the disease. This mouse line was the first to demonstrate that β -amyloid synthesis is necessary and sufficient for initiating Alzheimer's disease. The mouse line was subsequently used for screening various therapeutic drugs for blocking β -amyloid production, and was used by Elan Pharmaceuticals Inc. to test a vaccine for removing the toxic β -amyloid from brains to demonstrate its removal was beneficial for brain function (Schenk et al., 1999). The Elan Alzheimer's vaccine is currently in phase-III human clinical trials.

Parkinson's Fly

Parkinson's disease (PD) is a neurodegenerative disease caused by the decreased production and/or function of the dopamine produced by dopaminergic neurons. This condition eventually leads to muscle tremors, a slowing of body movement, and eventually a cessation of movement. Currently, there is no cure, although dopamine analogs have been used to temporarily retard symptom progression (Krantz, 2007). As with AIDS, research progress on PD has been hindered by a lack of affordable animal models other than primates.

Parkinson's fly was created by inserting a mutated gene for human parkin into a fruit fly. This causes the fly to demonstrate many of the neurodegenerative symptoms similar to the human disease, including the hallmark drop in dopamine. The flies experience a muscle and mesoderm degeneration which results in a diminished flying ability and grip (Greene et al, 2003). In this model, the males are sterile because the parkin gene is inactivated in sperm leading to a lack of mitochondrial function. To date, no Parkinson's drugs have been developed using this particular model, but hopefully will in the future.

Transpharmers

Transpharmers are one of the most interesting results yielded by the advent of transgenics. These animals present the possibility of a source of affordable and replenishable medicine for those abroad who really need it. Animals are engineered to produce various medicines or drugs in their body. The animals are usually made to secrete the drug in the blood or milk, allowing easy drug purification, thus transpharmers are essentially bioreactors.

Transpharming as a field has come a long way since its original inception. The original transpharmers were mice and other small rodents (Gordon et al, 1987); however, the protein

could not be produced in large quantities in small rodents, so scientists eventually began working with larger animals such as sheep, goats, and cattle (Houdebine, 1994). Mice were an important step in the field of transgenics, mainly because of the rapid rate at which they mature and begin to produce milk.

A mouse producing human tPA was the first transpharmer ever created (Gordon et al, 1987). This protein is an important clot-dissolving drug used to open arteries following heart attacks. Some very important sheep have also been created, such as a sheep producing an anti-hemophilic clotting factor in its milk (Clark et al, 1989), and a sheep which produces human α -1-antitrypsin an emphysema drug. The latter was a very important finding because it proved that transpharming could yield a large amount of functional protein (Wright et al, 1991).

Transgenic goats were the next step up the ladder of protein production, because they produce more milk, but they take slightly longer to produce it. A great example of a transpharming goat is the Genzyme Transgenics-Tufts collaboration project in Framingham, MA. These two prestigious organizations combined their efforts to create the first transpharming goats that produced human antithrombin III, an anti-coagulant (Genzyme Transgenics, 1999).

With respect to milk production, the field eventually focused on transgenic cattle. The world's first transgenic cow (now quite famous) was Herman the bull (Krimpenfort et al., 1991). Herman was created in 1990 by the Dutch company Pharming, and was engineered to pass on the genes required to create human lactoferrin (Cho, 2002), an antimicrobial protein which, as an example, interferes with the gp120 receptor in human cells decreasing the efficiency of HIV infection. Although Herman did not transpharm milk himself, he was a success in that his female offspring produced lactoferrin in their milk, but not in high enough quantities to be

useful. This eventually caused Pharming to drop the project, and move on to working on ways to improve the yield in cow transpharmers.

Scientists continue to try to improve transpharmer technology. In one study, researchers isolated a nearly immortal cell line (KIM-2) from the mammary glands of a pregnant mouse, and used the cells to insert a gene encoding a protein of interest, then inserted the transformed cells back into the mammary gland of the animal (Gordon et al, 2000). This process produces an animal that is transgenic only in the mammary gland, and is more efficient than creating an entire transgenic animal from a blastocyst. Although the technique can be applied to any of the transpharming mammals, it would not be inheritable.

Xenotransplanters

This specific branch of transgenics is of special interest to doctors and people around the world because if successful, organ donors will no longer be a rare commodity. The current well documented organ shortage causes countless lives lost, and has created an illegal market for human organs (Organ Transplants, 2008). With xenotransplanters, this market will no longer be viable since the transgenic organs produced by these animals will be much less expensive. Most animal organs are viewed a foreign inside humans, and immunorejected due to the presence of specific sugar residues on their surface. Xenotransplanters are engineered to not produce these glycoprotein markers on their organs which allow their organs to be used for donors no matter what antibodies the host has (Lai et al., 2002). The animals lack glycosyl transferase enzymes used to add the sugars to the cell surface. Pigs are chosen for these experiments because their physiology closely matches that of humans. Because of the sheer value of the organs contained in one of these xenotransplanting pigs, they can be worth more than a million dollars. Such

animals can be used to donate organs such as livers, kidneys, lungs, etc., and can also be used to donate tissue such as beta-islet cells for diabetic patients. UPMC, a medical company, is looking into commercializing these pigs soon, and distributing them to the 11 national organ storage locations so their organs can be readily available and the transplant wait lists can be cleared once and for all (Fabregas, 2006).

Food Sources

This branch is of transgenics is bred to provide food. The animals have had a growth hormone added to make them grow faster and larger with less food intake.

Superpig

Superpig is the common name for a group of pigs transgenically modified to include either human growth hormone (hGH) or bovine growth hormone (bGH) (Miller et al., 1989). The pigs expressed a higher production of their respective growth hormone with age (Miller et al, 1989). Ovine growth hormone (oGH) has also been tested. In all examples, the promoter for these genes is normally off until switched on by a high zinc diet. Although the Superpig, also known as the Beltsville pig, was a major leap towards improving a major world food source, it came with drawbacks. The altered pigs suffered from a variety of ailments including early onset arthritis, multiple organ failure, and sight issues caused by bugged out eyes. After the realization that these pigs would be plagued by health issues, a voluntary moratorium was placed on the transgenic addition of growth hormone to any mammalian host (Taylor, 1998).

Superfish

With respect to food sources, scientists have had better luck adding growth hormone genes to fish. Scientists have managed to create several transgenic fish which mature faster, and consume less food, which are very important for aquaculture. Contrary to popular belief, these transgenic superfish do not become monstrously larger than their wild-type counterparts, but grow to slightly larger than usual, but tend to reach that size more rapidly, and mature faster overall. Also, they don't need to eat as much food as their wild-type counterparts because their bodies metabolize food 10-30% more efficiently (Devlin et al, 2001). Optimists say this food is only a few years away from FDA approval for consumption.

One concern is that these engineered fish will escape their hatcheries, and outcompete the natural fish, so the geneticists who created them engineered them to be sterile. This is done with a moderate success rate by forcing the animal to be triploid (three sets of chromosomes) (Aken, 2000). This triploidy results in males or females with the genotype (XXY or XXX), and they lack genitalia. Satisfying environmentalists concerns will be important before such fish can be raised in large quantities.

Biological Models

Biological model is the name applied to any animal generated to increase our knowledge of the function of a specific protein. When a new protein is first discovered, key experiments often involve over-expressing it, or alternatively knocking it out, to determine the effects on the animal.

ANDi the Monkey

ANDi the monkey has a very specific name for a very specific reason: the name is iDNA backwards, standing for “inserted DNA”. This little rhesus monkey is the proof of concept for the possibility of eventually creating transgenic humans. ANDi is also the basis for a line of research involving the future creation of primate disease models, which scientists hope will allow them to more closely model (and create treatments for) human diseases. ANDi showed that primates can be the recipients of transgenic genes, but in this specific case he does not mimic a human disease or transpharm a drug. He carries a gene encoding green fluorescent protein (GFP), as a marker protein to prove the technology works. Unfortunately the gene was not expressed (Begley, 2001), but the fact that the transfection occurred was all that was necessary. Transgenic primates will be the subject of much controversy in the future, and will be discussed in Chapter-3.

Smart Mouse

According to Hebb’s Rule, when two neurons fire together, they form stronger synaptic bridges which allow them to subsequently fire more effectively. One way synaptic bridges can be made stronger is if they express the NR2b subunit of the glutamate receptor, as do most neurons in a newly forming brain. Scientists hypothesized that transgenic mice over-expressing NR2b might learn faster (Tang, 1999; Harmon, 1999). This process of facilitating bridging between multiple neurons is also called long term potentiation (LTP). Mice with an upregulated or overexpressed NR2b gene have an increase in LTP, which means that they learn and remember how to complete a maze test faster than a normal mouse.

This research on the glutamate receptor also led to the creation of a new disease model, when scientists realized that mice over-expressing the NR1 subunit of the glutamate receptor show schizophrenic behaviors. This shows that a possible gene therapy treatment (lowering NR1 expression) might be used to treat certain types of schizophrenia, and similar diseases (Bliss, 1999).

Supermouse

Supermouse is the generic name for any sort of mouse which has been altered to include a foreign growth hormone in with its natural genome. There have been several versions: a version with a rat growth hormone (rGH) (Palmiter et al., 1982), and a version with hGH (Palmiter et al., 1983). These ‘super’ animals are given growth hormone attached to a metallothionein promoter which is triggered by the presence of either cadmium or zinc in the animal’s diet. This process results in an animal with a much greater physical prowess than their wild-type counterparts, and that can undergo a large amount of physical activity over a long period of time, has a much faster metabolism (so much so that it requires >50% more food to live), and has no obvious quality of life sacrifices (Connor, 2007). This line of experimentation is important for helping demonstrate the function of the various types of growth hormones, and used the same promoter as the Superpig experiments. The difference is that the mouse is much more able to accept the higher metabolism and body mass alterations than the pig was.

Youth Mouse

Youth mouse is the term applied to a mouse which has been altered to include an over-expressed mouse urokinase-type plasminogen activator (MUPA) which has the interesting effect

of making the host mouse live about 20% longer, and grow to a smaller size (about 20% lighter and 6% shorter) (Miskin and Masos, 1997). It seems that the attenuation of the aging process in these mice is caused by the lack of excess calories metabolized by the mouse. When compared to a mouse that simply had a diet with fewer calories, very similar longevity was found (Miskin et al., 2004).

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Chapter 3: The Ethics and Morals of Transgenics

Ryan Clinton

The ethical and moral boundaries pushed by modern biotechnology is a hotly debated topic because of the many strong viewpoints on the topic. The Merriam-Webster English dictionary defines ethics as “the discipline dealing with what is good and bad, and with moral duty and obligation”; using that definition as my guide, I will discuss transgenic viewpoints from various organizations and people worldwide to document what many people feel about this field. Based on this research, I will then form my own opinion by first describing clear “good” and clear “bad” cases, and finally moving into the middle ground where the distinction is not as clear.

Various Organizational Views of Transgenics

Many organizations tend to have very strong opinions on the topic of transgenic animals, but their views vary a great amount based on the tenants of the organization. Some object to the idea based on the possibility that the world’s biomass might shift following the release of transgenic animals into the environment, while others debate the ethics behind causing possible harm to the animals. Others debate the religious implications of tampering with the genomes with humans and lesser species.

Greenpeace disagrees with the idea of creating a genetically modified organism because of the implications it has towards the natural biodiversity of the Earth. They worry that one day, genetically modified organisms (GMOs) will replace the natural animals of the earth, so in 1992,

Greenpeace initiated its “Cartagena protocol”, an agreement signed by over 150 countries (Greenpeace, 2005). This protocol imposed restrictions on the shipment and distribution of GMOs, and greatly impeded the release of any GMO into the environment. It requires shipments of GMOs to contain documentation stating the exact destination and intent of the cargo, as well as precise handling protocols to ensure that the contents are not accidentally released into the wild (USDA FAS, 2003). The main and very obvious argument by Greenpeace is that these organisms are not natural, and therefore should not taint the world with their unnatural genomes and artificial advantages.

There is also an interesting paper written by the Interchurch Bioethics Council, which debates the “ethical, spiritual, and cultural” aspects of this science (Jarvis et al, 2006). One of this group’s main issues is mankind’s ‘duty to be responsible for, and steward, our fellow creatures. Their analysis at one point focuses on New Zealand’s native Mauri people’s beliefs that the mixing of genes is offensive and insensitive. This is thought to be an important concept due to New Zealand’s highly intermingled multicultural core. Also, one important fact noted by the council, not previously considered by the author of this chapter, is the consumption of an animal containing a human gene could be seen as cannibalism. The Mauri tribe also has concerns about the consumption of transgenic animals because of previous issues they had with the neurological disorder Kuru, caused by the ingestion of neurological tissue containing prions. However, the ingestion of tissues contaminated with disease causing prions is very different than for example ingesting a transgenic salmon containing the human gene for growth hormone (not prions). I found this paper to be an eye-opening one which made me think more about different core belief systems, not just religious ones, and to broaden my perspective of what types of

circumstances might influence various countries to disagree with genetically engineered organisms in general, especially genetically engineered food sources (Jarvis et al, 2006).

A paper published on bioethics by a group of Seventh Day Adventists was very interesting because they actually accept the idea of genetic engineering and genetic testing, but with certain stipulations outlined by passages in the bible (Loma Linda University, 1997). With respect to genetic manipulation, they believe it should be limited only to critical health related enhancements, and approve nothing else (Loma Linda University, 1997). Based on this stance, transgenic animals such as Alzheimer's mouse, Oncomouse, Transpharmers, and Xenotransplanters would be allowed, but not Superfood.

In their Professional Ethics Report, the American Association for the Advancement of Sciences (AAAS) discusses many factors on the general American view on GMOs and genetic research. They logically address many of the issues that several of the aforementioned organizations brought up as key issues with the field of transgenics. They begin by addressing various fears about genetically modified crops. The commercially available crops have alterations giving them a resistance to a certain factor which kills many plants. They are typically either an herbicide resistance factor or a mild insecticide, and neither of these are harmful to humans. They also mention how these can have detrimental results to non-human members of the environment such as the insecticide being poisonous to non-targeted insects, and the possibility of creating resistant strains of insects (Siang, 2000).

This author found all of these various commentaries very informative about the scope and breadth of opinions on the topic of transgenic animals. While some concerns were fairly expected and could be addressed by a small amount of research into the subject, other concerns were encountered that I found unexpected. The Mauri tribe's concerns about the possibility of

prion development and symbolic cannibalism weren't something this author expected to see while researching this. It certainly brings a new viewpoint and set of concerns into plain sight.

Non-Ethically Justified Examples

One way to help determine whether a particular transgenic experiment is ethical is to consider the benefit of that particular experiment to society weighed against any detriments to the animal. We now turn our attention to various transgenic examples, some are clearly ethical in this author's views, others are clearly non ethical, and others are less clear.

In this author's views, there are only a few clear-cut examples of transgenics which fall into a negative ethical area. One of the best examples in this category is Superpig. As discussed in Chapter-2, this animal was engineered to overexpress either human or bovine growth hormone (Pursel et al., 1990) to allow it to grow larger with less fat, on less food intake. Unfortunately, the animal eventually experienced severe health issues, including lethargy, lameness, gastric ulcers, a lack of libido, and several other issues (Pursel et al., 1990; Rollin, 1996). So these pigs are in constant pain and they do not reproduce normally. The construction of these animals reminds us that it is impossible to predict the exact outcome of inserting a particular foreign gene into animals, so in some cases the only way to find out is to make the change and observe the effects. Because of this unpredictability, we must try to reduce the numbers of animals experimented on until the effects are known for a given gene, and in the case where an animal is observed to suffer, to reduce its suffering in any way possible including pain killers or euthanasia (Rollin, 2003).

In this author's opinion, the transgenic Superpig is the only example of a non-ethically justified transgenic animal; all other types have strong benefits to society and should be allowed.

Ethically Justified Transgenic Examples

Based on the criteria discussed above, a positive transgenic example would be a case in which the animal provides a strong medical benefit to society, and does not appear to suffer in any way. Based on the author's research, one example in this category are Transpharmers that are engineered to produce life saving drugs in their milk, but who appear to suffer no ill health effects. Based on the research performed so far in this type of animal, they over-express a protein of value in their milk, but this protein has no physiological effect systemically on the animal. This situation would be very different than expressing the protein in the animal's blood (Society Religion and Technology Project, 2001). Moreover, to obtain the medical benefit, the animals do not need to be sacrificed, they only need to be milked, so the medical benefit to society does not result in the death of the animal.

Another good example of an ethically justified transgenic experiment in this author's views is the Alzheimer's mouse. This animal is a disease model which presents the symptoms of Alzheimer's Disease (AD) by over expressing amyloid precursor protein (APP) which allows the buildup in the brain of amyloid plaques consisting of toxic β -amyloid ($A\beta$) which mimics human AD (Games et al., 1995). With respect to medical benefits to society, the construction of a disease model for this disease aids our understanding of the initiation of Alzheimer's, and serves as a model for testing new therapeutic drugs. Even though the mice show the physical and mental signs of Alzheimer's disease, and learn slower on a learning test, they do not present with any observable detrimental changes, and do not have an altered quality of life (Hsiao et al., 1996).

Ethically Unclear Transgenic Examples

Because of the complicated nature of some of these transgenic animals, and their complex intended uses, a few experiments fall into an ethically gray area in this author's views. I think the best example of this ethically gray area is the xenotransplanter pig, a pig whose cells have been genetically altered to not express specific glycoprotein residues on the surface that are viewed as foreign by the human immune system. This general type of animal is also called a knockout transgenic because its genetic alteration deletes the expression of one of its own genes instead of expressing a foreign human gene. The potential medical benefit of working with transplant animals, especially pigs due to their compatible physiology to humans, is immense for patients awaiting transplant organs.

Organ donations currently occur post-mortem, but the donor must be histocompatible with the recipient, which does not occur very often, leaving an organ shortage (Correa, 2001). The number of people awaiting transplants has reached record high levels. For example, in the United Kingdom patients awaiting organ transplantation reached an all time high of 7234 (Dobson, 2007). In the United States, there are about 100,000 people on the Organ transplantation waiting list, while only 9000 transplantations actually occurred between January and April of 2009 (U.S. Transplant Data, 2009). Thus, many feel that we are morally bound to try and find another way to procure these organs to save lives.

The ethical dilemma with xenotransplanters is the animal must be sacrificed to obtain its organs to save a human life. Many organizations such as the Animal Aid Youth Group feel that this type of activity is heinous, and possibly harmful to the world by increasing the chances of a porcine virus entering humans to cause a pandemic (Animal Aid Youth Group, 2006). However, arguments in favor of xenotransplantation come from the International Xenotransplantation

Association, in fact it is one of the rare groups this author encountered which provided a positive outlook on the field of xenotransplantation (O'Connell, 2003). The entire purpose of this organization is to promote xenotransplantation as a valid, ethically acceptable, practice for saving human lives. They aim to do this by advocating strong legislative oversight of the entire process to ensure no unethical activities occur, and making the technology and its virtues known.

In this author's view, these pigs are close to the borderline of 'ethically justified'/'not ethically justified experiments, but one factor rises above the others, the chance of saving human lives. Although I share the viewpoint of some groups, that animal lives are not worthless, I do not share the viewpoint that they are equivalent. And the animal suffering is not chronic as with Superpig, it occurs only at time of euthanasia. Therefore as long as the organs will function properly in the human body, and will not pass on non-human pathogens, I am all for xenotransplantation.

Oncomouse is another ethically questionable creature because we have given it one of the worst illnesses known to man. Oncomouse has been altered to make it more susceptible to developing the genetic mutations which result in cancerous growth. As discussed in chapter 2, these animals are often created by inserting a human oncogene into the mouse's genome which gives it a predisposition to develop tumors (Leder and Stewart, 1984). In this author's opinion, Oncomouse is the closest grey example to being non-justified because it was given a terrible disease, but the redeeming factor here is that they have been used to further our knowledge of cancer to save human lives (Alexander, 2000). This author believes that Oncomouse is an ethically justified transgenic experiment because of its potential for curing cancer, as long as the suffering of the animals is closely monitored and moderated, there should be no reason to ethically argue against this type of transgenic animal.

Chapter-3 Conclusion

The author of this chapter feels that transgenic experiments cannot all be lumped together into one group with one clear ethical outcome, but each experiment must be individually analyzed and judged. Some types appear clearly ethical, other types are clearly unethical, and others require deep thought and consideration. Personally, I think that Transpharmers are clearly ethical, as they provide strong medical benefit with no apparent animal suffering or sacrifice. This type of transgenic is possibly one of the most useful and beneficial for all of mankind. It has the potential to create a renewable source of drugs, while being relatively easy to maintain. Alzheimer's mouse is also in this category, providing a critical model for testing drugs to block this devastating disease, while causing no observable suffering to the animal. Xeno-transplantation in my opinion is the second most important transgenic category next to transpharming. When xenotransplantation is perfected, and the organs are immune-response-free and pathogen-free, it will save the lives of thousands of people annually awaiting organ transplants or dying from transplant rejection.

The most unethical and immoral practice in transgenics to date was the Beltsville pig (Superpig) which led to massive animal suffering and behavioral changes, while providing no strong medical benefit to society. To this day there is a voluntary moratorium instituted by scientists prohibiting mammalian growth hormone experimentation. Non-mammalian growth hormone experimentation on the other hand (Superfish), has great potential to help feed the world by creating a source of food whose population is ready for consumption much quicker and on less food than its wild-type counterpart. Though some are worried about these animals

escaping into the wild, they have been engineered to be incapable of growing genitalia by an imposed triploidy, so this is not a problem.

In this author's view, any transgenic research which is beneficial to society and does not cause outrageous or unmediated animal suffering, is not only ethically justified, but is almost immoral to not do such experiments. It is important to do all that is possible to save human life, and as long as no undue animal pain is caused, the world will be improved.

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Chapter 4: Transgenic Legalities

Gregory Richmond

As is typical for any controversial technology, laws have been enacted to control the construction of transgenic animals. From a legal stand point, one of the most controversial topics surrounding transgenic animals is whether an individual or company can patent life, and if so to what extent. Scientists want to patent their transgenic animals to claim their research as their own in the eyes of the law, and to perhaps make a profit to continue performing research, while activists don't want them to be able to do this arguing you can't put a price-tag on life. The purpose of this chapter is to discuss some of the early court cases involved with patenting life as an example of the impact of a new technology on society.

The First Patents on Life

The first patent of a *living* organism was granted in 1930 and was entitled the *Plant Patent Act*, which covered newly developed asexually reproducing plants. However, until 1980, no patents had been allowed on animals or microbes. The first patent awarded for a *microbe* was presented in 1980-1983 to Dr. Ananda M. Chakrabarty who developed a bacterium able to break down crude oil into shorter chain molecules (Diamond vs. Chakrabarty, 1980). This bacterium is not naturally occurring, so it initially appeared to satisfy the three main requirements of a patent: it was useful, new, and non obvious (Ladas.com, 2003). But because the case involved a *living* microbe the case was actually quite prolonged with appeals.

Dr. Chakrabarty's patent claim was in three parts: 1) the *process* of producing the oil digesting bacteria, 2) the system for *delivering* the bacteria to the oil, and 3) the bacteria

themselves. The US Court of Customs and Patent Appeals initially allowed the first two tenants, but denied the third tenant, arguing that the bacterium created by Dr. Chakrabarty was not a “product of nature” but was changed and manipulated by his own hands, and therefore the modified bacterium is not patentable under the U.S. patent law, which states:

“Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title.”

However, The Court of Customs and Patent Appeals in 1983 reversed the initial rejection allowing the third tenant, arguing the engineered bacterium is a new “manufacture” and “composition of matter” as outlined in the Patent Law of 1793. The Patent and Trademark Office in a now famous statement said:

“The Patent and Trademark Office now considers nonnaturally occurring non-human multicellular organisms, including animals, to be patentable subject matter. The Board’s decision does not affect the principle and practice that products found in nature will not be considered to be patentable subject matter. An article of manufacture or composition of matter occurring in nature will not be considered patentable unless given a new form, quality, properties, or combination not present in the original article...”
(Edwards, 2001).

This 1980-1983 case set a landmark precedence that multicellular organisms including animals can be patented, and acted as a framework for one of the most famous legal cases of all time, Harvard’s Oncomouse.

Oncomouse Courtcase in the United States

The first complex living organism to be patented in any country was Harvard and Dupont’s Oncomouse. Philip Leder of Harvard produced this transgenic cancer prone mouse in work funded by Dupont, by incorporating the human oncogene *myc* into the mouse’s genome,

which triggers tumor growth. As discussed in Chapter-2, Oncomouse provides us with the ability to research cancer origins and cures in a convenient lab model system, but the Oncomouse court case raised serious questions of whether animals should be patentable, and how should the morals of animal suffering be addressed in relation to allowing the patent. After several appeals, the patent was eventually granted in 1988 to Dupont and Harvard College for a process for preparing transgenic animals, and for cloning oncogenes. To address moral concerns that humans cannot be patented, the transgenic patent excluded humans and any modifications of the human genome (WIPO, 2006).

Oncomouse in Europe

In Europe, oncomouse had to answer to two articles of The European Patent Office's (EPO) guidelines. In Europe, patents are not awarded if "the publication or exploitation of which would be contrary to *ordre public* or morality." Also European patents at that time excluded "animal varieties or biological processes for the production of...animals" (WIPO, 2006). In 1989, Oncomouse was initially denied in Europe because the patent law clearly stated that no animal variety may be patented. But this was appealed under the premise that oncomouse is not an animal variety (new species), but is a new engineered animal. The appeal was awarded in 1992 (Sharples and Curley, 2009).

After the 1992 initial patent award, the next problem for the scientists was the opposition from third parties who claimed that the mouse did not fall under the "morality" *ordre public* clause outlined in European patent laws. To answer this morality question, the European oncomouse case addressed whether the transgenic animal's suffering outweighs the medical benefits to society. The EPO eventually found that the medical benefit of increasing our

understanding of cancer initiation, and screening potential cancer drugs, outweighed the mouse's suffering, so the European patent was finally awarded in 2004 (WIPO, 2006).

Oncomouse in Canada

Although the Oncomouse patent was eventually awarded in the US and Europe, Canada took a different approach. The Canadian and American patent systems are very similar in terms of what can be patented, but have one very significant difference. In America anything made by humans is patentable, including a mouse. In Canada, microorganisms can be patented, but anything considered a complex organism cannot (Bird and MacOdrum, 2008). Following Canadian patent law, Canada ruled that the mouse itself was not patentable, but the *processes* used to create oncomouse were (WIPO, 2006). Canada's laws state that a patent must be a "manufacture or composition of matter within the meaning of invention" (WIPO, 2006). From the Oncomouse findings, "manufacture" was defined as a non-living process, and "composition of matter" was defined as a mixture of substances combined by a person. The single cell mouse egg injected with the *myc* oncogene was patentable as a new composition of matter, but the multicellular mouse itself did not fall under Canadian patent law (WIPO, 2006).

In 2002, Harvard scientists appealed this decision as they had in Europe, and it was initially ruled that the altering of genetic material was a form of "composition of matter", and that Oncomouse fell within the patent law, but this appellate ruling sparked outrage with the animal rights activists and religious groups who argued that patenting animals would prevent the distribution of research materials in other fields of research, drug and treatment discovery (Check, 2002). The opponents also argued that the moral downfalls of allowing an animal to be patented outweigh any possible research benefits. So the Canadian government, after receiving

numerous letters from activists and religious groups in Canada, appealed the case for a second time, and the outcome was to overturn the patent as no longer valid in Canada. The Canadian Supreme court justified their decision by stating that life is not just a “composition of matter” (Mitchell and Somerville, 2002). To this date, Canada remains the only developed nation to reject the Oncomouse patent.

Recent FDA Regulations

With respect to patenting animals in the US, recent guidelines were released in January 2009 by the Food and Drug Administration for governing the use of genetically modified animals. These new guidelines consider the recombinant DNA in transgenic animals as a drug, giving the FDA the ability to regulate such patents. The FDA will thus investigate the safety and environmental impacts of transgenic animals.

In the original drafted guidelines released in September 2008, scientists and citizens were concerned with the lack of transparency in the patenting process. So the new guidelines, to provide more insight into the patenting process, mandate the holding of public advisory committee meetings before the FDA will approve anything. Citizens still have some concern over the transparency though, because the information released in the public advisory meetings will be restricted. The public also concerned over the FDA’s ability to evaluate risks of the transgenic animals, and the lack of labeling requirements on transgenic animal products (Ledford 2009). Preventing the consumer from knowing details doesn’t reduce any of their concerns.

Chapter-4 Conclusion

Transgenic animals, like other products, are an industry, which provide jobs and competition. In the case of transgenic animals, competition between scientists leads to faster and

better “products” ranging from disease cures to bigger and healthier food. The more scientists learn, the more benefits society gets. New technology can also lead to more investments, expanding research opportunities, and new jobs in the industry.

The new FDA regulations put in place are there to aid in the question morality. It isn't easy to obtain a patent for a transgenic animal. Oncomouse is a prime example of this; it still is not patented in Canada, and it took over a decade to be patented in Europe. The recent FDA regulations, while under criticism, are a step in right direction for the United States, as they attempt to bring openness to the process for public view. The patent office is there to decide what is patentable according to the law, and the author of this chapter agrees that another institution needs to weigh the morality.

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PROJECT CONCLUSIONS

The authors of this IQP believe that further development should be done with transgenic animals in the categories as superfish, Alhezimers mouse, and transpharmers. All of these examples provide strong benefits to society with no observable animal suffering.

Other cases are not so clear cut when some animal suffering is observed. In the case of superpig, the scientific community proved it can regulate itself with their voluntary moratorium on human growth hormone insertion into mammalian hosts, and the authors agree such experiments should be banned. With respect to xenotransplanters, the authors believe that with their strong medical benefit to society (transplants in a world of severely limited donors), this area of should be developed since the animals could be sacrificed humanely to obtain the organs, unlike with superpig where the pain was chronic. With respect to oncomouse, the authors feel that, as was stated at the conclusion of the European oncomouse case, the benefits to society outweigh any suffering by the mouse. And with the use of early euthenasia and painkillers, the pain could be minimized while still achieving the benefits. The documented court rulings that allow scientists to patent their transgenic animals represent a great stepping stone for scientists to protect and further their research. Although the authors agree with the patenting of transgenic animals, we also believe that as the industry grows, more regulations will be needed to control it.