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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

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ABSTRACT

This Interactive Qualifying Project (IQP) examined transgenic animals and their effects on society. The project first describes the technology used to create such animals, then categorizes the types of transgenic animals created to date. Later chapters focus on their benefit to society versus the ethical concerns of their creation, and documents current legislations regulating their use. Finally, the project authors provide a conclusion about which transgenic experiments should continue or cease. This IQP strives to relate the social needs for transgenic animals to the concerns raised by the development of this controversial technology.

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PROJECT OBJECTIVE

The purpose of this project on transgenic animals is to discuss the methods, means, and morals of this controversial new technology, to examine the effects of this technology on society. The methods for creating genetically modified organisms are explained in detail. Then the applications and directions of the science are reviewed. Moral and ethical issues are discussed regarding the benefits to society versus the cost to the animal. Next, the laws regulating the development and growth of the transgenic industry are investigated. The project aims to inform the reader of key relevant information regarding the debate on transgenic animals so that they may be able to come to a conclusion on their own about how this technology should be regulated.

Chapter-1: Transgenic Technology

A transgenic organism is genetically modified to have foreign genetic material for the purpose of giving it a new trait. The technology to create one has come about fairly recently in man's history. The first transgenic organism was created in the 1970's; using recombinant DNA techniques, scientists were able to take deoxyribonucleic acid (DNA) (the genetic material that makes up all living things and some viruses) from one type of bacteria and insert it into another completely different bacteria, using a plasmid as cloning vehicle. Although the end results lie inside an organism, most of the experimentation actually occurs *in vitro*, which in Latin translates to "in glass."

Currently, there are many different methods for making transgenic organisms. Chemically competent cells can be made that take up foreign DNA, DNA can be physically microinjected into a cell, or a virus can be used to deliver the DNA inside a cell. Although there are many different ways to create a transgenic organism, the basic ideas behind the techniques are the same; the DNA containing a gene, the basic unit of heredity in a living organism, must make it inside a cell where it can be expressed as a RNA and protein that dictate a specific function within the cell. And once these organisms get made, they must be screened to determine whether they are transgenic. Some screening methods test for the proteins made from the gene of interest, while others test to see if the DNA integrated into the host DNA, and still others can test the rate of expression of the gene. In the span of about four decades, recombinant technology has come extremely far. The purpose of this chapter is to describe the technology for

making and screening transgenic animals, as a prelude to subsequent discussions of the technology on society.

A Brief Transgenic History

In 1866, Gregor Mendel, a man know today as the "father of Genetics" published a paper called "Experiments on Plant Hybridization", where he studied inheritance and traits in peas. He concluded that there were two laws governing the inheritance of plants: the law of segregation, and the law of independent assortment (Mendel, 1866). In 1869, Fredrick Meischer discovered and isolated DNA from a cell nuclei, although it was unknown at the time that DNA was genetic material. Later, in 1928, Fredrick Griffith studied transformations between virulent (disease causing) and non-virulent bacteria in mice. Griffith discovered what he called the transformation principle, noticing that by injecting mice with a live non-virulent stain of bacteria mixed with a dead virulent strain, the mouse would die because something from the virulent strain was being absorbed by the non-virulent one to transform its characteristics into a virulent strain. In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty, using the work Fredrick Griffith had started, made a major break through by concluding the Griffith transforming substance was DNA, the inheritance molecule (discussed in Avery et al, 1979).

Then, in 1970, Hamilton Smith discovered restriction endonucleases (enzymes that cut DNA at specific sequences), giving us the tools to begin to work with DNA. Paul Berg, in 1972, was the first to join two strands of DNA from different sources into a single plasmid. Finally, in 1973, Stanley Cohen and Herbert Boyer made the world's first transgenic organism. Using recombinant DNA techniques, Cohen and Boyer altered the genome of *E. coli* by adding a gene from another type of bacteria (Cohen et al., 1973). In 1974, Rudolf Jaenisch created the world's

first transgenic animal by inserting foreign DNA (from an SV40 virus) into early stage mouse embryos; not only did the mice carry the modified gene but the mice also transferred the gene to their progeny (Jaenisch and Mintz, 1974).

Since those landmark transgenic experiments were performed, transgenic technology has emerged into the forefront of research, and along with it has appeared legislation for regulating it. In 1975, the Asilomar conference, lead by Paul Berg, was held in Pacific Grove, California to discuss the potential biohazards and regulations for recombinant DNA technology. The conference came to an agreement that strict guidelines should be set by the National Institutes of Health in the United States, and by comparable organizations in other countries, for regulating its use (Transgenic History, 2005). These laws will be discussed later in Chapter-4.

Recombinant DNA

DNA is made up of four different compounds called bases, adenine, thymine, cytosine, and guanine. They come together to form a double stranded chain (**Figure-1**). Adenine is normally paired with thymine, and cytosine is normally paired with guanine. The order of the bases (the base pair sequence) is what determines the biochemical function of the DNA molecule. Each organism has a specific DNA sequence that makes up their genome, and the differences in their genome allows each organism to express different characteristics.

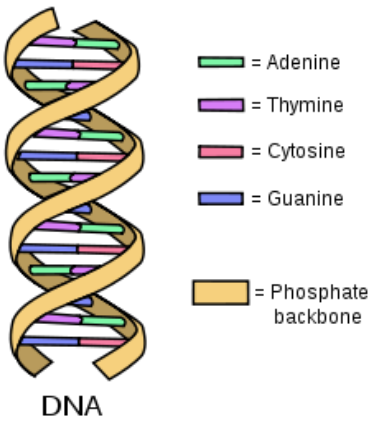

Figure 1: Diagram of the Structure of DNA. Diagram shows the pairing of bases (colored rungs on the ladder) and the overall helical structure of DNA. http://commons.wikimedia.org/wiki/File:DNA_simple2.svg

Recombinant DNA (rDNA) is an unnatural arrangement of DNA, made by combining different DNA stands (sequences) to form a new sequence that would not normally occur in nature. rDNA is often called "chimeric" DNA in reference to the mythological chimera, an amalgamation of several animals. The DNA is usually cut using various restcriction enzymes, mixed, annealed to allow compatible sticky ends to adhere, then sealed using DNA ligase.

rDNA is usually inserted into a "cloning vehicle" or "vector" whose purpose is to help amplify the material. Four different kinds of vector can be used: plasmid, virus, cosmid, or an artificial chromosome. Plasmids are used most frequently due to their ease of use and large copy number (**Figure-2**). Plasmids are small pieces of circular DNA that replicate in bacteria and are separate from the bacterial chromosome. The process of inserting foreign DNA into bacteria is termed transformation, since once inside the cell the plasmid DNA becomes expressed to give the bacteria new properties.

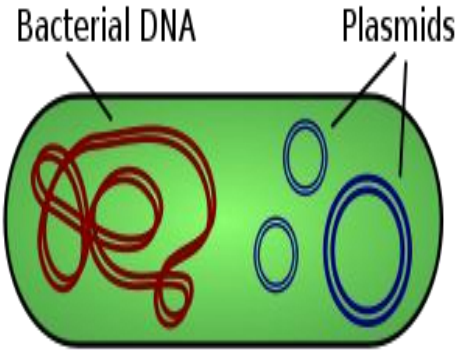

Figure 2: Diagram of Plasmid DNA. Figure illustrates an incorporated plasmid (blue circles) in a bacterial cell containing bacterial chromosomal DNA as its genome (red material). Plasmids are commonly used as DNA cloning vehicles. http://en.wikipedia.org/wiki/Plasmid

Plasmids usually contain an origin of replication, a specific DNA sequence where DNA replication is initiated. Also contained within the vector is a multiple cloning site (MCS) that contains many restriction sites to allow flexibility in inserting various types of cut DNA. Lastly,

plasmids contain a selectable marker that allows cells containing it to be selected for.

Methods for Inserting rDNA Into Cells

There are various ways for inserting foreign DNA into a cell, including microinjection, chemicals, electroporation, viruses, or sperm manipulation. When a plasmid, virus, or bacteriophage is used to transfer genetic materials it is called transduction. In higher-level organisms like eukaryotes (plants and animals), the genetic incorporation of DNA is called transfection.

DNA Microinjection

Microinjection is the most reliable method for introducing DNA into a cell. This technique was commonly used to create most of the early transgenic animals (Gordon et al., 1980) (although this technique was not used to create the very first transgenic animal which used virus delivery; Jaenisch and Mintz, 1974). Using a newly fertilized egg created by *in vitro* fertilization, an egg is held in place by a microtube suction device, and a separate glass needle approximately 0.1 μm in diameter is used to insert transgenic DNA into the male pronucleus (will be discussed in more detail below).

DNA Chemical Methods

Some bacteria have the ability to uptake extra-cellular DNA, but this process is rare in nature. However, some bacteria can be made "competent" to take up DNA by using various salts, especially calcium or rubidium. Chemical competency is a good method for transforming bacterial cells. For eukaryotic cells, chemical methods include complexing the DNA with a

positively charged polymer, then binding that polymer to the cell membrane. The DNA complex is released into the cell, where is can be taken it to the nucleus and expressed. Human and mouse cells can be efficiently and easily transformed when exposed to the packaged DNA (Segura and Shea, 2002).

DNA Electroporation

DNA is a negatively charged molecule due to the presence of phosphate groups in its backbone. So when it is exposed to a current of electricity, DNA will migrate towards the positive anode. Electroporation is performed by exposing a cell culture mixed with transgenic DNA to a pulse of high voltage electricity. The DNA is pulled through the cell membranes by the electric current. Once inside the cell the DNA moves through the cytoplasm and becomes incorporated with the genetic material inside the cell (Taconic, 2003).

DNA Viral Delivery

One of the most sophisticated methods for introducing foreign DNA into cells uses viruses. DNA viral delivery was used to create the world's first transgenic animal (Jaenisch and Mintz, 1974). Jaenisch's group infected mouse embryos with a virus containing SV40 sequences, creating the first transgenic animal. Normally a virus would destroy the host organism but using genetic engineering it was altered and allowed the viral DNA to incorporat into the genome. This method involves encasing the altered viral DNA in a protein coat that will allow the virus to infect the target cells. Viral delivery is efficient, and improves the chances of transgene expression. In some case, like the Adeno-associated viruses (AAV), the viral DNA inserts at specific host DNA locations. The two biggest limitations of viral delivery are the size of the

DNA molecule that can fit inside the virus, and the chance that a chimera will be made without the ability to pass on its transgene.

Sperm Manipulation

Some scientists are trying to develop new ways to incorporate modified DNA into a cell by using recombinant sperm. In 2004, Noriyoshi Sakai and Kayoko Kurita were able to create a transgenic fish by modifying sperm from a zebrafish to perform in vitro fertilization. The transgenic fish grew into a fully developed adult (Kurtia et al, 2004). This technique is being expanded to other animals.

Transgenesis by Manipulation of a Pronucleus

Now that we have described ways for making rDNA for for inserting it inside cells, we turn our attention to the two main ways for making a transgenic animal: 1) manipulation of the pronucleus of a newly fertilized egg, and 2) manipulation of embryonic stem cells. Traditionally the most common method used to create a transgenic animal was to manipulate the male pronucleus in a newly fertilized egg. The technique begins by harvesting eggs from a super-ovulating female made by injecting her with specific hormones. The eggs are fertilized *in vitro*, but before the male and female pronucleus fuse to become a nucleus, the male pronucleus because of its larger size is microinjected with the DNA containing the gene of interest (**Figure-3**). The fertilized eggs are then cultured to the blastocyst stage (about 5 days), where the embryo looks like a hollow ball of cells. Finally, the blastocyst is implanted into a pseudopregnant mother where it will develop into a transgenic animal.

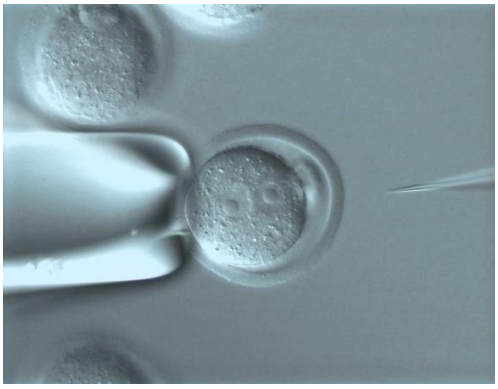

Figure-3: Microinjection of Foreign DNA into the Male Pronucleus. A large pipette (left side) is used to hold a fertilized egg in place with gentle suction. The micropipette for injecting DNA (right side) will target the DNA to the male pronucleus (center left) (Oregon Health, 2009).

The male pronuclear microinjection method is very useful with a wide variety of species, and it has been proven reliable. Although DNA microinjection into a male pronucleus works, there is no control over where the transgene will insert itself into the host genome. It could insert into a control gene necessary for cell function and result in a low survival rate for the transformed cells. If the transgene incorporates into an active region of the host chromosome, the surviving cells will express the transgene. Male pronuclear DNA microinjections create a pure transgenic animal in which all cells contain the transgene, while other techniques like embryonic stem cell microinjection create chimeras.

Transgenesis by Manipulation of ES Cells

The second main method for making a transgenic animal is to manipulate embryonic stem (ES) cells. An embryo is prepared by *in vitro* fertilization, then grown about 5 days to the blastocyst stage. ES cells are found in the inner cell mass of the blastocyst (**Figure-4**), and they are isolated using a microneedle. The harvested ES cells are cultured *in vitro* in a medium containing leukemia inhibitory factor, which prevents them from differentiating. These cells are then manipulated to take up foreign DNA using any of a variety of techniques. One of the

biggest benefits of this process is the ability to screen growing ES cells to verify they are actually expressing the recombinant DNA. For example, if the injected plasmid DNA contains the gene for neomycin resistance, the ES cells can be grown in neomycin and only those that grow will contain the plasmid (and its transgene). The recombinant screened cells are then microinjected back into a blastocyst which is implanted into a recipient uterus (Stem Cell Basics, 2006). Note that only the injected ES cells contain the transgene, not the other ES or surrounding trophoblast cells of the blastocyst, thus the founder animals produced using this ES technique are chimeras, containing some cells that are transgenic and some not. But usually each organ has some transgenic cells (Wheeler et al, 1991), including their reproductive organs, so by selectively breeding chimeras a pure transgenic line can eventuallly be created (Taconic, 2003).

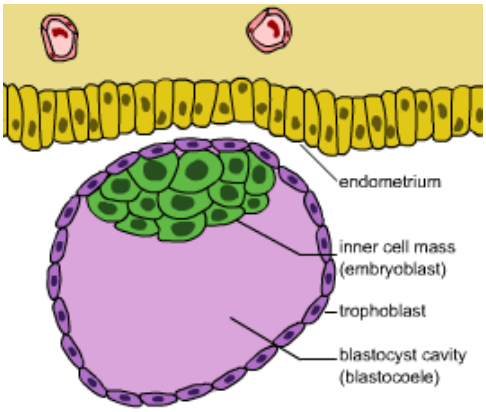

Figure-4: Diagram of a Cross Section of a Blastocyst. Shown are embryonic stem cells (green) which are used to create transgenic animals. http://en.wikipedia.org/wiki/Inner_cell_mass

DNA Homologous Recombination

Both male pronuclear and embryonic stem cell techniques are effective at producing a transgenic animal, but DNA microinjection generally allows no control over where a transgene inserts into the host genetic material. This problem can be overcome using the natural process of homologous recombination (**Figure-5**). In a eukaryotic cell, DNA is organized into

chromosomes that allow the genetic material to be condensed and regulated. In higher organisms like plants and animals that reproduce sexually, chromosomes are diploid (meaning they have two sets of chromosomes), one from their mother and one from their father. During the sex cell replication process of meiosis, matching chromosomes pair (diagram left), and can exchange small equivalent pieces of their DNA (diagram center) in a process called a crossover, to create new chromosomes (diagram right), allowing an increase in genetic diversity from parent to offspring.

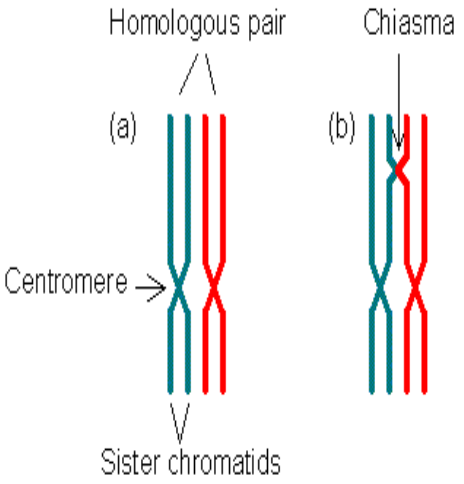

Figure 5: Diagram of Homologous Recombination. During meiosis, homologous chromosomes pair (diagram left), which sometimes allows crossovers to occur (diagram center) to create a new chromosome (diagram right). This process can be used to target DNA to a specific chromosomal location in transgenesis. http://www.web-books.com/MoBio/Free/Ch8D1.htm

During transgenesis, the use of this natural process of homologous recombination accurately targets the new foreign DNA to a specific host chromosome location (Bronson and Smithies, 1994). The vector DNA is modified to contain sequences identical to regions of host DNA constituting the desired location for insertion. The vector is engineered so these host DNA sequences flank the transgene. After microinjection of the vector, the host sequences in the vector exchange place with its matching region on a host chromosome, bringing along the transgene. The modified ES cells are then injected back into a blastocyst as described before.

Although homologous recombination allows site specific integration, to use this process

the sequence of an organism's chromosome must be known in advance to allow construction of the plasmid. Homologous recombination is also a good technique for performing DNA "knock outs" in which specific host genes are targeted and stuffed with useless DNA or removed. A "knock out" is where the DNA is engineered to remove a gene, while a "knock in" adds one. These are valuable tools when determining the function of specific genes in the development of an organism, because the expression of a protein can be removed and the effects of its absence analyzed. Knockouts and knock ins would not be possible without the precise gene targeting allowed by homologous recombination.

Transgenesis by Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer (SCNT) involves the removal of a nucleus from a somatic cell, most likely a skin cell, and the implantation of that nucleus into an enucleated egg by microinjection. After the nucleus has been transferred, the egg is developed into a blastocyst, and implanted into a surrogate mother. The resulting offspring is genetically identical to the donor of the skin cell nucleus. This SCNT process was used to create the worlds first cloned mammal, Dolly the sheep. With respect to transgenesis, the injected nucleus can also be engineered to be transgenic. The nucleus can be prescreened before the organism is developed to ensure its uptake of the transgene (Nuclear Transfer Technology, 2005). This method produces an incredible strain on a cell because it is completely reprogrammed, but the end result will be a 100% transgenic animal.

The SCNT technique has mostly been done in mice, but in 2001, SCNT was performed in human cells that developed into early embryos (Cibelli et al, 2001). However due to the controversial nature of egg collection in humans, the lack of human embryo survival to late

stages, and the current legal ban on the process, few further human SCNT experiments have been performed. An infamous experiment in 2005 by Hwang's lab in Korea claiming to have created human cloned cells by SCNT (Hwang et al., 2005) was subsequently withdrawn for fraud.

In 2006, scientists discovered how to reprogram somatic skin fibroblast cells back into ES-like stem cells by inserting DNA encoding 2-4 key transcription factors that induce de-differentiation (Vogel, 2006; 2008). This technology is especially useful in medicine because a patient's own skin cells can be used to regrow diseased tissues, with cells genetically identical to the patient eliminating graft rejection. And the cells can even be engineered to produce a product, so for example a skin cell could be reprogrammed to produce insulin and then implanted into the pancreas to treat diabetes (The Future of Cloning, 1998).

Methods for Transgene Detection

The process of making transgenic animals is not efficient. Many resulting offspring do not take up the transgene, or they take it up but do not express it, so it is important to screen offspring for transgene integration and expression. Many methods for the detection of a transgene or its by products have been developed. Some screen for the transgene DNA itself, some for the mRNA produced from the transgene, and some screen for the transprotein encoded by the mRNA. Some transgenic techniques even allow screening before the embryo is developed, but most screening occurs when the newborn founder animal is about 3 weeks of age. And even if a founder proves positive, it is still important to continually check for transgene expression through the course of the animal's life.

Southern blotting is a technique that detects a specific DNA sequence in a complex mixture of DNA. So for example, this technique can detect the presence and copy number of a

transgene inserted in the genome of an animal. The DNA most commonly tested is attained from tail, ear, or white blood cells. In the Southern process, the organism's genome is cut using restriction enzymes into smaller fragments (**Figure-6**). These fragments are then separated by size in a process called gel electrophoresis (diagram upper center). Using electricity to pull the negatively charged DNA through a gel, it can be separated based on size. The DNA in the gel is then denatured to form single stranded DNA (to allow it to anneal to a probe), and the DNA is transferred to a paper like membrane (diagram upper right). The DNA on the membrane is washed with a solution containing a probe that contains a complementary DNA sequence to the transgene sequence of interest (diagram lower center). The probe has either a radioactive or fluorescent label attached to it to allow visualization (lower right).

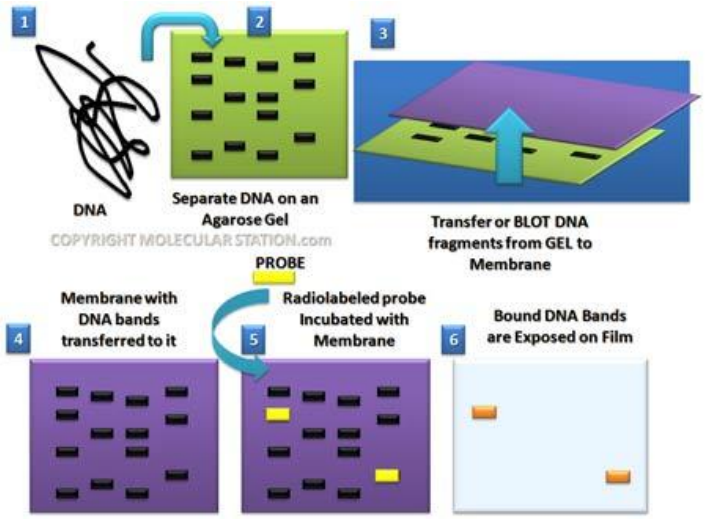

Figure 6: Diagram of the Southern Blot Process. This procedure is often used to assay the presence and copy number of a specific transgene in a founder animal's genome. http://www.molecularstation.com/images/southern-blot.jpg

Northern blotting is another technique used to determine the expression of a transgene. It is very similar to Southern blotting, hence its name, but cellular RNA is electrophoresed instead of cellular DNA. In this case, the RNA on the membrane is hybridized to a transgene probe, and a signal indicates the presence of transgene mRNA in the cell.

Another very effective method for detecting transgene mRNA is quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR). This method uses the mRNA from a cell and an enzyme called reverse transcriptase to create complementary DNA to the mRNA. DNA primers are engineered to bind a portion of the transgene sequence, then an enzyme called DNA polymerase binds to the primers and amplifies the target DNA sequence. The primer DNA is combined with a molecule called SYBR green that becomes extremely fluorescent when complexed with double stranded DNA. The increase in fluorescence is a measure of signal amplification from the transgene mRNA.

Western blotting is also very similar to Southern and Northern blotting, but in this case cellular protein is electrophoresed. The membrane is hybridized to an antibody against the transprotein, and a signal indicates the presence of the transprotein.

Western blotting is not the only technique that can be used to determine the presence of a transprotein. Enzyme Linked Immunoabsorbent Assays (ELISAs) also screen for the production of a transprotein. A small plastic well is coated with antibodies specific to the protein of interest, then the well is filled with a test solution potentially containing the transprotein (i.e. lysed blood from a founder animal). The antibodies coating the well hold the transprotein in place. Non-bound proteins are washed away, then a second antibody against the transprotein (but conjugated to a fluorescent or radioactive label) is added to the well to detect the presence of the transprotein. ELISAs are far more quantitative than Western blots.

In some cases, the presence of the transgene confers new visible properties on the founder animal, and these can be used to determine the uptake and expression of a transgene. In the case of bacteria, a gene encoding resistance to an antibiotic can be engineered and inserted into their genome. These bacteria will grow on a plate containing the antibiotic, only those who have taken up the transgene will survive. Or a transgene can be engineered to make a cell glow a different color. Green Fluorescent Protein (GFP) is the golden child reporter for expression. GFP can be fused with the transgene, so when the transgene is expressed so is GFP (**Figure-7**).

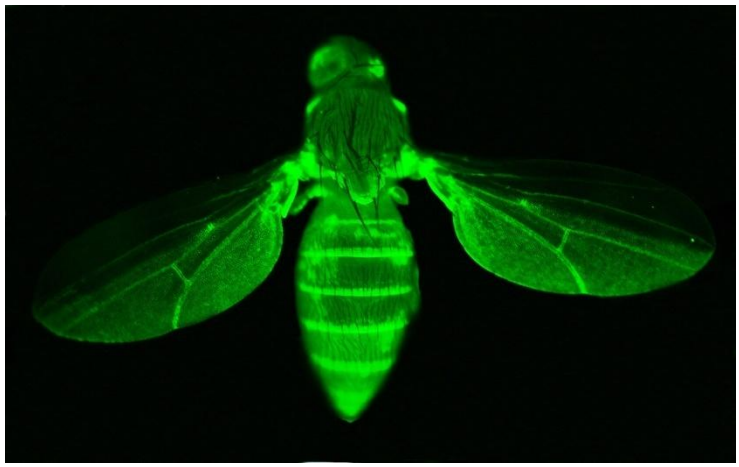

Figure 7: Picture of a Transgenic Fly Expressing Green Fluorescent Protein. Reporters like GFP can be used to help determine expression of a transgene. http://genetik.fu-berlin.de/institut/en_GFP_fly3.jpg

These techniques together constitute the main ways founder animals are screened for transgene integration and expression. Each transgenic experiment brings us closer to more fully understanding genetics, and creates new animals for aiding mankind. New techniques are always emerging to make the process more efficient. Transgenic technology has the ability to greatly assist in man's struggle, and with a caring eye and guiding hand it will.

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Chapter 2: Transgenic Applications

Benjamin Seicol

Introduction

Transgenic technology, the ability to recombine DNA between species, can be applied to almost every species on earth, ranging from complex mammals down to unicellular organisms. In the past century, many transgenic animals, plants, and bacteria have been created to explore this rapidly developing technology. Transgenic applications can be divided into five major categories based on the purpose of the transgene: scientific models, disease models, xenotransplanters, transpharmers, and food sources. Throughout this chapter, several examples of transgenic animals will be documented to help illustrate their classification and benefit to society, as a prelude to discussing their ethics in the next chapter.

Scientific/Biological Models

Commonly referred to as scientific models, or biological models, this very broad category of transgenic animals sheds light on specific protein functions *in vivo*. This is often done by either over-expressing a specific protein whose function is in question, or by knocking out its expression. Information gained from this class of animals on the function of a protein is used when creating transgenic animals of any other category. Understanding specific protein functions and mechanisms is vital for developing any successful transgenic animal to benefit society. As more of the mechanisms underlying biological systems become clear, our ability to regulate these natural pathways increases dramatically.

Examples of animals in this category include: a transgenic monkey engineered to express

jellyfish green fluorescent protein to study primate gene expression (Chan et al., 2001), a smart mouse that over-expresses the NR2B subunit of the glutamate receptor to learn faster and retain memories better than wild type mice (Tang et al., 1999), and knock out mice used to study the developmental effects of specific proteins.

Disease Models

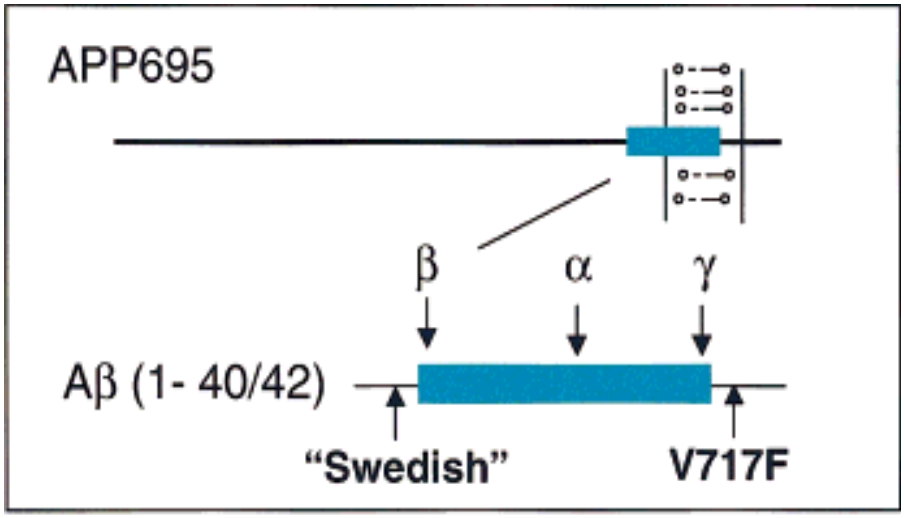
Disease models are created to mimic specific aspects of a human disease, to aid our understanding of disease onset, and to serve as a method of rapidly screening potential therapies on a model other than humans. In these models, human genes implanted animals to allow them to mimic certain aspects of a human disease. A transplanted gene must be introduced into the host animal to create a platform for the disease. This creates nearly identical symptoms in the host animal that a human would exhibit, allowing scientists to test new medications and treatments without risking human lives. Like other model organisms, disease models are often done on mice and flies since they are so readily manipulated in lab situations. Modeling human diseases with transgenic animals is among the most prominent category of transgenic animals, as they are required in order to proceeding to clinical trials, the final step preceding the release a new pharmaceutical. Both Alzheimer's disease and Huntington's disease have each been modeled in mice and other animals, paving the way for greater understanding of the molecular mechanisms underlying these devastating diseases.

Alzheimer's Mouse

Alzheimer's disease (AD) is a neurodegenerative disease that primarily leads to senile dementia. The pathology of this disorder is characterized by the formation of amyloid plaques,

neurofibrillary tangles, and brain atrophy. Amyloid plaques are primarily made of small proteins, usually 40-42 amino acids in length, called the β-amyloid peptide (Gurney, 2000). Tangles, on the other hand, are twisted fibers formed inside dying cells, and are composed of a protein called tau. These plaques and tangles show up mostly in the cerebral cortex and hippocampus where they disrupt the processing of information. This localization makes sense, since those regions of the brain are responsible for both memory and cognitive thought. The mechanism, by which neurotoxic β-amyloid is believed to be generated, involves the incorrect processing of amyloid precursor protein (APP) as shown in **Figure-1**. This modification is dependent on cleavage sites located on each end of the β-amyloid peptide region of APP (shown as blue in the figure).

Figure-1: Diagram of the Formation of β-Amyloid in Alzheimer's Disease. Neurotoxic β-amyloid (blue) is formed by the cutting of the beta and gamma secretases at sites located on each side of the peptide. Mutations on each side of the peptide, as shown below the sequence, can also accelerate production of the toxin. (Gurney, 2000)



Several different mutations are known to affect production of β-amyloid proteins in families prone to Alzheimer's. The figure shows two different mutations, one at each cleavage site.

These mutations accelerate the cleavage of APP to produce neurotoxic β-amyloid.

Based on these findings, scientists at WPI and the former Transgenic Sciences Inc, cloned the gene for an early onset APP mutation (the Indiana mutation, denoted as V717F and shown on the right in Figure-1) and inserted it into a mouse line (Games et al., 1995); thus creating the world's first Alzheimer's model. Earlier attempts to produce such models had failed, but the 1995 model succeeded, as the mice developed amyloid plaques and showed similar degeneration of neurons to an Alzheimer's patient. This model taught us that β-amyloid formation is necessary and sufficient for initiating the disease. However, no neurofibrillary tangles formed. In order to successfully model the disease, subsequent models inserted a tau mutation that is more easily hyperphosphorylated. Now, both hallmark lesions have now been modeled. These models have already been used to screen for drugs to block beta and gamma secretases from forming more β-amyloid. Specifically, the 1995 model was used by Elan Pharmaceuticals to create a vaccine capable of removing β-amyloid from brains (Schenk et al., 1999). The vaccine has already moved into human clinical trials, where other disease model-inspired treatments have joined it. These models could lead to a greater understanding of what causes Alzheimer's and, therefore, how to prevent it.

Huntington's Mouse

Huntington's disease is a neurodegenerative disorder classified as a "triplet repeat disorder" because it is characterized by excessive repetition of 3 nucleotides – CAG (Doherty et al., 1999). Normally, the *Huntingtin* gene (HTT) contains a segment within the coding region containing anywhere from 3-30 CAG trinucleotide repeats. Patients with Huntington's disease undergo an expansion of this section, resulting in 35-121 repeats, which leads to abnormal

production of polyglutamine and nuclear aggregates (Gurney, 2000). A mouse model of this disease can be generated by transgenesis of DNA carrying highly expanded CAG repeats. Transgenic mice model this disease better than the Alzheimer's models, as these mice replicate the movement disorder and weight loss experienced by people with Huntington's (Doherty et al., 1999).

Recently, a new model has been created using an animal more advanced than a mouse. Huntington's disease was successfully modeled in primates, marking one of the most major breakthroughs in the history transgenic technology (NIH.gov, 2008). The research team developed the rhesus macaque monkey model by introducing altered forms of the HTT gene into macaque eggs. For the first time ever, five monkeys were born mimicking a specific human disease (NIH.gov, 2008). The potential benefits of this transgenic application in monkeys will help scientists to accurately target Huntington's in humans.

Xenotransplanters

Over the past 2 decades, the number of organ transplants conducted in the United States each year has increased dramatically. As the demand for viable organs rises, the supply from donors cannot keep up. In 1996, 20,000 transplant procedures were done in the US, yet approximately 50,000 persons were left awaiting organ donations at the end of that year (Pearson and Chapman, 1998). Despite a significant increase in our capacity to perform transplantations due to improvements in medical technology, recipients awaiting organ donation have more than tripled since 1988. A possible alternative organ source exists that could potentially eliminate this demand for donated human organs: using transgenesis to create large mammals called xenotransplanters, which can be used as human organ generators. Xenotransplantation is defined as a "procedure that involves the use of live cells, tissues, or organs from a nonhuman animal for

transplantation" (Pearson and Chapman, 1998).

In order to successfully transplant a donated organ, it must be histocompatible with the recipient. Complete compatibility can rarely be found, so immunosuppressive drugs are usually given to the transplant patient to prevent an immune response. Normally, the body rejects organs (or any tissues) that lack histocompatibility. Antigens on the surface of foreign cells are recognized to induce the appropriate immune response. This same system results in the rejection of a transplanted organ, usually resulting in the patient's death. Therefore, xenotransplanters are engineered not to express antigens viewed as foreign in humans.

The most promising animal for xenotransplant research is the pig, mostly due to its closely matching physiology to that of humans. Also pigs are far cheaper and more established for large-scale production than monkeys, which are physiologically more like humans. The primary problem with organs from a pig is the presence of alpha-1,3-galactosyltransferase an enzyme that adds the sugar galactose onto proteins on the surface of the cells (shown as circles in **Figure-2**). This is the primary antigen that would be recognized as foreign by the human recipient resulting in immuno-rejection of the organ (Soin and Friend, 2000). Thus in 2002, scientists created pigs in which the galactosyltransferase gene has been knocked out, so the organs do not have galactose on their surface (Lai et al., 2002). These pig organs are currently being tested for transplants into baboon models, but the sufficient data are not yet available.

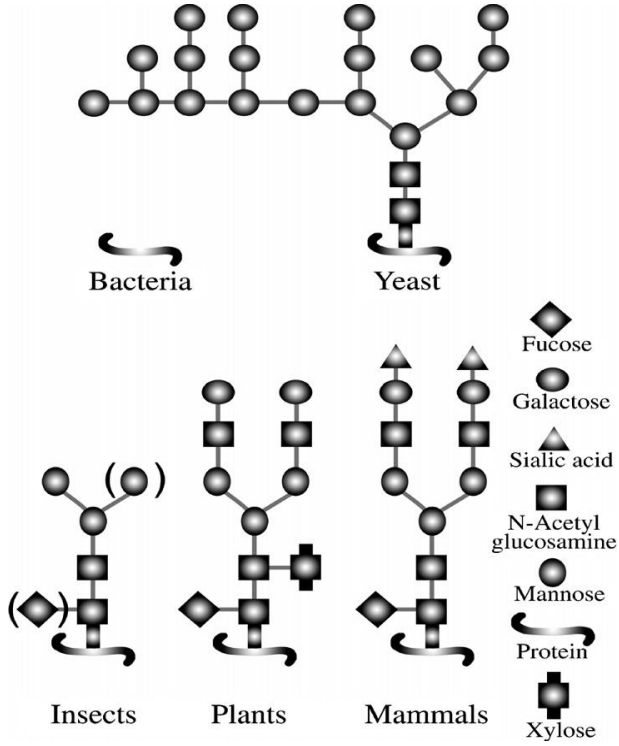


Figure-2: Diagram of Some Key Sugars Attached to Proteins Following Post-Translational Modifications. Shown are various sugar residues (key on the right side) generally found on proteins in various species. In regards to xenotransplantations, the specific sugars may vary between mammals. For example, galactose (denoted by circles) is removed to increase the histocompatabilty of organs from xenotransplanter pigs (Soin and Friend, 2000). Primarily, these unique structures related to transpharming, because scientists desire sources that are easier to work with in lab. However, the protein produced must be altered to mimic human's structures. So if many synthetic steps are required another, more similar, source is required (Houdebine, 2009).

Transpharmers

Proteins were first used as pharmaceuticals almost 100 years ago, with the use of insulin isolated from pig pancreas. Unfortunately, proteins like insulin are too complex to synthesize from amino acids for mass production, and tissue donations for protein isolation get far too expensive in large-scale operations. Living organisms, such as bacteria, offer a far more practical (and affordable) option, and have been used since 1973 with the first transgenic bacterium (Cohen et al., 1973). Although bacteria demonstrated the ability to produce a variety of active proteins, extensive use of bacteria is not the best option since they cannot accomplish the post-translational modifications of higher organisms. Without these modifications, some bacterially produced proteins are inactive in humans, and must undergo expensive activation in a lab. Synthetic reactions can activate the protein, but additional changes also drive up costs.

Animals and plants contain post-translational modifications similar to our own (Houdebine, 2009), so over the years the production processes have moved to higher organisms such as these. Many different biological systems are available for researchers, most of which are detailed in the Houdebine review and are shown above in **Table 1**.

Table 1: A Comparison of Six Methods for the Production of Pharmaceutical Proteins.

Points to consider	Production systems					
	Bacteria	Yeast	Insect cells + baculovirus	Animal cells (CHO cells	Transgenic plants	Transgenic animals
Theoretical production level	+++++	+++++	+++	+	+++++	+++++
Practical production level	++ (+)	++ (+)	+	+	++	++++
Investment cost	+++++	+++++	++	+	++++	+++
Production cost	+++++	+++++	++	++	+++++	++++
Flexibility	+++++	+++++	++	+	+++++	++++
Line conservation	+++++	+++++	+++	+++	+++++	+++++
Line stability	+++++	+++++	++++	+++	+++++	+++++
Delay for the first production	+++++	+++++	+++	+++++	++++	+++ (+)
Scaling up	+++++	+++++	++	+	+++++	++++
Collection	+++++	+++++	+++++	+++++	+++++	++++
Effect on organism	+++ (+)	+++ (+)	+++ (+)	+++ (+)	+++ (+)	+++
Post-translational modifications	+	++	+++	++++	+++	++++
Glycosylation	+	++	+++	++++	++	++++
Stability of product	+++++	+++++	+++	+++	++++	++++
Purification	+++	+++	+++	++++	+++	+++
Contaminant pathogens	+++++	+++++	+++++	++++	+++++	++++
Intellectual property	++++	+++	+++	++	+++	+++
Products on the market	++++	+++	+++	+++++	+	+++

The + signs denote relative levels of production for comparison purposes. Note that transgenic animals (the column on the far right) are well suited for post-translational processes, including glycosylation to produce biologically active proteins while preventing rejection type reactions (Houdebine, 2009).

The production of proteins in farm animals (and plants) is termed transpharming. Although animals have been produced that manufacture foreign proteins in blood, milk, and egg whites (**Table 2**), production in the animal's blood has mostly been replaced by production in the milk since the proteins in the former case directly affect the animal's physiology. Milk is the most promising production system that transgenesis can manipulate, and is definitely the highest yielding system available (Houdebine, 2009).

Table 2
Comparison of the different transgenic animal species to produce

Points to consider	Production systems		
	Blood	Milk	Egg white
Theoretical production level	+++++	+++++	+++++
Practical production level	++	++++	+++ (+)
Investment cost	+++	+++	+++
Production cost	++++	++++	++++
Flexibility	+++++	+++++	+++++
Line conservation	+++++	+++++	+++++
Line stability	+++++	+++++	+++++
Delay for the first production	+++	+++	+++
Scaling up	++++	++++	++++
Collection	+++++	++++	+++++
Effect on organism	++	+++	+++ (+)
Post-translatlonal modifications	+++++	++++	+++ (+)
Glycosylation	++++ (+)	++++	+++
Stability of product	+++	++++	++++
Purification	++	+++	+++
Contaminant pathogens	++	+++	+++
Intellectual property	++++	+++	+++
Products on the market	+	++++	++

Table II: A Comparison of Transgenic Protein Production in Blood, Milk, and Egg Whites. The + signs denote relative levels of production for comparison purposes. Note that production in milk is especially well suited for high levels of production of highly stable proteins, that are easy to isolate (Houdebine, 2009).

As an example of an animal in the transpharmer category, ATryn® is the world's first FDA approved transpharmed drug, produced in the milk of transgenic goats as engineered by Genzyme Transgenetics Corp (GTC) of Framingham, MA (Atryn, 2009). These goats produce the anticoagulant anti-thrombin at high levels in a form that is biologically active. Transpharming has also been applied to cattle, with the production of a line of cows producing lactoferrin in their milk, a key ingredient in human milk that is missing in cow's milk (Lactoferrin, 2008).

Transgenic Food Sources

The final category of transgenic technology to be discussed in this chapter is genetically modified food sources. The goal of this group is to produce animals (and plants) that grow to

large sizes on less food intake, without increasing costs. Transgenic food animals are not yet commercially available, due to the ethical and safety issues associated with their possible escape to interbreed with wild type animals, and problems associated with growth hormone production in mammals. In one such experiment, scientists spliced the gene for Human Growth Hormone (HGH) into a pig's genome. The pig, given the name "Superpig" (or the Beltsville pig), grew larger and faster than normal pigs, while consuming less food (Pursell et al., 1997).

Unfortunately, Superpig suffered from several painful side effects of the transgenesis, including arthritis, gastric ulcers, stomach lesions, lack of coordination, and severe muscle weakness. In response to this, scientists halted all further tests involving farm animal transgenesis with human growth hormone. While it seemed to be a good idea, the negative side effects of human growth hormone on pigs make it ethically wrong to continue such growth hormone experiments in mammals (Rexroad, 1994).

In fish, on the other hand, far less devastating side effects were observed when they were provided with growth hormone genes (Devlin et al., 1997). These salmon grow 3-6 times faster than normal salmon, with less food intake, so are very well suited for aquaculture (Fletcher and Shears, 2002). These salmon reach marketable size a year earlier than other commercially produced salmon. Although not yet available commercially due to ethical concerns if they escape and interbreed with wild type salmon, Aquabounty Technologies the company marketing them, hopes they will be approved by the FDA within the next few years, giving hope for a new food source (Aquabounty, 2009).

Conclusion - Chapter 2

Many of the applications discussed have become quite well established, or at least have shown a lot of potential. Transpharming to produce pharmaceutical proteins, and xeno-transplanters for producing organs for transplants, are becoming completely practical ideas. Additionally, disease and biological models using transgenic animals have already proven vital in the development of new pharmaceuticals. Animal testing has become very socially accepted, partially due to the incredible benefits that have already been observed. Future treatments, derived from this research, may alleviate many "incurable" diseases that humans face today. The broadest application to transgenic technology has been in the production of food crops. Thanks to our infrastructure for mass production of these plants, commonly observed low yields are less of a concern. Most of the ethical questions raised by the controversial research surrounding transgenesis do not apply to plants, with exception of environmental concerns. But transgenic plants hold the promise of feeding billions of people around the world.

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Chapter–3: Transgenic Ethics

Benjamin Parkhurst

Humans have relied on animals for millennia for food, clothing, transportation, protection, and many other reasons. Animals have also been used to produce a wealth of knowledge in the field of science, from anatomical and medicinal practices to neurological applications. Despite the vast advancements we have made in scientific knowledge, many argue against animal testing for reasons of cruelty, religion, morality, and the environment. As intelligent beings we are obligated to consider both the benefits as well as the costs of using living creatures as tools. For transgenesis, the subject of this IQP, the power to manipulate the genome of plants and animals comes with great responsibility. This chapter will examine the ethics involved in animal research in the field of transgenics.

Any time emotion is the primary ingredient involved in a situation there will never be a completely right or wrong answer, there will only be opinions. Some people see *any* use of animals for human benefit as wrong, and that under no circumstances should animals be used for experimentation. Others will always view human needs above those of an animal, and would permit *all* uses of "lower" beings for experimentation. It is obvious that without the use of animal experimentation we would not have nearly as much scientific knowledge as we currently do. It is important to understand both sides of the argument to make a solid ethical argument.

To understand the modern day animal rights arguments, a brief history of animal research, along with early animal experimentation opposition must be understood. Then different ethical perspectives including religion will be investigated. Some of the specific animal experiments described in previous chapters will be morally evaluated to give us a better

understanding of the ethical concerns involved with transgenics. Although the legalities involved with transgenic animals will be reviewed in the next section, this section will briefly discuss the ethics behind the laws. For transgenics, with such great scientific power left up to the politicians to determine how far it will go, it must be considered *who* actually holds the moral standing to determine what types of transgenic study will be allowed.

Brief History of Animals in Research

Animals were first used in experiments in ancient times that mainly focused on anatomical curiosity. Most of the early research done with animals was vivisection (dissection), and was done without anesthesia and was occasionally conducted while the animals were still alive. Some of the first experiments were conducted in the third century BC by Alexandrian physicians, Herophilus and Erisistratus. It has been recorded that they examined the functional differences between sensory nerves, motor nerves, and tendons (Singer, 1957). From the beginning of animal experimentation, records indicate scientists reactions to working on animals. Galen of Pergamum (129-199 AD) preferred to vivisect pigs to "*avoid the unpleasant expression of the ape*" (Maehle and Trohler, 1987).

There is little documentation of animal vivisection from the Dark Ages. In a time when animals were experimented on, as well as human criminals, often in public lectures on anatomy, few concerned themselves with the morality of these actions. The Christian Church at this time viewed only humans as possessing a soul and the power to reason. Animals existed only for human needs and were bereft of moral status (Linzey, 1989).

It was not until the 16th century that rise of modern biomedical studies began. At this time, many things previously not understood about the body such as circulation, digestion, and

respiration were seen to have physiological explanations. With this in mind the use of animals for experimentation began to increase and continued into the 20th century. Another landmark of physiology came from Claude Bernard (1813-1878), when he declared that a precise approach to experimentation must involve the study of one parameter while holding extraneous variables constant (this remains a fundamental approach in modern science). In one paper he presented his opinion on the experimental use of animals; "*It would be strange indeed if we recognized man's right to make use of animals in every walk of life, for domestic service, for food, and then forbade him to make use of them for his own instruction in one of the sciences most useful to humanity*" (Bernard [1856], 1957). Around 1850, the anesthetic properties of ether were discovered which would allow the operation of animals to be conducted without putting them into any pain. This made surgical procedures involving animals a norm in many facilities across Europe. Records show that the number of animal procedures involving research animals increased from 311 in 1880 to over 95,000 in 1910 (Monamy, 2000). The discovery of the bacterium responsible for tuberculosis in 1882, and the diphtheria antitoxin in 1894 (which rapidly reduced infant mortality from 40% to 10% in those afflicted), whose breakthroughs were accomplished through animal research, led to a broader public acceptance of animal experimentation (Turner, 1980).

In the twentieth century, the continued use of animal experimentation along with the relatively low restrictions on animal use, lead to numerous medical breakthroughs. In 1989, the American Medical Association on Scientific Affairs published an impressive list of medical advances made possible through research using animals. This list includes expanding our knowledge of autoimmune diseases, acquired immunodeficiency syndrome (AIDS), anesthesia, behavior, cardiovascular disease, cholera, diabetes, gastrointestinal surgery, genetics,

hemophilia, hepatitis, infant health, infection, malaria, muscular dystrophy, nutrition, ophthalmology, organ transplantation, Parkinson's disease, rabies, radiobiology, reproductive biology, shock, the skeletal system, toxoplasmosis, yellow fever, and virology.

Thus, animals have been used for generations in various scientific experiments, from the most basic dissection to the current science of transgenic genetic alteration. And there has been an element of human emotion involved that has made it unclear exactly when the ends justify the means, so the next section will examine both the positives and negatives of transgenic research.

Cost Versus Benefit of Transgenic Animals

The relatively recent discovery of transgenic technology is a great tool that has already been used to make significant advancements in medical and food resources, as discussed in Chapter-2. Just as the medical advancements of previous centuries came at a price, so do transgenic experiments. To make a valid ethical judgment on transgenic animals, there must be an understanding of what is gained and lost from the research.

The Cost of Transgenesis

People against animal experimentation argue for animal welfare, religion, and morality, and the environment. Animal rights groups such as the ASPCA (The American Society for the Prevention of Cruelty to Animals) and PETA (People for the Ethical Treatment of Animals) have lobbied for years for limited and regulated animal experimentation. From the outset it must be noted that some types of transgenic research do cause some animal pain or even death. The super pig, whose genome incorporated HGH (human growth hormone), experienced a very

painful period of time before it was euthanized. Disease modeling, in which an animal is given a certain human disease, or aspects of a disease, can create similar ethical issues.

Many different arguments are made about the use of animals in today's society. Societal and religious beliefs cause much debate about exactly how animals should be used. Some people believe that the use of mice creates less of an ethical dilemma because they are not sentient creatures such as monkeys. Many people have great concern when it comes to any testing done on cats and dogs because they have more direct contact and experience with those types of animals. This same type of animal bias is true in religion. An ancient Hindu verse says he who kills, eats, or permits the slaughter of a cow will "rot in hell for as many years as there are hairs on the body of the cow so slain!" (Karanth, 2009). In Buddhism, all animals are seen as sentient beings and are capable of the same amount of suffering as humans. In Judaism, certain animals may be killed but only if prepared a certain way. Christianity is not very clear when it comes to animal rights, however some say that because everything is God's will, then so is animal experimentation.

With respect to transgenic environmental issues, the possible environmental impact that could from the accidental release of transgenic animals and plants is a clearer issue. Augmenting an animal's DNA can leave it bigger, faster, stronger, healthier, and more fertile than its natural counterparts. Thus, the risk of these animals escaping from research facilities and going into the wild could have devastating effects on natural species.

The Benefit of Transgenesis

Individuals who are in favor of transgenesis argue mainly for the benefits of animals to medical research and food sources, both of which have the potential to save and prolong both

human and animal life. The benefits of using animals in transgenic research have already been greatly significant, and will continue to be in the future.

As already stated in Chapter-2, hundreds of medical discoveries have come from the use of transgenic animals. Scientists using transgenics are continuing to use animals in a very creative ways, and with today's medical knowledge most animals are put through minimal amounts of pain, if any at all. For example, transpharming is a great example of animal transgenics because there is a high scientific yield (the cheap production of human medicines) and virtually no cost in terms of suffering to the animal. It is also important to note that not all medical studies serve purely a human purpose, veterinary medicine and all of its animal vaccines and treatments would not be anything without the use of animals in experimentation.

Disease Model Ethics

A transgenic disease model is an animal that has incorporated a transgene that will allow the animal to have some of the symptoms of a human disease. This is a great tool that allows us to study a human disease and test potential cures or vaccines directly through an animal, before proceeding to test the treatments in humans. The use of animals in this respect means that time, money, and side effects on human test subjects can be kept to a minimum.

The Alzheimer's disease model (discussed in Chapter-2) serves as an excellent case for discussing transgenic ethics for this class of animals. Some quick facts about Alzheimer's can show just how serious of a condition it is. In the US, as many as 5.3 million people in the US are living with Alzheimer's, in fact every 70 seconds someone develops Alzheimer's (Alz.org, 2009). It is the 7th leading cause of death, and the direct and indirect costs of Alzheimer's and other dementias amount to $148 billion a year. The Alzheimer's mouse was developed at WPI

by Prof Adams in collaboration with the former Transgenic Sciences Inc. (Worcester) (Games et al., 1995). The animal proved that the production of toxic β-amyloid protein is sufficient for initiating the disease in the brain, and provided a model for rapidly screening potential therapeutics. Elan Pharmaceuticals used this model to create the first test vaccine for Alzheimer's disease (Schenk et al., 1999). In phase-I clinical trials, the vaccine was safe in most individuals tested, but a small percentage of patients experienced inflammation, so Elan is currently testing a second generation vaccine. The research conducted using the Alzheimer's mouse line is easily seen as having a solid moral foundation, considering so many people are drastically affected worldwide, while there is no evidence of physical pain brought on by this genetic alteration. To the author of this chapter, it is very clear in this case that this was an ethically acceptable practice of transgenic animal research.

Xenotransplantation Ethics

Xenotransplantation is the use of animals to grow tissues or organs that can be used for transplantation into humans. Many people in this country die every year waiting for an organ transplantation that they never receive. One problem with using organs from another human is that the recipient's body will reject the foreign organ unless the donated organ is histocompatible with the recipient. Surprisingly, pig organs are very similar to humans, and have been used for transplantation research. However, implanting organs from another animal does not come without its risks, including immunorejection or viruses. The biggest concern of using pig organs is spreading pig viruses to humans, for example as we currently worry about the current H1N1 swine flu outbreak. This means that implanting a human with a pig organ could allow cross species infection (already proven for influenza virus), to create a new human strain of a virus

previously present only in pigs. However, this might be prevented by screening the donated organs for known viruses beforehand. Another argument against xenotransplantation is whether the pigs should be raised solely for human purpose, as they would be sacrificed to obtain the organs. However, since animals are also raised to be eaten and therefore save lives, it can hardly be argued that animals cannot be raised to have their organs implanted instead of ingested, especially if the animals are humanely sacrificed.

Transpharming Ethics

Transpharming is the use of animals to produce a desired a chemical or protein for pharmaceuticals. This technology is used most efficiently when the desired chemical is produced by the animal in their milk, as the produced protein has minimal effects on the animal's physiology. This method requires almost no further purification of the product, and does not physically harm the animal. Animals such as cows, sheep, and goats have been successfully used as transpharmers. The author of this chapter believes there are no real ethical arguments against this type of trangenics, as there are strong medical benefits to society, with no observable animal suffering.

Transgenic Food Source Ethics

In today's poor economy the high price of food is something that could be lowered for some items by the use of transgenic animals. Superfish are farm raised fish that have had their DNA altered so that they will grow larger, faster, on the relatively the same amount of food. Superfish appear to undergo no pain and are far better adapted to aquaculture than normal fish. A similar situation is seen with for maize that has been transgenically altered to grow faster and

yield a larger crop than the normal strain. World hunger continues to increase with an increasing population.

The alteration of plant genes has been going on for several years, and has had both positive and negative results. As a positive example, plants such as tomatoes have been designed to grow bigger and faster with less watering, and to be less likely to bruise during shipping. People are typically less concerned with the well being of plants than with animals, but are seriously concerned with their environmental impact. Much has already been accomplished altering a plant's DNA to allow it to benefit us. As a negative example, Monsanto created "Round-up Ready Tomatoes" who's DNA was recombined to make it resistant to a certain type of herbicide. This meant that the herbicide could be sprayed on a crop of tomatoes and kill other weeds but not the tomato plants, yielding a larger crop. But the environmental concern is that these genetically stronger plants will spread and wipe out the unaltered species. It is hard to control pollen spread by the wind, and to keep these genetically modified plants from spreading.

Ethics and the Law

Ultimately it is left up to the politicians as influenced by society and their ethical beliefs to legislate laws on animal experimentation. This topic will be discussed in detail in Chapter-4. One group will continue to lobby for more research funding, while activists lobby for illegal use of all animal experiments. Transgenics is not just a US issue, but is truly a global issue, so key questions will revolve around what persons or groups of people have the power to enact the laws that will control transgenic animals? Transgenic science is happening very rapidly and new discoveries are made every day. But with great knowledge comes great responsibility, and as a society we must make sure that our morals keep up with this new technology.

Chapter-3 Conclusions

In this chapter, the positives and negatives of transgenic animal use have been examined, and it is clear that in most cases the good outweighs the bad. Animals have been used to better mankind for centuries. The author of this chapter feels that transgenesis should continue, but with strong oversight to minimize animal suffering. The use of animals for research must be continued, but under careful regulation. Transgenic animals can be a great benefit to our planet and can save the lives of generations to come. The next chapter will go into depth on the laws and regulations involved with transgenic animals.

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Chapter 4: Transgenic Legalities

Katherine McCormack

Introduction

Research involving transgenic animals has been on the forefront of the biotech industry since 1973 when the first genetically modified organism was created (Cohen et al., 1973). In the form of recombinant bacteria, the first genetically modified organism immediately brought many ethical concerns to the public eye, including the safety of transgenic organisms and the processes used to create them. Much of this controversy comes from the consumer side, questioning the possibilities of new and unnatural diseases or allergens that may be introduced though the production and consumption of the animals. Alongside consumer concerns are also industrial concerns. Advances in transgenic technologies may allow some industries and even nations to thrive more than others when the medical benefits come fully to fruition. Industries can gain recognition and boost the value of their patentable materials by integrating transgenic technologies into their work.

Coinciding with these industrial and consumer concerns, many governmental bodies have been involved in overseeing transgenic research. Governments have limited the use and patenting of genetically modified animals. Investigating the advantages and disadvantages of transgenic animals helps governmental entities decide whether to patent these animals or restrict their use. Looking at the outcome of various cases involving genetically modified organisms around the world helps in understanding the legalities surrounding this new technological process. This chapter will document and describe the effects of transgenic technology on the

legal system, specifically the process of patenting life forms, and will use a specific landmark case (Oncomouse) to view various strategies when trying to patent a transgenic animal.

Should Animals Be Patented: Advantages and Disadvantages

Types of Patents

When justifying the patentability of transgenic animals, it is best to first understand what can legally be classified as patentable material. In the United States, the Patent and Trademark Office defines three types of patents: utility patents, design patents, and plant patents. Utility patents are granted to "anyone who invents or discovers any new and useful process, machine, article of manufacture, or composition of matter, or any new and useful improvement thereof. Design patents may be granted to anyone who invents a new, original, and ornamental design for an article of manufacture. Plant patents may be granted to anyone who invents or discovers and asexually reproduces any distinct and new variety of plant" (USPTO, 2005). These three types of patents clearly outline what a patent is, and what can be patented.

Patentable Material

Along with the types of patents granted by the United States Patent and Trademark Office, patent laws govern how to obtain a patent and what material is *patentable*. The patentable criteria do not state anything against animal patenting, but do not directly permit it either, so the controversy lies in this area. The courts have to decide whether a genetically modified animal can be classified as patentable material. The United States Supreme Court has focused on special categories not represented in the "patentable subject matter requirements. These categories include: 1) laws of nature, 2) natural phenomena, and 3) abstract ideas"

(Cornell University Law School, 2009). Determining whether transgenic animals lie within these special categories is an important decision that directly impacts many people, domestically and globally.

Effects of Transgenic Patents on Consumers

When deciding whether transgenic animals should be patented, it is important to look at the effects of such decisions on all parties that could be affected. To do so, I have come up with four different areas to be addressed: the effects on consumers, industries, animals, and the environment. Analyzing the possible advantages and disadvantages in relation to each of these four categories will aid in an overall conclusion whether animals should be patented. In a later section I will discuss specific patent cases where various decisions have already been made to patent and not patent specific transgenic animals.

Transgenic animal production will likely have a large effect on consumer products in the future. Transgenic animals "can be classified as: (1) models for the study of nutrition or diseases, (2) sources of modified food products, and (3) bioreactors that produce ingredients for nutritional products" (Prieto et al., 1999). As we discussed in Chapter-2, we also document two additional classes of transgenic animals, xenotransplanters for creating transplant organs, and scientific models for studying the effects of a newly discovered proteins on physiology. The possibilities to the consumer are endless, from salmon that grow to larger sizes with less food intake, to cows that produce life saving drugs in their milk. The benefit to consumers was discussed in detail in Chapter-2, but some good examples are provided briefly here. A good example is a transgenic cow that "produce(s) more milk, or milk with less lactose or cholesterol"

(Margawati, 2009). A disease model for Alzheimer's disease (Games et al., 1995) has already served as a model for testing vaccines (Schenk et al., 1999) that are in human clinical trials. Another disease model is Oncomouse that develops cancer, was the world's first patented animal, and has already taught us information about cancer formation and has served to help screen drugs. The ability to create a mouse that has cancer has opened up many doors in the medical field for testing possible cures. The use of transgenic animals may lead to breakthroughs that could potentially save the lives of many people suffering from various diseases. For all these various purposes, patenting transgenic animals looks positive for the future.

Effects of Transgenic Patents on Industry

Pharmaceutical, agricultural, and material industries are among those who can benefit from transgenic animals. In the material industry, animals can be used to produce a variety of products. One example would be a transgenic goat that secretes spider silk in its milk. The President and CEO of Nexia Biotechnologies stated that:

> "spider silk is practically the world's strongest material…It's much stronger than steel -- five times as strong. We're going to make fishing lines out of it…Yes. Biodegradable fishing lines. Or maybe tennis racket strings…You could make hundreds of things out of spider silk, if only you could produce enough of it. Biodegradable sutures for surgery . . . replacement ligaments or tendons . . . hemostatic dressings . . . fashion. We call our product BioSteel" (Osborne, 2002).

There are many applications for transgenic animals in industry. There are unlimited ways that these animals can help us in the future. Janine M. Benyus wrote a book entitled "Biomimicry" in 1997. Over ten years ago she "observed that while humans create synthetic materials by means of high temperatures and pressures ("heat, beat, and treat" methods, as they are known), nature

does so under life-friendly conditions. That is to say, in water, at room temperature, and without harsh chemicals" (Osborne, 2002). This observation is interesting, making us think about the many factories we have, and all of the pollution that is seeping into our world. Transgenic animals can be altered to produce many valuable industrial materials as well as medical products. Without the use of factories and the "heat, beat, and treat" method that Janine speaks of, production efforts, specifically those using transgenic animals, can be more efficient and less costly.

Looking at the medical industry, there are a vast number of applications for transgenic animals. One that is particularly interesting is the use of transgenic pigs as possible organ donors. When any of the major human organs fail, the treatment is to replace them. This is extremely difficult because of a lack of donors. Although there are many concerns about rejection of such organs, using transgenic pigs as donors is becoming more feasible. These pigs could be made to carry human antibodies that will lessen the possibility of rejection. Xenotransplantation, the transfer of tissue or organs from one species to another, "has been carried out at various times using non-human primates as donors. Although use of non-human primates has enjoyed some very limited success, the very large number of organs needed impels consideration of non-primates, such as the pig as organ donors" (Houdebine, 1997). Perfecting this method using pigs by engineering them to not produce a sugar viewed as foreign to our immune system, will be a great advance in the medical technology industry. There is no doubt that transgenic animals will have a key role in the industrial world in the future, where patenting regulations are necessary for these animals.

One might question the usefulness of transgenic animals in developing countries that may not have the technologies to create such animals. However, transgenic technologies can be

beneficial to developing countries. The use of transgenic technologies to create vaccines and medicines allows industries to make more of the product at a cheaper cost, for use worldwide. Also, some vaccines may prove to be edible, which greatly facilitates their administration in countries lacking extensive healthcare systems. John McClellan, Director of Marketing at ProdiGene in College Station, Texas, says that "edible vaccines offer the ability to produce large volumes of proteins at very economical costs" (Mann, 2001). Because of this economical cost of production, developing countries can purchase vaccines and medicines at a cheaper cost. Moreover, once the animals producing the vaccines are created, they can easily be bred to expand production, including in third world countries.

Effects of Transgenic Patents on the Environment

Although creating transgenic animals can benefit consumers and companies, they might pose threats to the environment. The environmental concerns associated with transgenic animals include the possibility that transgenic animals could escape from their containment, outcompeting or interbreeding with wild type populations. Studies have been conducted by the United States National Research Council (NRC), along with other groups, to investigate the potential environmental impact of transgenic animals and technologies. "These studies conclude that GM animals may have either positive or negative effects on the environment, depending on the particular animal, trait and environment into which it is introduced" (Green Facts, 2009).

Taking into account the many different transgenic animals that could be created, the impact on the environment could eventually be staggering, unless controls are rigidly enforced, such as engineering them to die off unless a special nutrient is provided. Researchers are analyzing each case individually. One case gathering special environmental interest is the

breeding of rapidly-growing salmon, as these fish are close to being ready for commercial aquaculture. The environmental concerns related to this type of fish include possibilities of the salmon escaping and breeding with non-transgenic salmon, and various ways the salmon could respond to different levels of environmental stress. "The ability of fish to respond to stress was not dramatically influenced by growth hormone (GH) transgenesis in the present study since neither physiological stress response nor the cellular stress response was affected by elevated temperature treatments. By contrast, GH transgenic mice carrying an MT promoter appeared to experience a heightened physiological stress response" (Jhingan et al., 2003). Unlike the GH mice, the superfish did not respond to different levels of stress, so GH animals are not all alike in their stress responses. This creates a level of uncertainty when considering what will happen if transgenic animals are introduced into a natural habitat and encounter various stresses.

With respect to the salmon escaping to interbreed with wildtype salmon, although Aquabounty Inc. (the company nearest to marketing superfish) has enforced rigorous rules for preventing fish escape, accidents could still happen. "The potential spread of transgenes to native populations of fish is of high concern should transgenic fish escape into open waters" (Wong 2008). The outcome cannot be predicted with certainty, but there is a large concern that the salmon will wipe out native salmon. Containment arrangements for transgenic animals, including fish, must be regulated and thoroughly tested. Engineering to hinder survival in the wild would add an extra safety feature.

Effects of Transgenic Patents on the Animals

Transgenically modified animals, and indeed animal research in general, has become a fundamental tool in the field of biomedical research. Similarities between humans and animals

have helped in finding cures for many diseases, but there are many characteristics and diseases that humans do not share with any other species. The use of transgenic technologies allows researchers to use animals to study and test cures for human diseases that animals may not have. While the benefits for humans look to be very positive, there are many negative ways in which these animals are affected by the gene transferred from another species. As discussed in the previous chapter, many ethical concerns arise as people feel it is unfair to submit these animals to potential harm or a shortened life span.

GlaxoSmithKline is a company that uses transgenic animals in their research. According to their position on the Global Public Policy regarding transgenic animals:

> "Transgenic animals suffer more abnormalities than regular research animals. The introduction of DNA into an animal can be very complex, and the possible side effects can be difficult to predict. Possible harms might arise from surgical techniques used to harvest and re-implant embryos; the collection of tissue from the tip of the tail for genotyping; and non-specific effects caused by damage to genes adjoining the altered area of DNA. Also reduced fertility and/or oversize fetuses may result from this technology. In most cases the mutations impact highly specific metabolic processes or cell receptors without actually causing disease, discomfort, pain or malformation in the animals. The legal controls for their use are very stringent and GSK devotes considerable resources to monitoring these animals" (GlaxoSmithKline, 2009).

Thus, there is a high concern about the welfare of the animals affected by transgenic technologies. Although transgenic technologies allow companies to use fewer normal animals in their research, the possible animal defects and death rates are alarming. **Figure-1** shows the survival rates of transgenic mice (open circles and triangles) in comparison to non-transgenic mice (black circles and triangles). It is clear that for this particular example, transgenic animals do live a shorter life, suffering a low survival rate. Factors such as this complicate the decision whether to patent transgenic animals.

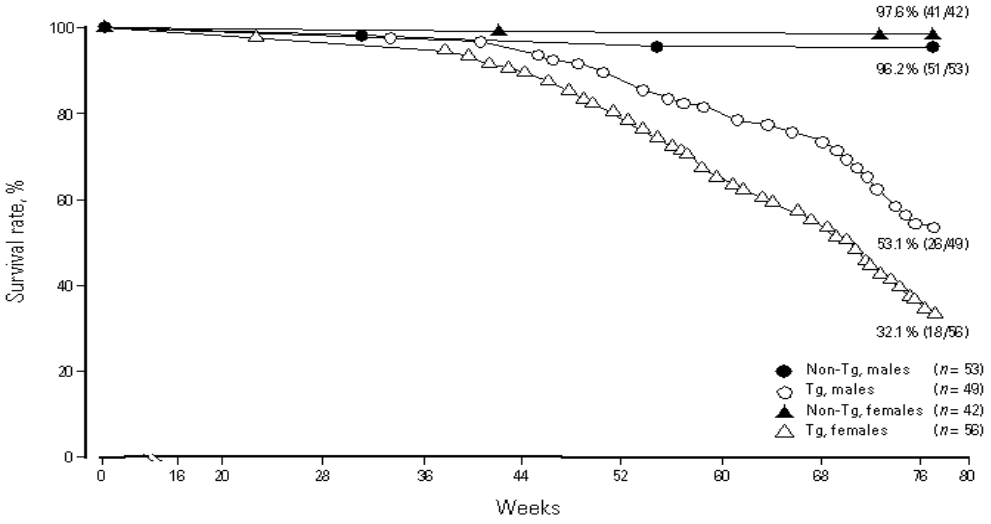

Figure 1. Survival Rate Comparison of Transgenic Versus Non-Transgenic Mice.
http://www.ehponline.org/members/1998/Suppl-1/57-69yamamoto/yamamotofig2big.GIF

Transgenic Regulations

Considering all of the advantages and disadvantages for consumers, industries, the environment, and the transgenic animals themselves, decisions must be made whether to patent transgenic animals. Organizations around the world are coming up with guidelines to help the decision making process. In this section, I will discuss patent making decisions made by different countries. We will see that transgenic animal use and research are valued on different levels throughout the world.

U.S. FDA Guidelines

The United States FDA released its guidelines on January 15th, 2009. These guidelines address the way that transgenic animals should be considered under United States Law. Currently, three organizations are responsible for biotechnology products in the Unites States:

> "The FDA is responsible for regulation of food, feed, human drugs, and animal drugs; the EPA regulates pesticides and toxic substances; and the USDA oversees

> meat, poultry, and egg products, plant pests and noxious weeds, and animal biologics…Transgenic animals may be regulated by the FDA under the theory that the genetic manipulation is an animal drug, and if the genetic change produces either a drug, food, or biologic, then that product may also be regulated by the FDA. The potential for EPA regulation exists if the transgenic animal produces potentially toxic chemicals" (Biotechnology, 2009).

The FDA has chosen to categorize a transgenic animal as a food, drug, or chemical. They have determined that transgenic animals will mostly be classified as an animal drug, the drug being the piece of DNA inserted into the animal. The newly categorized transgenic animals must fall under the existing requirements for regularly bred animals, given hormones or antibiotics, before they can be authorized for public use.

However, some concern comes from the public about the decision of the FDA to categorize transgenic animals in this manner. The FDA does not regulate the *processes* used to make the product, but regulates the nutritional value of the food. This means that "if the food product is 'materially different' from a conventional product, then the FDA can require that it be labeled. But the FDA does not currently require that a pork chop be labeled whether it came from a pig produced through artificial insemination or by conventional breeding" (Adams, 2009). Similarly, the FDA is not required to label food products from a transgenic animal if it is nutritionally the same as a conventionally bred animal. These FDA guidelines are a great start to categorizing transgenic animals, but there remains a sense of public uneasiness about transgenic products in the future, especially if they are not labeled.

After the release of the FDA guidelines, there was a 60 day commenting period where anyone could share their thoughts about the guidelines. Thousands of comments and questions were raised about the safety of the animal products and how these products get to the market.

Questions like these have come up around the world, not just in the United States. Product acceptance studies were conducted in the Netherlands and it was found that:

> "Acceptance problems with the introduction of a new technology, like biotechnology, cannot be solved by public information campaigns alone. True enough, public information is a clear necessity, but there are two complicating factors: first, higher levels of knowledge about biotechnology do not necessarily coincide with higher levels of acceptance and, second, it is not reasonable to expect that the public will adapt totally to the new developments" (Hamstra and Smink, 1996).

It is clear that despite the efforts around the world to regulate transgenic technologies, further efforts must be made to make the public aware of the safety of the animals, and the benefits they can have for humans around the world.

Canadian Council for Animal Care Guidelines

Another effort to regulate the integrating of transgenic animals into the world is to clearly regulate how the animals are contained, and requiring procedures in case of a transgenic animal breach. In Canada the Canadian Council for Animal Care (CCAC) has provided guidelines for transgenic animals, of which one section is specifically dedicated to their containment. The guidelines read as follows:

Containment for Transgenic Animals:

i. All proposals for creation or use of transgenic animals must assure the ACC that risks to human health and the environment are minimized to an acceptable level. For transgenic animals created using micro-injection or replication-defective viruses, the containment risks are limited to those associated with the escape of the animal and interbreeding with wild stocks. Proposals should include information about:
    - containment and security procedures in animal facilities and, if applicable, during transportation when importing the animal;
    - plans for recapture should a breach of containment occur; and
    - The consequences to human health or wild populations should containment fail.

ii. For commonly-used transgenic species, each animal facility should have SOPs for containment, which can be referenced by proposals.

iii. ACCs should discuss with the institutional Biohazard Committee any proposal which raises biohazard containment concerns. (CCAC Guidelines, 2005)

These Canadian guidelines when followed should show that procedures dealing with transgenic animals and technologies have been devised and aim to protect the public against any problems that may occur if a transgenic animal is integrated into the wild by accident. The CCAC understands that transgenic technologies are new and rapidly developing. For this reason, the guidelines presented above are subject to review at least every two years. This constant review and updating is a great way to stay on top of regulating the rapidly developing issues surrounding transgenic animals.

Transgenic Case Study: Oncomouse

After investigating the advantages, disadvantages, and actions taken to regulate transgenic animal use, the specific case of Oncomouse can now be used to observe the decision making process about whether to patent a transgenic animal. Oncomouse was produced at Harvard medical school in the 1980's (Leder and Stewart, 1984). This transgenic mouse was created to have a high potential for getting cancer. Since the mouse had great value in the growing search for a cure for cancer, Harvard sought to obtain a patent in the United States, and eventually in other countries. Currently in the United States and Europe, universities are legally obliged to patent and publicize any intellectual property that comes from governmentally funded projects. As discussed in the previous chapter, the Oncomouse case was one of the first to raise the issue of morality concerning transgenic animals. Is it right to patent "animals or animal

varieties, particularly for higher-order animals such as mammals, even if they do otherwise meet patentability criteria" (Bioethics and Patent Law, 2006). In the United States, Europe, and Canada, Oncomouse patent decisions were made, all with different processes and outcomes.

Patent law covers inventions and discoveries, but there is a clear distinction between the two. If one makes a scientific discovery of a naturally occurring phenomenon, the discovery is not patentable. If something is invented that has been constructed using artificial materials and processes, the invention is patentable. Because genes are naturally created, it is hard to decide if they are patentable. A transgenic animal is created using artificial processes, but holds natural genes that were discovered, not created by any process. One can argue that because the gene is not specifically something that is created or invented there should be no patent granted. When trying to obtain a patent on a gene, a scientist must "isolate it from its natural state and identify an industrially useful property for it" (Exploiting Abstract, 2004). In the case of Oncomouse, the gene is useful because it aids in finding a cure for cancer.

U.S. Oncomouse Case

In 1988, the United States Patent Office granted a patent to Harvard University. The patent was granted for a "transgenic non-human mammal whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal" (Bioethics and Patent Law, 2006). The oncogene could be isolated and it was proven to be useful for the biomedical industry. "Harvard initiated the commercial cycle of credit in 1988 when the oncomouse patent was granted and licensed to DuPont giving the firm an exclusive license to the sweeping coverage of the transgenic landscape embodied in the patent. The license gave the firm the control it needed to start the transformation of property rights into financial revenues"

(Murray, 2007). Since the patent was initially licensed to DuPont in 1988, transgenic technologies have subsequently evolved, and now hold a prominent role in the biomedical field as well as farming and material industries.

Oncomouse Case in Europe

While the United States Patent Office chose to grant a patent for Oncomouse, the European Union prolonged the decision making process. In May of 1985, the president of Harvard College applied for a European patent, specifically for the method of creating transgenic animals. In 1989, the European Patent Office rejected the patent for Oncomouse. There were two main reasons for this rejection. First, the European Patent Convention (EPC) states in "Article 53b...animal varieties are not patentable" (Dutfield and Suthersanen, 2008). Second, Article 83 of the EPC says that the application must be specific enough for somebody skilled in the field to be able to repeat and carry out the process. The application was not specific enough. The wording in the application included 'all non-human animals', not specifically mice.

In 1990, under an appeal, the decision was made that transgenic animals were not an animal variety. The Examining Division was asked to reconsider how they had interpreted the patent application. They also took under consideration Article 53a of the EPC, which considers public morality and ethics. If the patent would be contrary to the public and their morality, then the patent would not be granted. The Technical Board of Appeal (TBA) came up with a balancing test to help decide if the public was generally for or against the patenting of transgenic processes on animals. The study also investigated whether the technologies were harmful to the animals and the environment. The conclusions were in favor of the patent application, and in October of 1992, a patent was granted by the EPO. Since then there has been several situations

where the TBA has had to review the Oncomouse patent because of questions on its validity, but each time they decided the patent is still valid. This ongoing process shows that transgenic technologies are still a growing issue, with many different jurisdictions.

Canadian Oncomouse Case

Contrary to the decisions in the United States and Europe, Canada chose *not* to patent the Oncomouse. The case in Canada is very interesting because they faced the decision with a different strict approach. The rejection of the patent claim was caused specifically by the language of the claim. The words 'manufacture' and 'composition of matter' were interpreted differently by the Canadian court. The Canadian Patent Office originally rejected the claims for the animal product, but approved the *process* used to create the animals.

After the original rejection of the patent, the Federal Court, on an appeal, ruled that Oncomouse was in fact a composition of matter and granted a patent to Harvard College. Since the process used to create the mouse could easily be duplicated the Federal Court decided that it was patentable material. But by 2002, on another appeal, the case was brought to the Supreme Court for a ruling. They decided that the two terms, manufacture and composition of matter, were too broad and did not eliminate the use of higher life forms. This led to the court drawing a distinct line between what life forms were counted as higher forms and what life forms could be patented. The court decided that a mouse could not be considered a composition of matter.

The Supreme Court did not grant a patent because they did not find sufficient reasons to deviate from the original patent law as they interpreted it. From 2000 until 2003, many arguments were made for and against patenting Oncomouse. After a long process of patent

claims, approvals, and appeals, the final decision in Canada was Oncomouse and the processes used to create Oncomouse, are *not* patentable material

Looking at these three different cases regarding the patenting of Oncomouse, it is clear that different governmental entities are going to have different opinions and interpret animal patenting in different ways. Recommended regulations as discussed in the previous section will help serve as landmark cases to lessen subsequent legal controversy surrounding transgenic animal processes. Transgenic technologies have a rising importance in the biomedical field as well as agriculture and other industries. The case of Oncomouse has paved the way for many other transgenic animal patents to come.

Chapter-4 Conclusions

When considering whether to patent life forms it is important to take into consideration all parties that will be affected by the decision. Over the past few decades transgenic technologies have been of rising importance in the biomedical, pharmaceutical, farming, and material industries. The use of transgenic animals as disease models and pharmaceutical producers shows a promising positive impact for mankind in the future. It is important that these animals are patented to protect the industries and consumers alike. Continuing growth of transgenic animal research and use around the world shows that these animals are going to hold a strong position in the lives of future generations. However, regulations should be rigorously enforced to help ensure transgenic animals are not released into the wild, and if so, show diminished survivability relative to wild populations. But regulations will not be enough to contain public uneasiness toward the new technologies. Education about these animals can ease some of the controversy, but can never make it go away. There will be many legal cases to come

from these animals and technologies, many courts will have to make decisions whether to patent a particular animal. More regulations and laws are inevitably going to be made to aid in this decision making process.

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PROJECT CONCLUSIONS

In biological and medical research, transgenic animals have already proven to be invaluable, serving as models for testing new therapies for diseases, serving as bioreactors for producing human drugs, and providing information on the function of newly discovered proteins. In the near future, transgenic animals may also provide organs for transplant, and may provide aquafarmed salmon for human consumption. In spite of these vast benefits to society, transgenic animals also have a variety of ethical issues, including the welfare of the animals themselves, which must be balanced against societal gains. Based on the research performed in this project, the authors conclude the following about the field of transgenesis. We believe that all of the above mentioned types of transgenesis should continue, as each type mentioned provides strong benefit to society. In most cases, the animals do not suffer, but in those cases where animal suffering is observed (i.e. for Oncomouse) we believe in strong IACUC and FDA oversight to ensure suffering is minimized by using painkillers or euthenasia. Although the growth hormone experiments with fish provide a transgenic success story, the same types of GH experiments with mammals (i.e. Superpig) failed by not providing strong benefits to society, and the animals suffered needlessly, so we agree with the current moritorium on such experiments. We applaud the new FDA guidelines for patenting transgenic animals and their products. They are long overdue, and should help standardize the industry, while providing strong oversight to help ensure animal health and helping prevent environmental disasters.