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Transgenic Animals

Paul N. Cupido
Worcester Polytechnic Institute

Philip Declan O'Sullivan
Worcester Polytechnic Institute

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TRANSGENIC ANIMALS

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By:

Paul Cupido

Phil O'Sullivan

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APPROVED:

Prof. David S. Adams, PhD
WPI Project Advisor

ABSTRACT

A transgenic animal carries foreign DNA deliberately inserted into its genome for specific scientific purposes. Transgenic animals can be engineered to improve human welfare in agriculture, industry, and medicine. Although the use of these animals in research provides benefits and new hope for discovery in many scientific fields, there still exist questions and ethical concerns regarding the usage and creation of such animals. The purpose of this project is to provide a brief understanding of what DNA is and how it works within a cell, along with dwelling on the specific methods scientists use to create transgenic animals. This project also aims to highlight the most notable transgenic animals created to date, and also to examine the legal and ethical ramifications of this technology. It is concluded that despite public criticism, transgenic animals are an important technology of the future and offer society very strong benefits.

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PROJECT OBJECTIVES

The objective of this project is to explore the quickly developing technology of transgenic animals while gaining an understanding of the benefits, ethics, and legal issues associated with these animals. The report will define transgenic animals, explain the most popular methods used to create them, highlight each major category using examples, discuss ethical concerns, and examine current laws that regulate transgenesis and animal patenting. In both scientific and lay communities, transgenesis is considered one of the more controversial bio-technologies. Therefore this report aims to accurately provide sufficient information to the reader to help them decide whether to support transgenic research.

CHAPTER-1: TRANSGENIC ANIMAL TECHNOLOGY

Paul Cupido

A transgenic animal is an animal that carries foreign DNA deliberately inserted into its genome. The inserted gene is incorporated into the DNA of the host and is eventually expressed by its cells, creating an organism with new properties that normally would not exist in nature. Transgenic animals are a valuable tool for exploring many biological questions, for better understanding diseases, and for producing new drugs for saving human lives. Without the use of animals as models for human disease, the likelihood of medical breakthrough is significantly reduced. Transgenic animals allow for more efficient scientific advancement due to the inexpensive cost of housing lab animals, their short life cycles, and their rapid reproduction rates. Although such animals benefit society, what is the cost to the animals? To gain a better understanding of this technology, it is important to first become familiar with the process of transgenesis, the most common ways of creating transgenic animals, and how to screen for the transgenic positives.

The two most common ways transgenic animals are created are by pronuclear microinjection and embryonic stem cell manipulation, each providing its own advantages. But before understanding how scientists use these processes to create a transgenic animal, we must first understand the foundation of biology, DNA.

DNA: The Molecular Basis of Life

DNA is not only considered the molecular basis of life, but also the molecule that ties all life together. DNA, once transcribed to mRNA, is then translated to proteins which perform all

the functions necessary for life. DNA, regardless of its source from bacteria to human beings, is composed of the same basic subunits, making transgenics possible. These subunits, also known as nucleotides, are paired like the rungs of a ladder against a sugar and phosphate backbone **(Figure-1)**. Weak hydrogen bonds hold each nucleotide base pair together in a double helix structure.

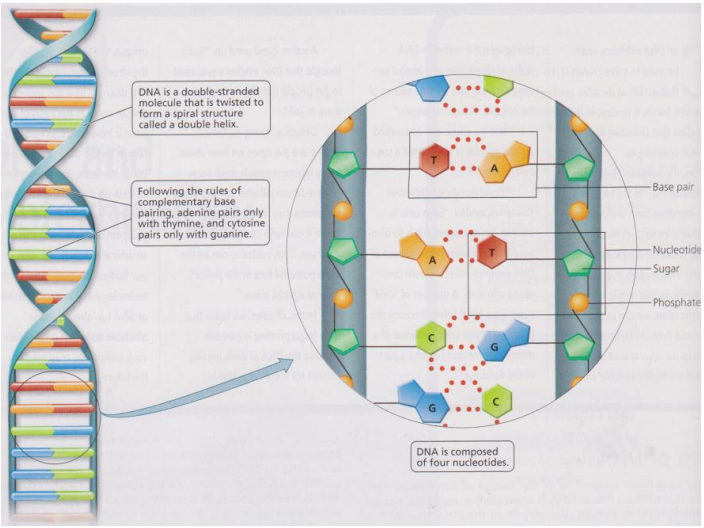

Figure-1: DNA Structure. Shown on the left is the unique shape of DNA, with its double helical structure. On the right is shown the specific base pairs held together by hydrogen bonds . (Goodenough and McGuire, 2010).

The four nitrogenous bases which make up the core code of DNA are adenine (A), guanine (G), thymine (T), and cytosine (C). These bases follow the rules of complementary base pairing discovered by two scientists, James Watson and Francis Crick circa 1953 (Crick and Watson, 1953; Kimball, 2011). These rules, based on structural constraints, state that only adenine can pair with thymine, and guanine can only pair with cytosine (**Figure-2**). This bonding of base pairs is so specific, that the bases on one strand of DNA are always complementary to the bases on the other strand (Goodenough and McGuire, 2010). Since each DNA strand is complementary, DNA can be replicated using each parental strand as a template

for a complementary daughter strand, then its message can be read by other functioning molecules in the cell.

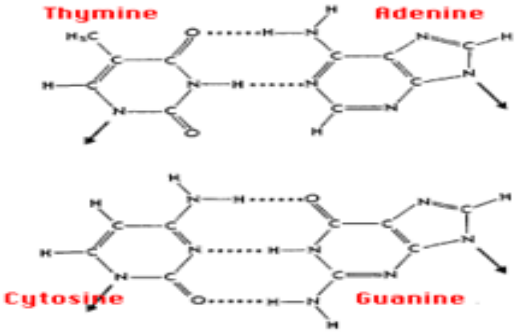

Figure 2: Nucleotide Bonding. Specific bonding occurs between certain purine-pyrimidine base pairs due to their ability to form hydrogen bonds with one another (Kimball, 2011).

So how are phenotypic traits related to an organism's DNA? The exact order of a DNA strand's nucleotide code determines the amino acid sequence for making a protein. Gregor Mendel (**Figure-3**), an Austrian monk/scientist who published a paper in the Proceedings of the Natural History Society of Brünn in 1866, was the first to help prove that physical characteristics of an organism were determined by genotype. In an experiment with pea plants, he bred two different strains and formulated three laws of inheritance:

(1) each trait inherited by an offspring is determined by a specific element, today called genes, (2) each trait or gene is inherited separately, and (3) each inherited trait is determined by the intersection of two genes, one from each parent, with one trait always dominant over the other (Gregor Mendel, 2002). These three ideas opened the flood gates for further research and discoveries in genetics.

Figure 3: The Mendel monument in the garden of the Brno Monastery, honoring Mendel for his work with nature as the father of genetics (Edelson, 1999).

In 1928, Frederick Griffith used Mendel's work to discover the phenomenon of transformation. He was able to convey specific traits from dead bacteria by mixing them with living bacteria that took up the "trait molecule" and expressed it. Sixteen years later, in 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty identified DNA as the molecule capable of this transformation, rather than protein which was strongly suspected by scientists at the time based on its greater complexity. In 1952, DNA was confirmed as the molecule controlling heredity by Alfred Hershey and Martha Chase (**Figure-4**) who used a system composed of a DNA virus infecting a bacterium to show that the infecting material was DNA and not protein. Using this previous evidence as the foundation for their work, Watson and Crick discovered the structure of DNA to be further supporting evidence of Mendel's theories. Powered by the knowledge and understanding of the structure of DNA, and how the genetic code worked, scientists figured out ways to isolate, manipulate, and clone DNA thus opening up a revolutionary door to the world of transgenics.

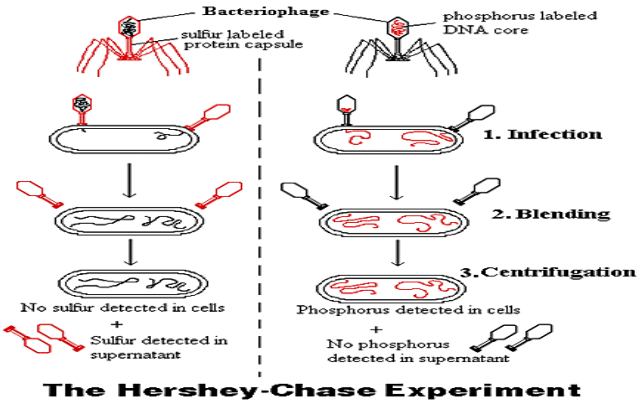

Figure-4: The Hershey-Chase Experiment. Using bacteriophages composed of both protein and DNA, in 1952 it was proved that DNA enters and infects the host cell as the genetic material. Sulfur was used as an indicator for protein, and phosphorous as an indicator for DNA, and the phosphorus entered the host cell. (The Hershey-Chase Blender Experiment, 2009).

Creating the Transgene

Transgene Structure

The first step in creating a transgenic animal is to isolate and create the foreign gene that will be inserted into the animal. A transgene is an artificial gene, and transgene structure must contain all the critical elements for gene expression (**Figure-5**). A transgene contains a promoter, an intron, a protein coding sequence, and a stop sequence (Wallace et al., 2011). These critical structure elements exist in 3-adjacent nucleotide long sequences known as codons. The promoter is a segment of DNA which facilitates transcription of a particular gene. It creates a secure initial binding site for RNA polymerase, allowing for transcription into mRNA. The promoter sequence is also responsible for determining in which cells and at what time the transgene is active (Wallace et al., 2011). Introns are the non-protein coding region of the transgene, linked to gene-regulation function. They can be spliced out in alternative ways during DNA reproduction, allowing for a single gene to encode several varying versions of the same protein under different circumstances.

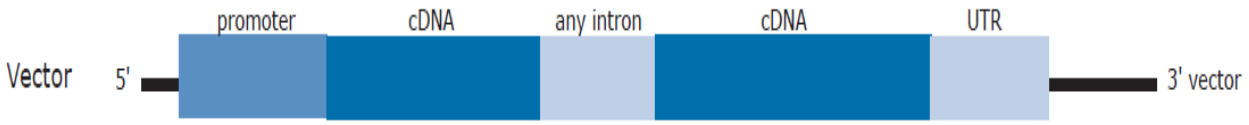

Figure-5: Transgene Structure. Basic structure of a transgene contains a promoter upstream, cDNA of the gene of interest, intron sequences and a stop codon. The untranslated region (UTR) must be included for proper gene regulation. Intron sequences do not necessarily need be from the gene of interest (Charles River, 2005).

The protein coding sequence, also known as an exon, is the main part of the transgene a researcher is focused on. Exons code for a specific portion of a protein and are joined together when the introns dividing them are removed in the RNA. These multiple exons, when edited to

make mature RNA, are translated to form complete proteins. The stop sequence following the coding sequence signals termination within the messenger RNA to halt protein translation.

Isolating and Manipulating DNA

To create a transgene, scientists must be able to isolate a particular gene of interest and then have the ability to add, delete, or rearrange a section of it for study. Creating these DNA sequences not normally found in biological organisms is called making recombinant DNA (rDNA). Once rDNA is prepared, it is inserted into a vector (plasmid or virus) and is ready for insertion into a cell. The process for manipulating DNA is a "cut and paste" method requiring the use of restriction enzymes and DNA ligase (**Figure-6**). A restriction enzyme binds to a DNA molecule at a recognition site in the DNA and cleaves it. Restriction enzymes are exclusively chosen to generate DNA fragments with "sticky ends" that are capable of being linked to other compatible DNA segment ends.

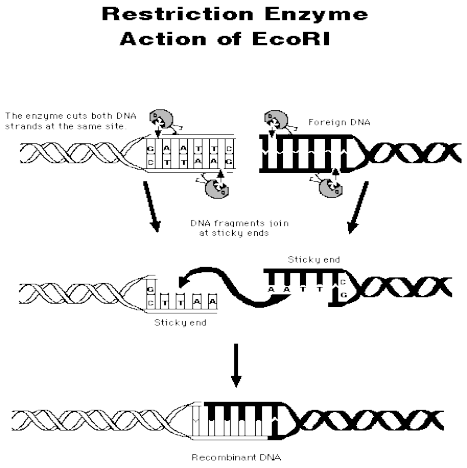

Figure-6: Cutting DNA With EcoRI Restriction Enzyme. Shown is an example of a restriction enzyme, EcoRI. The enzyme has a precise shape that allows it to run along the grove of the DNA double helix, scanning for the base letter sequence GAATTC. When it recognizes that sequence, it cuts the strands between the G and A, leaving sticky ends for recombinant DNA to be formed (Thompson, 2010).

Typically the cloning vector is treated with the same restriction enzyme as the recombinant DNA to ensure compatibility. Once the vector is ready to receive the foreign rDNA, the two are mixed together, and sealed with DNA ligase. The resulting mixture contains

vector DNA linked to the foreign DNA. The mixture is sorted out in the cloning process once the DNA mixture is introduced into the host cells.

Gene Cloning

Experiments involving transgenic animals require a relatively large amount of rDNA, so the DNA is usually cloned and amplified prior to use. Viruses/plasmids used for transgenesis are normally mutated to create specific properties suitable for cloning (Cohen et al., 1973). For example, a plasmid can contain an ampicillin-resistant gene (amp^r) used to select for positive cells containing the plasmid. The host cells are treated with chemicals (such as calcium-chloride) to make them permeable to the DNA molecules. Only a few of these cells take up a recombinant plasmid, which is why a marker gene such as amp^r is so useful. The cells are poured onto a nutrient agar plate containing an antibiotic, in this case ampicillin. The host cells which have not accepted the plasmid, lack the ampicillin-resistant gene and die. Cells that have transformed with the plasmid are resistant to ampicillin and should produce successful colonies. As the host cell's chromosome replicates, the plasmid also replicates and segregates to each daughter cell, forming a clone. This method of DNA cloning can be applied to the DNA from virtually any organism including bacteria, yeast, plant and animal cells.

Methods of Transgenesis

Once a specific rDNA sequence has been created, it must be inserted into an animal and expressed by its offspring. This is called the process of transgenesis. Various methods have been discovered to create transgenic animals, but the most common methods are DNA

microinjection into pronuclei, and embryonic stem cell-mediated gene transfer (Margawati, 2003), each has its own advantages and disadvantages.

Pronuclear Microinjection

The pronuclear microinjection technique is most commonly used for creating transgenic mice. The first successful genetically modified animal with inserted genes in its offspring was created in 1981 using the microinjection method (Gordon and Ruddle, 1981). Today, mice are the most important models for mammalian genetics, much of the technology developed in mice is potentially applicable to humans (Griffiths, 2008). The principles of pronuclear microinjection in mice have been applied to other species such as rats, rabbits, birds, fish, sheep and pigs.

Pronuclear injection involves injecting the foreign DNA directly into the male pronucleus of a fertilized egg. The process begins by collecting female eggs for fertilization. To produce the eggs in mice for example, the female donors are given two hormone injections spaced 46-48 hours apart (DNA Microinjection Services, 2011). The hormonal injections induce the female to super-ovulate, releasing more than the usual amount of eggs while becoming more receptive to mating. Successful super-ovulation protocols consider the species, age, and weight of the animals (Charles River, 2005). Corresponding fertile male animals are then used to mate with the superovulating females. Breeding should be monogamous, as the presence of other animals creates additional technical challenges (Charles River, 2005). The fertilized eggs are then collected before mitosis begins. It is important that the microinjection occurs before the genetic material in an egg replicates, preceding the first cleavage phase in cellular reproduction. The fertilized eggs have a pronucleus from the male and female gametes, which become microscopically visible for several hours following the entry of the sperm into the developing

egg (Charles River, 2005). The eggs are examined and visualized using Differential Interference Contrast (DIC) optics (**Figure-7**) in order to locate the injection locus (DNA Microinjection Services, 2011). Using a fine glass micropipette, the cell membrane is delicately penetrated with caution to avoid cell damage. Numerous copies of the desired DNA are injected into either the male or female pro-nucleus with approximately 2 to 3 pico-liters of volume. The male pro-nucleus is usually best for microinjection because it is larger, more visible, and closer to the egg cell's surface.

Figure-7: DNA Microinjection into the Male Pronucleus. A female egg (center) is held in place by a suction pipette (left) while a fine glass pipette carrying the foreign DNA (right) prepares to inject the DNA solution. As seen using DIC optics (Mullin, 2010).

If the injection is successful, the DNA can integrate into the chromosomes of the pronucleus. Once the DNA in each pronucleus has duplicated, the male and female pronuclear envelopes break down, and the chromosomes align on the metaphase plate to prepare for cell division to make the fertilized zygote. The fertilized zygote divides to form a two-cell stage embryo ready for re-implantation into a pseudopregnant surrogate (DNA Microinjection Services, 2011). In order to create a pseudopregnant host capable of receiving the embryos, vasectomized male mice are mated with the fertile females. The process of mating stimulates the female reproductive tract to support the development of eggs following surgical implantation. Since the males are sterile, the host animal does not become pregnant by that male. The

resulting offspring, known as the founder generation F_o, sometimes possess the transgene. An F_1 generation of offspring is usually produced by mating specific founder animals together with the goal of creating a homozygous genotype.

The disadvantage of the pronuclear microinjection technique is that the desired gene integrates within the host DNA randomly. The integration site is a critical determinant of the transgene's expression, so its function may be impaired even though the transgene is present (Harper, 1999). Similarly, the integration of a transgene can occur between functional segments in the host DNA, disrupting it. This can cause an insertional mutation which can negatively affect the host animal. Also, difficulties occur if the transgene is not integrated fast enough into the host DNA, or too many copies of it insert into the genome. The animal will only have some of its cells contain the new genetic code, creating an animal known as a "mosaic animal". The advantages of this method lie in its reliability and widespread use. Pronuclear injection can be used in an abundance of animal species and is often the quickest method.

Embryonic Stem Cell Manipulation

Another popular method for creating transgenic animals involves the insertion of the desired gene into a culture of embryonic stem (ES) cells. ES cells are undifferentiated cells that have the potential to differentiate into any type of cell in the animal, giving rise to a complete organism (Buy, 1997). ES cells are taken from an *in vitro* fertilized egg which has been allowed to grow for approximately 5-7 days into a blastocyst. ES cells are located in the inner cell mass of an animal blastocyst (**Figure-8**), and are extracted for culture.

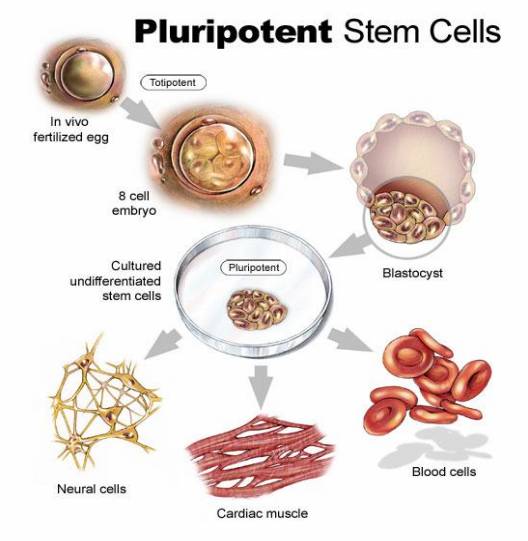

Figure-8: Isolation of Pluripotent Stem Cells. Pluripotent stem cells used for ES cell manipulation are collected from the inner cell mass of the blastocyst stage (upper right), and have the ability to develop into any cell in the body (Kochar, 2004).

The ES cell culture is treated with the prepared rDNA containing the gene of interest. The vector can be introduced into the ES cell nucleus in a variety of ways including retroviral infection, electroporation (the use of electric current to enhance cell permeability), and microinjection. It is important to maintain the stem cells ability to develop into more than one type of cell, so the stem cells are co-cultured with feeder layers of embryonic fibroblasts and growth factors to help maintain their de-differentiated state (Mudgett and Livelli, 1995).

In terms of retroviral insertion of a gene, this type of virus is sometimes used as a vector to infect the ES cell lines. A retrovirus can be "gutted" out to remove all the disease causing genes, and that DNA is replaced with the transgene of interest. The virus, when cultured with the ES cells, infects them, releasing its DNA inside the cell for integrating in the host DNA. Transgenesis by means of electroporation passes an electric charge through a sample of layered DNA and ES cells. Since DNA is negatively charged, it becomes attracted to positive electrodes and passes through the cells made permeable by the electric current. Microinjection in ES cells is similar to the pronuclear method, but is not as widely used.

The advantage of ES cell manipulation is that unlike pronuclear microinjection, integration of the DNA into the cell is not random. The process relies on homologous recombination (**Figure-9**) which is an exchange process that occurs during DNA replication if the vector DNA has significant regions of host DNA. The exchange allows the transgene to be inserted at a specific location in the host DNA sequence, lessening the change of transgene silencing by integrating in an inactive area. A transgene is constructed to be flanked by sequences homologous to the targeted integration site (Charles River, 2005). After insertion into the nucleus, DNA replication begins and the homologous regions are exchanged.

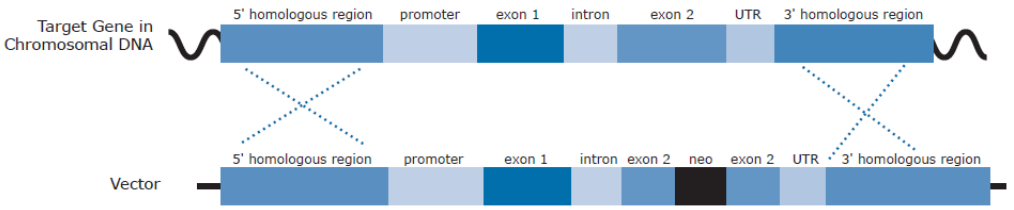

Figure-9: Homologous Recombination. The targeted gene of interest (exons 1 and 2) is flanked by analogous host DNA to promote homologous recombination. The dotted lines represent the "cross-over" point of insertion of the vector DNA into the analogous regions of the host cell genome. Some exons can be replaced with a selectable marker, such as neo^r (black) to help select for the transgene (Charles River, 2005).

The treated ES cells are then screened using methods discussed below to determine whether transgenesis was successful. If the transgene successfully inserted, the modified ES cells are re-injected into a blastocyst, and the embryo is inserted into a pseudopregnant surrogate mother. The adult offspring are initially mated with normal males, but the resulting progeny are chimeric, having some tissue derived from the original blastocyst cell lines, and some from the transplanted ES cell lines (Griffiths, 2008). From here, the offspring are mated with their siblings to eventually produce homozygous animals for the transgene.

Unlike pronuclear microinjection, only one copy of the transgene is inserted into the host genome using homologous recombination, helping to dissolve problems with over-expression. Unfortunately, this method of creating transgenic animals is very time consuming. Although the site of integration can be highly controlled, the DNA target sequence must be known. ES cell manipulation costs are a lot higher, but testing for the presence of the desired transgene in the treated cell line does not require live transgenic offspring (Margawati, 2003).

Figure-10 compares the two main methods for creating a transgenic animal. The pronuclear method is more reliable, and produces animals with all cells in the body containing the transgene, but you cannot control the integration site. ES cell manipulation allows the targeted use of homologous recombination, and screening for transgenic positives at the cell line stage prior to implantation. ES cells can allow for a researcher to use the strategy of *gene targeting* for study; when a normal host gene is substituted for an inactive gene, the targeted inactivation is called a *gene knockout* (Griffiths, 2008). Pronuclear micro injection is more likely to be used to study the "gain-of-function" of multiple copies of a transgene. Examining the phenotype of knock-out or knock-in animals can allow researchers to deduce the function of a gene in a biological process (Mullin, 2010). In addition, it can create models for testing new drugs and designing new therapies.

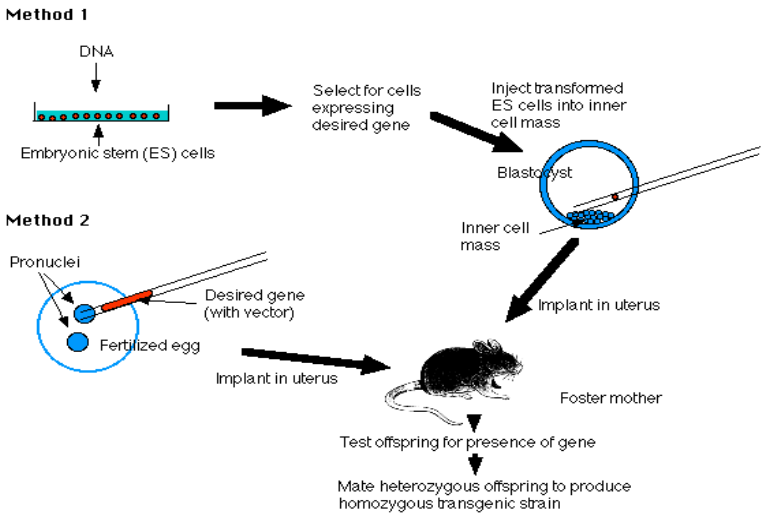

Figure-10: Comparison of the Two Main Transgenic Methods. Method (1) represents ES cell manipulation while Method (2) displays pronuclear microinjection (Kimball, 2011).

Transgenic Animal Screening

Since transgenesis is not an efficient process, it is important to screen each transgenic animal to determine whether the foreign DNA integrated into its genome. Screening is most often performed using Southern Blot assays, RT-PCR, Westerns, or ELISAs. As one of the more reliable methods, a Southern Blot assay (**Figure-11**) can detect the presence of specific DNA sequences in a mixture of DNAs. Restriction endonucleases are used to cleave the host DNA into small fragments. Then the DNA fragments are electrophoresed on a gel to separate the fragments by size (Panel-2 in the figure). Next, the DNA pattern of fragments is blotted from the gel to a membrane (Panel-3 in the figure). The DNA is fixed onto the membrane, and then hybridized to a single stranded DNA probe containing the transgene (Panel-5). The probe contains a marker, usually radioactive or fluorescent, so it can be identified once it binds to the target sequence (Panel-6).

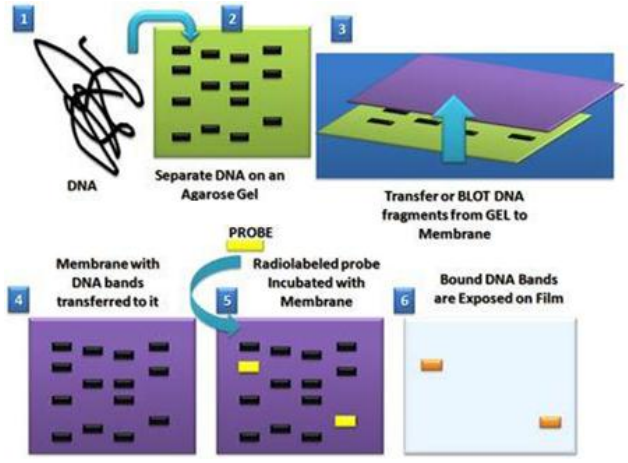

Figure-11: Southern Blot Assay Process. Simplified steps used for identifying the number of copies and presence of a transgene in an animal (Southern Blot, 2007).

A Western Blot assay is a similar screening method, but instead of analyzing for the presence of a transgene DNA sequence, this assay examines whether the transgenic protein is

produced in the host cell. Cellular proteins are isolated from the host tissue being screened, separated by electrophoresis, and blotted to membrane. Then the membrane is treated with an antibody against the transgenic protein. If the transgenic protein is present on the membrane, the antibody binds to it, allowing detection. A secondary antibody, linked with a marker enzyme, is exposed to the samples that binds the primary antibody to enhance the detection signal.

Another approach to detecting transgenic proteins is an Enzyme-Linked Immuno-sorbent Assay (ELISA). This technique uses antibodies against the transgenic protein bound to wells in a plastic micro-titer dish (**Figure-12**). If the transgenic protein is present in the animal's blood or urine, the antibodies in the well capture the protein, anchoring it to the well. Non-related proteins are then washed from the well, and a detection antibody (green in the figure) is used to locate and quantitate the transgenic protein in the well. The detection antibody or its secondary antibody, is conjugated to an enzyme that facilitates color formation from a substrate.

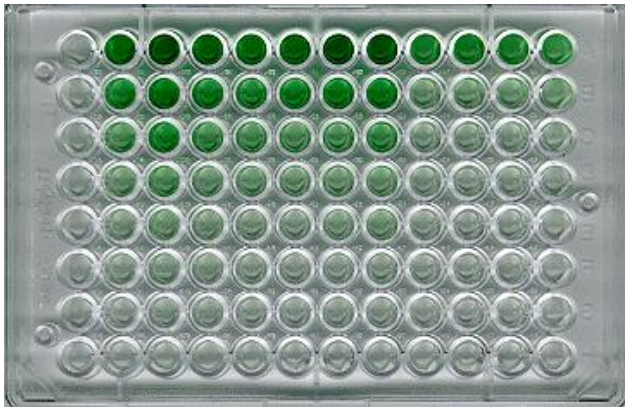

Figure-12: ELISA Assay. An example of an ELISA plate with the darker colored wells providing evidence that more of the targeted transgenic protein is present (ELISA, 1998).

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Chapter-2: Transgenic Applications

Phil O'Sullivan

The objective to genetically modify or enhance certain animals indicates that each transgenic animal is intended for a purpose. Although hundreds of transgenic animals have been created to date, they can be divided into five main categories based on their purpose. By analyzing each category, this chapter will discuss, in depth, the various transgenic animals that have been created to date and their purpose, to introduce their benefits to society. This benefits information is important when discussing transgenic ethics in a later chapter. Transgenic animals can be divided into five main categories: disease models, transpharmers, xeno-transplanters, food sources, and scientific models.

DISEASE MODELS

Transgenic disease models are animals that have been created to provide information on how human diseases initiate and progress, and are also used as test subjects for the development of possible cures before human testing. These animals are paramount to disease research because drugs cannot be FDA approved without animal testing on their safety and efficacy, yet many animals do not acquire human-specific diseases. Thus, genetically modifying animals to serve as a model for a particular disease allows them to exhibit some of the symptoms and the progression of that specific disease so that it may be observed and understood, to facilitate research and development of cures.

AIDS Mouse

The human immunodeficiency virus (HIV) is a lentivirus that causes acquired immunodeficiency syndrome (AIDS). AIDS is a mostly human-specific condition that causes progressive failure of the immune system. Some species of monkeys are infectable with simian immunodeficiency virus (SIV), but SIV is not HIV. And some chimpanzees have been shown to be capable of supporting HIV replication (Bunce and Hunt, 2004), but they are expensive. Thus, less expensive animal models for HIV infection would be useful for HIV research. The creation of an AIDS mouse would replace the need to use large, rare, and expensive animals as test models, effectively reducing the cost for research exponentially.

In 2001, the AIDS rat was created at the University of Maryland by microinjecting the HIV-1 genome into fertilized mouse eggs (Reid et al., 2001). An interesting benefit of the AIDS rat is that the HIV-1 genome used as transgene does not include two important genes directly linked to the spread of HIV in humans, so the rats are much safer than previous monkey models infected with HIV, the risk of the disease spreading is minimized (Reid et al., 2001). Rats allow bigger blood samples and organ extractions than mice, and also have certain proteins that facilitate HIV replication better than mice (Reid et al., 2001). With a nearly limitless supply of these rodent HIV models, researchers are now able to more effectively study how to identify early-onset symptoms of the disease, confirming proper diagnoses, and administering early treatment processes.

Alzheimer's Mouse

Alzheimer's disease (AD) is the most common form of dementia and affects 26.6 million people worldwide. It is a progressive neurodegenerative disease that induces problems with language, decision-making ability, judgment, and personality (Kantor, 2010). This progression

has been linked to the abnormal formation of amyloid beta (Aβ) and its deposits in the brain (senile plaques), the fundamental cause of the disease. Most animals do not get Alzheimer's disease. Occasionally, 60 year old orangutan monkeys develop it, but waiting 60 years to get your data is not a good model.

The first AD disease model that developed a robust AD related phenotype was developed in 1995 in part here at WPI by Professor Adams in collaboration with Exemplar/Athena Neuroscience group (currently Elan Pharmaceuticals) (Games et al., 1995). This mouse was the first of its kind to express a mutant version of the human amyloid precursor protein (APP) in the brain, which is associated with an early onset type of Alzheimer's disease found in an Indiana pedigree. This allows the mice to exhibit human-like Alzheimer's symptoms in about 8 months (Games et al., 1995). The model's true worth can be seen in how AD research has progressed in recent years. Companies such as Elan Pharmaceuticals and Wyeth have used AD mouse models to develop Alzheimer's vaccines. Although Elan Pharmaceutical's initial vaccine showed adverse side effects, their subsequent second-generation vaccine appears to show no inflammation. The benefit of an Alzheimer's vaccine was discussed by Israeli researcher Dr. Alon Monsonego who stated:

> *"Stimulating an immune response to Aβ in these humanized mice not only resulted in a highly efficient clearance of Aβ (plaque) from the brain, but also in a markedly reduced inflammatory reaction. The team was also able to predict that the characteristics of the immune response in mice were the same as in the human subjects"* (Sheva, 2009).

Interestingly, a recent article published in *National Geographic News* found that cell phone radiation has positive effects in reducing the development of Alzheimer's in mice. The study found that if cell phone exposure began before the genetically engineered mice started showing symptoms, they were less likely to develop symptoms later in life (Than, 2010). In the case of the AD mouse, fundamental research barriers such as the leap from the animal test

subject to humans were enabled due to the extensive testing allowed by the model.

Oncomouse

The oncomouse was the first transgenic animal to be patented. This mouse was created at Harvard in 1984 by Philip Leder and Timothy Stewart (Stewart et al., 1984), and was genetically engineered to model many forms of human cancer by replacing the normal mouse *myc* oncogene with a *myc* fusion transgene under the control of a strong promoter to over-express the oncogene. As the mice and their offspring grew, they developed carcinomas, confirming that oncomouse was a successful transgenic model for cancer (Stewart, 1984).

In 1988, U.S. patent 4,736,886 was granted to Harvard College for, *"a transgenic non-human mammal whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal…"* (Noonan, 2010). This patent specifically excludes humans, which reflects the concern about patents on human life versus animal life, which will be discussed further in Chapters 3 and 4. This mouse led the way to creating many other types of cancer mice, including those that develop cancer in specific organs. Currently, the biomedical research field has at its disposal an ever-expanding set of mouse models of organ-specific cancer (Hanahan, 2007).

Parkinson's Fly

Nearly 75% of known human disease genes have a recognizable match in the genome of fruit flies. The *Drosophila* genus, whose members are commonly referred to as fruit, pomace, vinegar, or wine flies, is currently being utilized to serve as a disease model for Parkinson's disease, and other diseases. Parkinson's disease is a neurodegenerative syndrome resulting from

the death of dopamine-generating cells in the *substantia nigra*, a region of the midbrain. Although the cause of Parkinson's disease is unknown, mutations in the alpha-synuclein gene have been determined to be a major cause of Parkinson's in genetic cases. The Parkinson's fly model contains two point mutations of the alpha-synuclein gene (A30P and A53T), which are directly linked to the disease's inheritance (Feany and Bender, 2000; Vogel, 2000). The fly's nervous system formed normally, but after 30 days, the dopamine-generating neurons had deteriorated completely. Thus, the model mimics three important aspects of Parkinson's disease: adult onset, nervous system involvement, and anatomical specificity (Feany and Bender, 2000). In recent studies, tests on the fly model have developed methods on how to effectively block dementia. Dr. Paul Shaw stated:

> *"Thanks to this model our labs have created, Dr. Galvin and I can not only quickly test potential new treatments for these symptoms of Parkinson's, we can also move up our treatments in terms of the timeline along which the disorder develops. That may give us a real chance to change the course of the disease."* (Galvin and Shaw, 2009)

TRANSPHARMERS

Transpharmers are animals engineered to produce important proteins in the blood or milk. Milk has become the most common site of production due to its ease of harvesting. Inserting a transgene into the animal under the control of a promoter for a milk protein ensures its production in milk. Prior to the development of transpharmers, proteins such as Human Growth Hormone (HGH) and insulin were usually harvested from human cadavers. Transpharmers drastically facilitate the production of these important proteins with no observable adverse effects on the animal itself.

The first transpharmer models were mice engineered to express the clot dissolver drug tissue plasminogen activator (tPA) in milk (Gordon et al., 1987). Rats were later used, and could

produce proteins such as human alpha-antitrypsin (hAAT) and human alpha-lactalbumin, which help treat emphysema (Fujiwara et al., 1997).

As effective as transpharmer mice were, due to their size they did not yield nearly as much milk as companies desired, so farm animals such as goats, cows, and sheep were then used. The first successful transpharmer sheep were created in Edinburgh, Scotland by pronuclear injection of a fusion transgene containing a sheep milk protein promoter β-lactoglobulin (BLG) to drive the expression of the blood clotting protein factor IX (Clark et al., 1989). The trait proved to be heritable. Transpharmer goats were first produced in 1991 at the Tufts University School of Veterinary Medicine in Massachusetts to produce the clot dissolver drug tissue plasminogen activator (Ebert et al., 1991; Ebert et al., 1994). Like the sheep, the goats also passed the transgene to their offspring. GenPharm International engineered the first transgenic cow, dubbed "Herman" in 1990, and his first transgenic offspring were bred at GenPharm's lab in the Netherlands (Krimpenfort et al., 1991). Herman was modified to contain the gene for the multifunctional protein human lactoferrin, which is not found in either cow's milk or synthetic milk. He fathered 8 calves, all containing the gene, and was eventually put down in 2004 for health problems unrelated to his genetics (Sterling, 2004). The transpharming field is arguably the most ethical form of transgenic practice, as no observable side effects are present in the subjects when compared to naturally born animals of the same species.

XENOTRANSPLANTERS

Organ transplantation is the procedure of moving an organ from one body to another for the purpose of replacing the recipient's damaged organ. Until recently, human organ transplant operations could only be conducted if another human died with healthy organs intact, or a living

donor gave an organ that is not vital to their survival such as a kidney. These are known as allotransplants, as they are performed between the same species. Donated organs must also be histocompatible with the recipient or the body will reject the foreign tissue. For this reason, many of the 112,000 people in America awaiting organ donations will die without ever having an operation (OPTN, 2011).

Xenotransplanters may be the solution to a very low supply of human organs. The most compatible animal has proven to be a pig due to its similar physiology and organ size compared to humans. The major obstacle for a compatible pig organ is due to the presence of a protein, alpha (1,3)-galactosyltransferase present in pig cells that catalyzes the production of galactose on the surface of pig cells. This causes the human body to reject the introduced tissue or organs. The most effective way to ensure compatibility with pig organs was discovered in 2002 at the Department of Animal Science at the University of Missouri where four pigs were created with the alpha(1,3)-galactosyl gene effectively knocked out (Lai et al., 2002). The first "knockout pigs" showed successful eradication of the gene for one allele (mammals usually contain two copies of each gene), so the animal was heterozygous (Lai, 2002). Later, in 2003, the pig line was bred to homozygosity (Sanchez, 2003). But due to other immunologic problems that arise during human-pig xenotransplantation, further development is needed. Dr. David Cooper of the Thomas E. Starzl Transplantation Institute of the University of Pittsburgh Medical Center remains hopeful, and states:

> *"Advances in these areas might allow the initiation of clinical trials of xeno-transplantation, at least for cell or islet transplantation or for the use of a pig organ to 'bridge' a patient until a human organ is obtained. The potential benefits of successful xenotransplantation to large numbers of patients with very differing clinical conditions remain immense, fully warranting the current efforts being made to work towards its clinical introduction."* (Xenotransplantation, 2007)

TRANSGENIC FOOD SOURCES

The purpose of transgenic food sources is to provide animals that grow larger and mature faster on less food than their naturally born counterparts. This was accomplished by introducing growth hormones to the zygote for two types of food animals.

Superpig

The infamous Beltsville pigs, made in Beltsville, Maryland were created by genetically modifying them with an ovine metallothionein-1 alpha (oMT1a) promoter fused to ovine growth hormone genes. The MT promoter is constitutively always on, so the animals produce more growth hormone than usual. As expected, they expressed higher levels of growth factors (Miller et al., 1989). Unfortunately, despite growing radically faster and larger than naturally born pigs, the superpig developed many health problems after 6 months of age, including kidney and liver malfunction, ulcers, heart disease, and near immobilization due to extreme arthritis. For this reason, the pigs were euthanized, and further transgenic experimentation on mammals involving growth hormones ceased.

Superfish

Similar growth hormone modifications were performed on fish, and have been far more successful. Aqua Bounty Technologies, based in Massachusetts, has generated transgenic AquAdvantage salmon that grow to market size twice as fast as regular salmon while requiring less feed (Aquabounty Technologies, 2011). These genetically modified Atlantic salmon have two foreign DNA sequences inserted into their genomes. One encodes a growth hormone from Chinook salmon, and the other is the on-switch promoter used by an antifreeze gene from ocean pout, an eel-like fish found in the Northwest Atlantic Ocean. When placed alongside the growth

hormone, this on-switch promoter makes the salmon produce the growth hormone in cold weather when they otherwise would not, so the salmon produce growth hormone year round rather than seasonally. Importantly, the modified salmon do not grow larger than regular salmon; they just achieve their size in sixteen to eighteen months rather than three years. The Food and Drug Administration (FDA) is considering whether to approve this salmon based on safety grounds alone (Gitig, 2010).

SCIENTIFIC MODELS

These transgenic animals are created with the intent to further understand genetic development and protein function through overexpressing certain genes or knocking them out.

ANDi

ANDi was the world's first transgenic primate born on October 2, 2000. To create ANDi, researchers injected 224 unfertilized rhesus eggs with a virus carrying the gene for green fluorescent protein (GFP) (Chan et al., 2001). The virus's job is to integrate the gene into a random site on one of the chromosomes. Six hours later, each egg was artificially fertilized by sperm injection. Roughly half of the fertilized eggs grew and divided, reaching the four-cell stage. Forty embryos were chosen and implanted into twenty surrogate mothers—two per mother. Of these, three healthy males were born and two twin males were stillborn. ANDi was the only live monkey carrying the GFP gene (Trivedi, 2001). GFP direct fluorescence was detected in his toenails and hair, however ANDi does not glow (Chan et al., 2001). It has been recently proven that primates can pass the transgene to their offspring. Scientists of the Central Institute for Experimental Animals in Kawasaki, Japan, gave marmosets the GFP that made them glow green under UV light. When the single male of four transgenic newborns was sexually

mature, he successfully fathered a single offspring, which also glowed green, showing the trait was heritable (Coghlan, 2009). ANDi has proven that primates are able to accept foreign genes and has possibly opened the door to future human transgenic procedures.

Smart Mouse

The Smart mouse, dubbed "Doogie", was created at Princeton University in 1999 (Tang et al., 1999). Doogie is a strain of mice that is genetically modified to have improved learning and memory. In a novel object recognition test, the mice were given the chance to become familiar with two objects. Later, when one object was switched for another new object, Doogie mice quickly recognized the switch and devoted time to exploring the new object instead of the old one. Normal mice spent equal time exploring the new object and the old one (Tsien, 2000). The improved cognitive ability is accredited to the overexpression of NR2B receptors in synaptic pathways. This change means that the mice have juvenile-like brain features with regards to memory retention, which are believed to be more efficient at retaining large amounts of new information.

Supermouse

Supermouse was created using the same parameters and principles as the previously mentioned Superpig and Superfish, and represents the world's first expressing transgenic animal (Palmiter et al., 1982). By microinjecting mouse newly fertilized eggs with rat growth hormone genes, the newborn transgenic mice grew faster and much larger than their naturally born littermates. The Supermouse was the first transgenic animal ever created with observable changes in its physical traits and development, and paved the way for producing other transgenic animals. The success of Supermouse also showed potential as a means to take preventative

measures against dwarfism, gigantism, and other genetic diseases affecting height (Palmiter et al., 1982).

Youth Mouse

Youth mouse was created at the Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel in 1997. The mice overexpress the urokinase-type plasminogen activator, which acts as a clot dissolver. The mice are smaller, eat less, and live nearly twenty percent longer than normal mice of their type (Miskin and Masos, 1997). It is believed that the overexpression of the plasminogen activator extends the lifetime of the mice by preventing atherosclerosis, a process that develops plaques in the arteries of an animal as it ages. Recent experiments have also succeeded in reversing the effects of age, using telomerase, an enzyme that synthesizes small segments of DNA that seal the tips of chromosomes, which prevent genes from deteriorating. When scientists increased the levels of telomerase in mice, their organs began to rejuvenate (Hastings, 2010).

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CHAPTER-3: TRANSGENIC ETHICS

Paul Cupido

The human-animal relationship has existed for millions of years and continues to evolve to this day. Without the use of animals and science, our species would have struggled to survive, as throughout history animals have provided us with means of food, transportation, fertilizer, clothing, and protection. It was during the time of the Roman physician Galen (AD 129 – *c.* 216) that using animals for obtaining knowledge about human biological processes first became popular. By dissecting apes, sheep, pigs, and goats, Galen developed skeletal anatomy and an understanding of nerves, along with more epochal disciplines of medicine (Porter, 2003). Since then, the human-animal relationship has evolved to the pinnacle of biotechnology, as transgenic animals and genetic modifications now help to further our knowledge pertaining to our species. Despite all the advancements being made with this technology, the use of transgenic animals has received a lot of scrutiny regarding ethical beliefs. Many argue against using animals for testing due to reasons of cruelty, morality, and religion. Ethical concerns regarding jeopardizing the safety of our environment also creates arguments, as genetic engineering is viewed by many as an act of "playing god". This practice is nothing new, as selective breeding to create more useful varieties of plants and animals has been a form of biotechnology that human beings used even before Galen's time.

Since the dawn of man's existence on Earth, we have been selectively breeding animals and plants to better serve us for work and domestication purposes. On the large timescale of human history, our race has just recently been granted the power of changing an organism's genetic code at will. Ethics and science have always gone hand in hand. Now more than ever,

ethics should be considered, as genetic engineering continues to become known as "the most powerful technological tool ever possessed by humanity" (Rollin, 1996). Still, the importance of ethics within the scientific community often goes unseen, therefore hindering the advancement and development of controversial technologies. Genetic engineering especially has distanced itself from the public, as most people are misinformed and define the issues dealing with transgenesis erroneously (Rollin, 1996).

As with any argument pertaining to ethics, it is important to understand conflicting opinions in order to be most informed when making a conclusion. With transgenic animals some people believe that animals have their own rights, and using them for human benefit is unjust. Others hold the need of humans above that of animals, and turn a blind eye to all animal experimentation. There is no right or wrong answer to any ethical question, but a compromise in between these two opposing views appears to hold a rational solution. Forming an extreme opinion on transgenic research seems to be based on a lack of knowledge on the subject.

In terms of injustice, it is not fair to form a blanket policy on such an important piece of biotechnology. A key matter often overlooked is that each category of transgenic animals differs from one another. Disease models, transpharmers, xenotransplanters, food sources, and scientific models, each induce independent ethical discussions. If transgenesis is first examined as a whole and then broken down into its individual categories, the ethical balancing act that takes place between the benefit to society and the detriment to the animal will become more apparent.

The Costs and Benefits of Transgenic Animals to Society

Opponents of transgenesis claim that animal experimentation is detrimental to society, to the environment, and to the animal. Despite the importance of transgenic animals to science (discussed in Chapter-2), common misconceptions raise concerns about the use of animals in the laboratory. The first belief critics hold is that transgenic animals suffer more when compared to a regular research animal. It is believed that the introduction of foreign DNA often causes unpredictable and painful mutations not intended by the researcher in an experiment. While the process of successfully transferring foreign DNA into a living animal is unpredictable, inefficient and complex, mutations often impact highly specific metabolic processes or cell receptors without actually causing disease, discomfort, pain or malformation in the animals (GlaxoSmithKline, 2007). The opponents of transgenic research are correct in that the process of successfully expressing a gene in a living animal does not have a high success rate. Thus, a high percentage of the animals that do not express the foreign DNA are eventually destroyed, as they are no different than wild type mice used at the beginning of the experiment. This matter is most alarming to organizations such as PETA (People for the Ethical Treatment of Animals) and the ASPCA (American Society for the Prevention of Cruelty to Animals), who relentlessly campaign to stop procedures such as transgenesis with statements like the following:

> "*More than 100 million animals every year suffer and die in cruel chemical, drug, food and cosmetic tests, biology lessons, medical training exercises, and curiosity-driven medical experiments*" (PETA, 2011).

There are some forms of transgenic research that do cause pain or even death to the animal subjects, for example the Oncomouse and Superpig. The Oncomouse was created by inserting an oncogene that increased its susceptibility to cancer, while the Superpig was given HGH (human growth hormone). Superpig suffered immensely before inevitable euthanasia. However, not all disease model animals exhibit this misfortune contrary to popular belief and will be discussed later on.

Some skeptics believe that transgenesis comes at the cost of violating religious beliefs. Four major religions: Hinduism, Judaism, Islam, and Christianity surprisingly seem to leave animal rights open for individual interpretation. Hindus believe that non-human animals are inferior to human beings, but the cow is considered to be sacred (British Broadcasting Corporation, 2010). To Hindus, to kill or harm a cow is a horrible crime as Hinduism teaches that cows have feelings similar to humans. Judaism places great stress on proper treatment of all animals, as they are accorded the same sensitivity as a human (Rich, 2011). However, there is no definitive opinion in Judaism as to whether animals experience psychological and physical pain in the same way humans do. The Qur'an shares similar beliefs that animals have feelings. Islam does in fact support testing on animals as long as the goals of improving human health or human safety are respected. Christianity on the other hand does not have clearly defined beliefs when it comes to animal rights and using animals to benefit mankind.

Members of activist groups such as PETA believe all animals deserve moral consideration. Such a view creates problems when society is trying to resolve cases where the moral interests of different animals are in conflict. An interesting and reasonable way to distinguish which animals are suitable for experimentation is to arrange the organisms in a moral hierarchy. This approach is what philosophers call consequentialism (Singer, 2011). Organisms are arranged in three tiers, each containing organisms that deserve different amounts of moral consideration. The lowest tier consists of inanimate objects and simple organisms which deserve no moral consideration. Examples in this tier are insects and plants. The second tier is comprised of sentient organisms that are not self-aware and don't have any idea of continuing to exist in nature (Singer, 2011). Fish and rodents are occupants of this tier. These are animals that can feel pain and pleasure, but prefer to avoid pain. It is still considered wrong, under this

ideology, to cause pain to the members of this group. Killing and replacing individuals in this tier however is not significant because one individual is not significantly different from another (Singer, 2011). The third grouping includes humans and more complex and cognitive animals. These species are fully aware. This tier attracts the most moral value for animals. This ranking of hierarchy helps to justify using transgenesis on the second and third tiers of animals in a humane way.

Another potential negative aspect of transgenesis is that it poses a huge threat to humanity, even with as many problems it can solve within our species. Tinkering with nature and the natural order of evolution could drastically disrupt biodiversity. If a transgenic animal bred to be stronger, faster, and healthier ever escaped and mated with the wild population, the effects it would have on the species could do unimaginable damage. "Playing God" has its consequences, but is it necessarily wrong? The argument against this thought is that the human race has evolved to where it is today by altering the environment and nature. It is hard to understand why damming rivers, eradicating smallpox, and building cities and civilizations, is not also intrinsically wrong in this sense (Rollin, 1996).

Overall, it is appropriate to recognize the benefits of transgenic research as being monumental for science. The contributions to human welfare created through transgenic research can be grouped into three categories: Agriculture, Medicine, and Industry. Transgenic animals have helped farmers by providing them with animals equipped with better milk production and higher growth rates (Margawati, 2003). Transgenic cattle and pigs now exist with more meat on their bodies along with sheep that produce more wool. The quality of animals used for agriculture has significantly increased as genetic engineering can now provide disease resistant animals such as influenza-resistant pigs. In medicine, animals modified for

xenotransplantation provide new hope for patients on organ-donation waiting lists. Products such as insulin, growth hormone, and blood anti-clotting factors used as supplements and pharmaceuticals can be obtained through the milk of transpharmer animals. Herman the transgenic bull was produced in 1991, and is known as the first genetically modified bovine in the world. At the embryo stage Herman was microinjected with human gene coding cells for lactoferrin, a key protein in the human immune system. He was allowed to reproduce and fathered 55 calves, all containing the lactoferrin gene. Transgenic cows like Herman, have been used widely to produce hormones and human protein-enriched milk for treating those with digestive and other needs. It is hard to even fathom the potential transgenesis has when it comes to curing and treating genetic disease. The world's first spider-goats, Webster and Pete (Highfield, 2002), produced spider silk in their milk for industrial applications. They inspired a breed of offspring which can produce spider silk for use in military uniforms, medical microsutures, and tennis racket strings (Margawati, 2003). Today, the Netherlands Forensic Institute has combined its work with Randy Lewis, the creator of the spider-goat to make bulletproof skin (Fattah, 2011).

Transgenic animals can be divided into individual categories and further examined to provide full insight on ethical issues dealing with this subject. When analyzing the costs and benefits of this technology as a whole, the differences between each group of animals are often not realized. Disease model animals are the most controversial as they appear to the public as the class that exhibits the most pain and suffering. While they raise questions pertaining to animal welfare, transpharmer animals on the other hand almost seem to escape all ethical scrutiny.

Disease Model Ethics

Transgenic animals created to be disease models incorporate genes which will cause the animal to exhibit some symptoms of human disease. This allows scientists to learn more about significant disease and test potential treatments before exposing a human to the dangers of preliminary testing. Disease model animals save money, time, and most importantly lives. Giving an animal a partial human disease seems cruel, and can put an animal through a great deal of suffering. Disease model animals perhaps are victims of the harshest ethical judgment. Not all disease model animals suffer though. For example the Alzheimer mouse experiences no pain, yet it grants scientists the hope to cure the 6th leading cause of death in the United States (Alzheimer's Association, 2011). Developed at WPI by Professor Adams and the former Transgenic Sciences Inc., the Alzheimer disease model mouse expresses the human mutant β-amyloid precursor protein (APP) linked to autosomal dominant forms of Alzheimer's disease. This mouse is the first successful animal model to be transgenic for APP, and shows signs of neuro-degeneration. The only suffering this animal exhibits is a slower cognitive speed. This disease model led to the development of the first testable vaccine for Alzheimer's disease made by Elan Pharmaceuticals (Schenk et al., 1999).

The Oncomouse however, clearly can endure pain as a transgenic animal, especially if the tumors are grown to the advanced stage. Genetically modified to develop cancer, the mouse is extremely susceptible to tumor growth. This is done by introducing an oncogene specific to triggering the development of tumors into the mouse's genetic code (WIPO, 2006). This controversial animal generated many ethical issues as the creators sought patents in numerous countries. They succeeded in obtaining a patent in the United States, Europe, and Japan. But the

Canadian Supreme court denied the patent in 2002, claiming that the only major issue with the Oncomouse was that life should not be patented.

Xenotransplanter Ethics

The xenotransplanter branch of transgenic animals is an interesting topic for ethical discussion. The need for organs rises annually, and genetically modified animals can help resolve this problem. There are approximately 111,000 individuals currently in the U.S. awaiting organ transplants (Organ Donor, 2011), while only about 14,000 individuals donated organs in 2010 (Donate Life America, 2011). Transgenic animals can be tailored and customized to grow vital organs and save thousands of lives. Implanting organs into humans from animals does have its risks. The organ can be rejected by a patient's immune system and animal viruses can be spread to humans. The cross species infection of a virus can create a very dangerous situation. This can create a new human strain of a virus which would require time to create vaccines and treatments for. However, pre-transplant pathogen screening can prevent the potential outbreak of a new strain of virus.

Food Source Ethics

An animal whose genetic code has been altered to increase the rate at which it grows is considered a transgenic food source animal. The 'Beltsville pig', was one of the most controversial transgenic food source animals, as it was obvious how much pain it endured. The Beltsville pig, also known as the Super pig, was given a gene for a human growth hormone with the goal of creating larger animals with leaner meat. The pig reached enormous size, but it became deformed as it could not support its own weight, and it also suffered from severe arthritis

and various organ failures. Many people view this transgenic case as a complete failure. It is not normally recognized that this animal actually led to the development of the Superfish. Altered in a similar manor, Superfish have an immaculate growth rate, gain size quickly, suffer no apparent illnesses, and require less feed. Superfish are considered to be safe by the FDA, but congress shot down the first genetically altered animal ready to be marketed for consumption in the U.S. (Rain, 2011). Food source animals are very important to the progression of humanity as they can help combat seemingly impossible problems to solve, such as world hunger. Food source animals can be brought to slaughterhouses quicker because they require less time to grow. This can help drive down the price of certain foods as companies can increase the amount of animals processed for consumption daily. As long as animals in this transgenic category do not suffer from the presence of the transgene (like Superfish), there appears to be no other ethical concern besides sacrificing the animal for food, which already occurs daily in order for our species to survive.

Transpharmer Ethics

Transpharmer animals are engineered to produce large amounts of a particular hormone or protein in their blood or milk. This category of transgenic animals receives not nearly as much ethical debate as the other animal groups. No direct harm or pain has been proved to be inflicted on these animals. Transpharmers provide a high yield of product at a low cost. The morality of transpharmers should not be questioned as they possibly could increase the availability of drugs for people who can't receive such treatments.

Chapter-3 Conclusions

After examining each main division of transgenic animals it is clear that transgenic research needs to continue pushing forward. The negative side effects seem harsh in some cases, but the possibility of creating a new viral disease or disrupting biodiversity by breeding with wild type animals can be prevented by taking the proper precautions. When considering using animals for medical purposes, it is necessary to consider whether the pain and suffering of the animals is justified by the potential benefit to human beings (Nuffield Council on Bioethics, 2011). The potential transgenic technology provides human beings is enormous, and can justify experimentation in its own. When pertaining to animal welfare, inhumane treatment of animals cannot be overlooked, and institutional Animal Care and Use Committees (IACUC) should continue to provide strong oversights to prevent needless suffering. Animals should be considered to share common rights with humans, but shutting down animal research because of this belief would be detrimental to society. Humanity has been granted a gift from hundreds of years of hard work and study. It would be a shame to waste such technology, that when used correctly could solve the world's most challenging problems.

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Chapter-4: Transgenic Legalities

Philip O'Sullivan

Controversial technologies, including transgenics, are regulated by society through laws. Subjects such as whether animals should be patented are highly controversial, and often blend ethics with legal issues, especially regarding determining whether legal precedents have been established, or determining whether transgenic animals should be sold for profits. The legal cases that decide whether animal life can be owned are complex, and do not always turn out the same way in various countries. This chapter will discuss transgenic legalities, and investigate where lines should be drawn.

PATENT ISSUES

In general, patents serve to protect intellectual property, providing benefits to the invention creator, and blocking competition for a period of time. Title 35 of United States Code 101 states that: "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title" (Bitlaw, 2000). So patent law requires the invention to be new, and to be a process, machine, manufacture, or composition of matter. So the question becomes where do animals fit in this language. The U.S. Patent and Trade Office (PTO) also requires that a patent submission must satisfy the three requirements of novelty, utility, and non-obviousness. All guidelines considered, there is no evident regulation that distinguishes that patentable property can be either living or non-living.

In addition to the PTO controlling patenting, the government's Federal Drug Administration (FDA) is also a major player in the control of transgenic products. The FDA

decides how genetically engineered animals will be evaluated to benefit the public, and decides whether their products, such as transpharmed drugs or Aqua Bounty's superfish are suitable for consumption. Such animals will be regulated under the Federal Food, Drug, and Cosmetic Act; the recombinant DNA in the animal is sometimes considered the "drug", and its safety and environmental impact are investigated by the FDA (FDA to Regulate the Use of Transgenic Animals, 2009). Strong FDA involvement is evident in the recent developments regarding the market release and distribution of Aqua Bounty's genetically enhanced superfish (Marris, 2010).

PATENTING LIFE, DIAMOND V. CHAKRABARTY

The first case of patenting any living organism occurred in 1980. Genetic engineer Ananda Chakrabarty had developed a bacterium capable of breaking down crude oil, and proposed it would be a major benefactor to the rapid treatment of oil spills. The presiding patent examiner initially turned down his request, as at that time no legal precedent existed for patenting life. However, Chakrabarty persisted, and eventually the United States Court of Customs and Patent Appeals later overturned the original negative decision in Chakrabarty's favor dictating that, "the fact that mico-organisms are alive is without legal significance for purposes of the patent law" (Diamond v. Chakrabarty, 1980). This case served as a landmark for later patents for genetically modified animals and stem cells.

THE ONCOMOUSE COURTCASE

ONCOMOUSE IN THE UNITED STATES

Harvard and DuPont's Oncomouse was the world's first patented animal. Originally filed in 1984 (Leder and Stewart, 1984) it finally received patent number 4,736,866 on April 12,

1988, and continues to be the center of the animal patent universe. The original patent covered all mammals containing oncogenes, and specifically included the original animal that first received the oncogene and the process used to create it (Leder and Stewart, 1984). DuPont's licensing fee was originally high, and many researchers were concerned that DuPont's licensing could slow the testing of new cancer therapies, as only the rich labs could afford to pay the licensing fees (Smaglik, 2000). To overcome this problem, DuPont and the US National Institutes of Health (NIH) negotiated a deal giving non-profit researchers free access to the mouse with the stipulation that any commercial users must pay for the mice (Smaglik, 2000). Still, researchers argue that the use of licensing fees will deter research by causing an economic burden. The Oncomouse patent also drew controversy from many animal rights organizations, arguing that animals should not be patented. To date, the authority to grant a patent on life has been subject of many court cases.

ONCOMOUSE IN EUROPE

The success of the Oncomouse patent in the US was followed by DuPont's patent filing in Europe. DuPont's 1989 European proposal was initially refused, but the case was later appealed and granted on the grounds that while Article 53(b) of the European Patent Convention 1973 (EPC) states that animals are excluded from patentability, Article 52(1) states that patents are available for all inventions capable of industrial application, and therefore under this article, a transgenic animal could be seen as capable of industrial applications (European Patent Convention, 1973). Following this case, a debate led to the adoption on 6 July 1998 of EU Directive 98/44/EC on the legal protection of biotechnological inventions, known as the "Biotech Patent Directive" whose purpose is to clarify the distinction between what is patentable and what

is not. For instance, an invention relating to individual human, animal, or plant genes and gene sequences, and their functions, can be patented in Europe as long as the other patentability criteria are also fulfilled. However, the directive rules out the patenting of an entire human body, and eliminates applications for procedures designed to allow human cloning, human germ line engineering, or the use of embryos for industrial or commercial purposes (EPO – Biotechnology, 2011). The European Oncomouse case was officially resolved in June of 2004 in favor of DuPont. Today, European court cases continue to debate the future of patents for stem cells and human genes.

ONCOMOUSE IN CANADA

In Canada, the patent examiner initially rejected claims to transgenic animals on the basis that they were not included in the patent definition of an invention. Once again, DuPont appealed this decision. But in this case, the Supreme Court of Canada finally ruled in 2002, through a close 5 to 4 vote, that higher life forms were not patentable because they are not a "manufacture or composition of matter within the meaning of invention" of the Canadian Patent Act (Harvard College v. Canada, 2008). Manufacture was interpreted as a non-living mechanistic product or process, while "composition of matter" was understood to be ingredients or substances that had been combined or mixed together by a person. So while microorganisms, or in this case, an oncogene-injected egg capable of maturing into an Oncomouse, may be a mixture of ingredients and thus in theory patentable under Canadian Law, the body of a mouse, which was not combined or mixed together by a person, was not (Bioethics and Patent Law, 2006). The denial of Oncomouse in Canada has been a rallying point for activists who argue that animal patenting can create barriers preventing the free and rapid dissemination of scientific research materials

which, in turn, affects the discovery of drugs and treatments (Check, 2002).

BENEFITS OF ANIMAL PATENTABILITY

When determining whether a specific patent is fair or ethical, it is important to understand the basis behind the patent itself. When applying for a patent on a transgenic animal, the individual or company is attempting to secure protection for their idea. In the case of Oncomouse, Harvard and DuPont devoted time and money to the research and development of a transgenic animal that serves as a model for screening anti-cancer drugs and aiding our understanding of oncogenesis. Their attempts to patent their creation strive to secure financial benefits for their pioneering work, and to ensure proper recognition as the original developers of this idea. Competition has always been a main driving force in the business world. Michael E. Porter proposes that the threat of new entrants, the power of buyers, the power of suppliers, the threat of substitute products and services, and the threat of current competitors are "the five forces that shape industry competition" (Porter, 2008).

DuPont, above all, is a business striving to ensure financial gain and product success. Without proper protection of their ideas, other companies or research teams could easily replicate the necessary processes to derive their own benefits from DuPont's research, effectively leveling the competition barriers between companies and severely constricting the returns from DuPont's original investment. Proponents of business competition would argue that patenting drives the business world, edging businesses to strive for more cost-effective, durable, quality products.

A further stimulus for allowing the patenting of animals is that financial return has the potential to be reinvested and thus promote further research and development, boosting medical research. Without patent protection, these economic incentives would be non-existent, and the

biotechnology market would suffer. If patented animals prove to be profitable, more companies will fund this type of medical research. So as to the benefits of patenting transgenic animals, patent protection essentially motivates companies to create great new technologies and ensures their security as they are entitled to the rewards of their investments.

HINDRANCES OF ANIMAL PATENTABILITY

On the negative side to allowing animal patenting, entitling an idea to only one benefactor can make it difficult for others to utilize the product. In the Oncomouse case, the scientists at Harvard required that anyone looking to use the mouse must obtain a license to do so (Marshall, 2002). Thus, if researchers wish to use mice as cancer disease models, they have to either create a variation in DuPont's process to create a new model, or buy directly from DuPont. Financial strain can hinder research, especially if the animal is not readily available for shipment or if experiments are time-sensitive.

Attaining patents on animals could also act as a gateway for allowing human patents. The idea of patenting human life may initially seem ludicrous, however allowing patents on human cell lines could help create universal donor stem cell lines, or transplantable tissues and organs. Despite laws restricting the patenting of humans, a portion of a human, the human oncogene was placed inside Oncomouse, and the patent was allowed. Organs reside inside humans, and genetic engineering could be performed on them. Loopholes such as these could easily be exploited as patent grants become more available for establishing precedence.

Much resistance of the transgenic animal research field comes from animal rights groups such as People for the Ethical Treatment of Animals (PETA) and the Anti-Animal Vivisection Society (AAVS). These groups are morally opposed to the idea that any animal be created to be

used in research. More importantly, animals born with defects that cause them pain, such as the Superpig, or hinder their functionality such as the "Dry-Eyes" rabbit (born with hindered eyesight so that it can serve as a model for the dry-eye human condition), have to endure a miserable existence before being ultimately killed (Stopanimalpatents.org, 2011). The idea of animals being created merely for their own suffering is why many people advocate for the protection of animals so that none are created under such circumstances. This point is the driving force balancing the ethics of the needs of humanity versus the needs of the individual animal.

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Project Conclusions

In order to deepen our understanding of the controversial topic of transgenic animals, and form an opinion on whether to support it, it is first important to understand what exactly a transgenic animal is and how they are made. A transgenic animal is an organism containing foreign DNA inserted in its genome. It does not have to express the inserted DNA to be considered a transgenic animal, but the transgene must be expressed to give the animal a new phenotype or characteristic. Transgenic animals can and have already offered great advantages to the agricultural, industrial, and medical fields of science. However, this topic as a whole is considered very controversial as animal experimentation is viewed by some to be detrimental to the animal. Therefore, a thorough discussion of this topic must include transgenic ethics and legalities.

There are numerous methods for creating a transgenic animal. The two most widely used methods are pronuclear microinjection and embryonic stem cell manipulation. Each method first requires the creation of a transgene, or the piece of foreign DNA that will be inserted into the animal. A transgene consists of three main structural components, the promoter, the protein coding sequence, and a stop codon. The promoter helps signal in which tissue the foreign gene will be made in the animal, while the protein coding sequence dictates the new properties given to the animal. In the case of pronuclear injection, many copies of the transgene are directly injected into preferably the male pronucleus of a fertilized egg. Although pronuclear microinjection is a relatively reliable method of transgenesis, the desired gene integrates within the host DNA randomly. The integration site is critical in determining the transgene's expression, so its function can be impaired if the transgene integrates in an inactive area of the

chromosome, even though the transgene is present in the genome. Thus, pronuclear microinjection does not present the most efficient method of transgenesis. Embryonic stem cell manipulation involves inserting the transgene into the stem cells of a blastocyst. The transgene can be inserted via a virus, by microinjection, homologous recombination, or with the use of specific chemicals. Using some viruses (AAV-5) or homologous recombination allow integration site control, and the ES cells can be screened for positives before injecting them back into the blastocyst making this method more efficient. Both transgenic methods use a polymerase chain reaction (PCR), or a Southern blot analysis to screen the newly born offspring for successful integration of the desired gene. The advantage with embryonic stem cell manipulation is that the screening for the presence of the transgene can be performed to the cultured cells before re-implantation. This method is time consuming but has a greater efficiency.

Transgenic animals are divided into five categories: disease models, xeno-transplanters, transpharmers, food sources, and scientific models. Disease model animals are engineered to express the symptoms of a particular disease or illness for further scientific study. The Alzheimer's mouse for example, grants scientists the privilege of having an inexpensive and easily maintainable test subject for suspected treatments. Having this animal model available can help reduce the required time and cost of research for treatments. Xenotransplanters are used to grow valuable organs compatible for human transplantation for helping with the organ shortage. Transpharmers express a human drug in their milk, for example insulin or clot dissolver proteins. Food source animals are altered to grow bigger and more quickly to increase the efficiency of food production, to help drive down the price of food. Superfish are perhaps the most promising food source animal created to date.

The topic of transgenic animals receives a lot of criticism regarding the ethics behind the technology. Animal rights protection organizations such as PETA are constant opponents of animal experimentation. Although this organization states some valid arguments pertaining to the overall low efficiency of transgenesis, they often overlook the fact that not all transgenic animals endure a life of cruelty. When analyzed by individual categories, transgenic animals are not as unethical as opponents would like one to believe. Some disease model animals suffer the most pain, while the other divisions of transgenic animals do not appear to suffer at all.

It is concluded that the benefits to society that transgenic technology provides human beings justifies this experimentation on its own. When considering animal welfare, inhumane treatment of animals cannot be overlooked, and institutional Animal Care and Use Committees (IACUC) should continue to provide strong oversights to prevent cases of needless animal suffering. Instead of considering all transgenic animals to be unjustified, each type of altered animal should be considered separately. It is encouraging to see laws continually forming to provide strong oversight of this technology, creating situations in the laboratory which minimize animal detriment as much as possible.