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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

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ABSTRACT

This project examines transgenic animals in society today. The project details the methods of creating transgenic animals, explores the purpose and reasons they are created, and investigates the legal and ethical dilemmas that stem from this controversial practice. The particular use of each transgenic animal in science served as a prelude to a discussion of ethics. Views on the ethics of each type of transgenic animal were investigated, as well as the legal debates being waged regarding transgenic regulations. It is clear that the creation and use of transgenic animals, and the accompanying moral and legal debates, show the effect of technology on society. While it may continue to be a hotly contested, controversial practice, the benefits of transgenic animals are enormous and should continue long into the future. We believe that although animals are jeopardized for human benefit, these benefits are just to great to be ignored and transgenic technology should be allowed to continue.

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PROJECT OBJECTIVE

The objective of this IQP project is to inform the layreader about the topic of transgenic animals to allow a discussion of the controversial laws and ethics surrounding the topic. The report details what transgenic animals are, how they are created, and the uses of the various transgenic animals that have been created to date. Once this base of knowledge is established, the project goes on to discuss the ethics involved in animal experimentation and the laws that oversee their use. With all the complex aspects that transgenic technology encompasses, this has led to a plethora of misinformed opinions on the topic creating more controversy. This project attempts to inform the reader with information on how transgenic animals are made, used, and handled so that they are able to form their own opinion on the topic of transgenic technology.

CHAPTER 1: TRANSGENIC ANIMAL DESCRIPTION AND CONSTRUCTION

A transgenic animal is an animal that carries foreign DNA along with its original DNA. The foreign gene is inserted into the animal's genome by methods of recombinant DNA techniques. This allows the animals to have an altered DNA that will produce chemicals that it otherwise would not produce through natural means. This process can yield many benefits such as creating disease models for mimicking human disorders, to providing organ donors for transplantations, to serving as food, to enhancing animal milk that produces life saving pharmaceuticals in it. This chapter will discuss the methods for creating transgenic animals, and the assays used for their screening.

DNA

DNA, or deoxyribonucleic acid, is one of the two types of molecules that encode genetic information (RNA being the other). DNA is the material that stores and transfers genetic characteristics in all life forms, and is the primary component of chromosomes. DNA is a long double-stranded polymer that is held together by hydrogen bonds between base pairs of nucleotides (Figure-1). DNA is polymerized from monomer units called nucleotides, denoted as rungs in the ladder in the figure. These nucleotides are found in four different structures: adenine and guanine are purin bases; thymine and cytosine are pyrimidine bases. The adenine always pairs with thymine, and guanine always pairs with cytosine. The nucleotides form a paired structure which creates a double helix. The DNA is compacted with proteins, including histones, to make chromosomes (DNA, 2005).

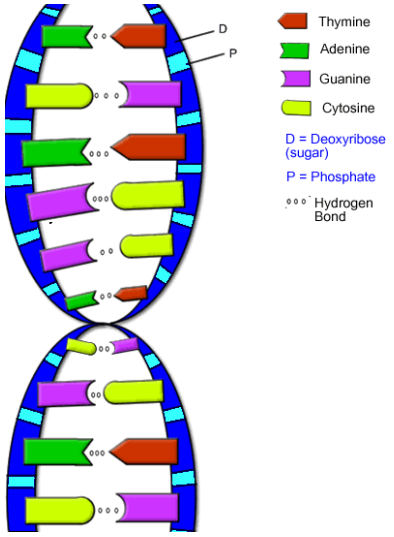

Figure-1: Diagram of DNA Double Helix Structure. This structure is formed by paired nucleotides, denoted by the rungs in the ladder. The nucleotides are represented by: Thymine –red, Adenine-green, Guanine-purple, and Cytosine-light green. (http://www.biologycorner.com/resources/DNA-colored.gif)

Genes are the basic hereditary factor in DNA. It is a segment of DNA that contributes to the organism's phenotype or function. Genes have distinct sequences of nucleotides that allow the DNA segment to produce its necessary proteins. The region of the gene that codes for the production of a protein are called exons. These exons contain codes that are used in specific portions for the completion of the protein. Introns are the parts of a gene that are initially transcribed into the primary RNA transcript along with the exons, but are subsequently removed to make the mature protein are called introns. The introns are removed when the exons are spliced together.

RECOMBINANT DNA TECHNOLOGY

Recombinant DNA technology was first developed in the early 1970's by Paul Berg, Herbert Boyer, and Stanley Cohen, when they were investigating ways to recombine the DNAs of microorganisms. In order to produce a transgenic animal, its DNA must be manipulated along

with foreign DNA. This method of genetic engineering, manipulation of DNA, is called recombinant DNA (rDNA). This transgenic methodology has now been applied to many other organisms such as fungi, mice, plants, pigs, sheep, fish, and even cows. Recombinant technology may be used to better understand human illnesses (as with Alzheimer's mouse) or even to have animals produce essential life saving drugs by inserting genes encoding pharmaceutical enzymes into an animal genome. This technology is the first step in cloning genes.

Recombinant DNA technology allows for short specific portions of DNA to be inserted into a vector which aids the DNA amplification. Plasmids are commonly used as vectors since they are easy to manipulate. Plasmids are molecules of DNA that are found in bacteria but occur separately from the bacterial chromosome (Figure-2). Plasmids are small, circular, and usually carry only few genes. The inserted DNA (shown as blue in the figure) replicates in the plasmid's cytoplasm, so a large number of DNA copies are created. The DNA may then be purified from the bacteria, and inserted into the host animal.

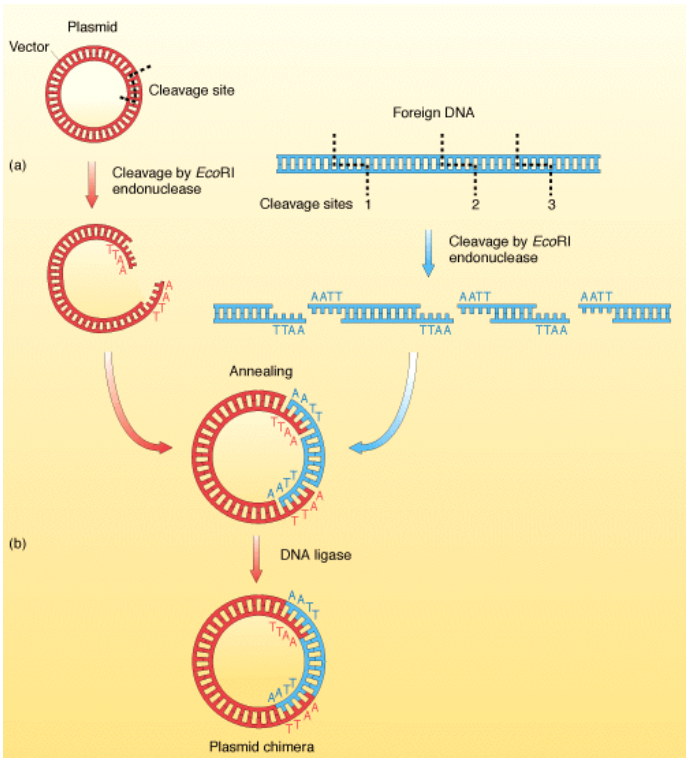

Figure-2: Diagram of DNA Cloning into a Plasmid. Both plasmid DNA and the foreign DNA to be inserted into it are cut with restriction enzymes (upper part of diagram) to create complementary "sticky ends". The cut foreign and plasmid DNAs are then mixed (diagram center), and the foreign gene inserts in the proper orientation. The joined DNAs are then treated with DNA ligase to seal the circularized the DNA into a functional plasmid (lower). (Introduction to Cloning and Biotechnology, 2005)

Restriction enzymes are used to excise a piece of human DNA encoding the transgene (upper right in the figure). The restriction enzymes cut the DNA at specific sequences, and this allows the DNA to be inserted at the proper position in the vector. A ligase enzyme is then used to seal the two pieces of DNA together, creating the recombinant DNA molecule. To better understand this process, think of the restriction enzymes as enzymatic scissors for cutting the DNA, and the ligase enzyme as glue to hold the mixed DNAs in place.

METHODS FOR CREATING TRANSGENIC ANIMALS

Microinjection of DNA into the Male Pronucleus

The method of DNA microinjection into the male pronucleus was the first successful transgenic technique utilized in mammals (mice in this case) (Wortman, 2000). Soon after, other species proved successful such as rats, cows, pigs, chickens, fish, birds, goats, and sheep. One of the benefits that contributes to the popularity of this microinjectionn technique is the variety of animals this can be applied to. Due to its success and reliability, pronuclear microinjection has become the most popular technique for making transgenic animals.

These transgenic animals are made by cloning the transgene of interest, and then inserting that gene into the genome of a newly fertilized egg. To do this, foreign DNA is inserted into an egg by using a microsyringe. The male pronucleus is usually used due to its larger size than the female pronucleus. The eggs are matured using hormone injections to increase amount of

ovulation in a group of animals. Once the eggs are harvested, they are injected with hundreds of copies of the desired DNA using a micropipette (Figure-3) (Transgenic Animals 2003).

Figure-3: Microinjection of DNA into a Male Pronucleus. The glass micropipette (on the right) is inserting the transgene into the egg. The device on the left is a microtube suction device that holds the egg in place. (http://www.medecine.unige.ch/transgenese/microinj.jpg)

When the microinjection procedure is successful, the animal will have the altered DNA in every cell of its body. But this procedure is not always successful (Table 1), the larger the species, the less efficient the process. The process of DNA integration into host DNA is still very random. Where the transgene integrates into the host genome cannot be controlled, or sometimes the DNA doesn't integrate at all. In some cases, only some of the animal's cells have the new DNA sequence, called a "mosaic animal". The offspring of a "mosaic animal" will sometimes carry the gene and other times not.

Animal Species	Number of Ova Injected	Number of Offspring	Number of Transgenic Offspring
rabbit	1907	218 (11.4%)	28 (1.5%)
sheep	1032	73 (7.1%)	1 (0.1%)
Pig	2035	192 (9.4%)	20 (1.0%)

Table 1: The Low Eficiency of the Microinjection Method. Figures in parentheses denote percent efficiency compared to original number of ova injected. (Transgenic Animals and Plants, 2005)

Microinjection of DNA into ES Cells

A second technique for making transgenic animals places the transgene into embryonic stem (ES) cells. These ES cells are isolated from the inner cell mass of blastocysts prepared by in vitro fertilization (IVF). ES cells are undifferentiated, which means they have not yet generated more specialized structures or protein characteristics. ES cells are able to renew themselves, and later develop into almost all major specialized cell types. So, if the transgene can be inserted into an ES cell, then that cell can be microinjected into a blastocyst, to create a transgenic embryo, which can be implanted into the host uterus to create a transgenic animal (Stem Cell Basics, 2006).

The first step in this technique is to perform *in vitro* fertilization and culture the embryo for 5-6 days to obtain a blastocyst. ES cells are then obtained from the blastocyst (Figure-4). To help prevent differentiation of the ES cells, the cells often co-cultured with an embryonic fibroblast feeder layer that produces leukemia inhibitory factor. The inner cell mass of the harvested embryos is retrieved from the blastocyst, co-cultured, and selected for those cells showing undifferentiated morphology to indicate their likelihood of pluripotency. Then ES cells are allowed to grow and multiply. Foreign DNA is introduced into the ES cells using any of a variety of techniques, such as electroporation, microinjection, viruses, or chemical transfection. This ES technique has been growing in popularity because of the variety of techniques that can be used for incorporating the transgene into ES cells.

Electroporation is a process that uses a pulse of high voltage to make cell membranes permeable to introduce new DNA into the ES cells. An electric charge passes through a plate consisting of DNA layered on top of cells. DNA contains phosphate residues that cause the DNA

to have a negative charge, and this is attracted to positive electrodes. As soon as the DNA is absorbed into the cell, the DNA moves into the cytoplasm, and eventually enters the nucleus to integrate into the nuclear DNA (Taconic, 2003). The cells become immortal ES cell lines that can be grown to large quantities to be evaluated for transgene incorporation. Due to evaluation process, only ES cells that actively took up DNA are reimplanted. This process allows for higher efficiency of the process. The ES cells can now be injected into a blastocyst, and the embryo implanted to make the transgenic animal.

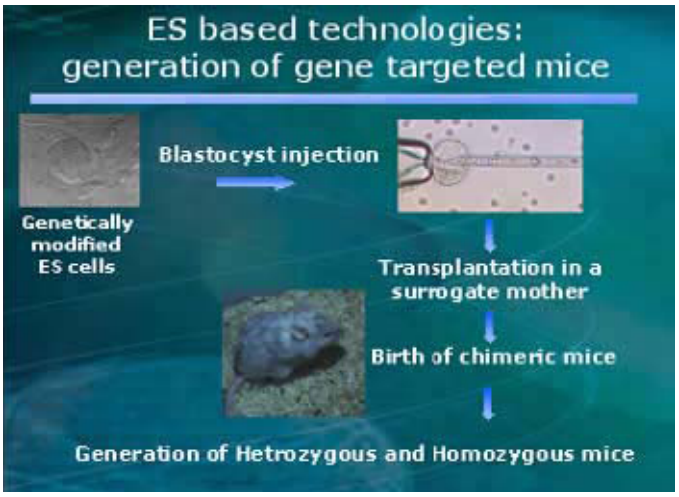

Figure-4: Summary of the ES Method for Making a Transgenic Animal. Cultured ES cells are transfected with the cloned transgene (upper left). Cells are then injected into a blastocyst (upper right). The blastocyst is implanted into a foster mother to produce transgenic pups. (Genoway, 2003)

This technique may be highly effective, but ES cell culturing is still very difficult. The survival rate of ES-injected blastocysts is low. And there usually is no way to control the location of where the DNA integrates, except with specific types of viruses. It is also hard to determine whether the implantation of the embryo into the uterus will be successful. According to tests, no more than one third of embryos will have successful implantation into the uterus (Transgenic Animals, 2003).

DNA Homologous Recombination

An advantage of using ES cells to create transgenic animals is the use of homologous recombination. Homologous recombination, or gene targeting, is a method for inserting the DNA that has control over where it integrates in the host DNA. The DNA of the transgene attaches to a known portion of the host's chromosome and then exchanges with it (Figure-5). Through genetic engineering, the transgene is inserted within the known cloned host gene. After the host cell incorporates the DNA, the flanking host DNA sequences recombine with their homologous sites in the host cellular DNA to incorporate the transgene into that location. The DNA is targeted to a specific location of the genome through this method (Bronson and Smithies, 1994).

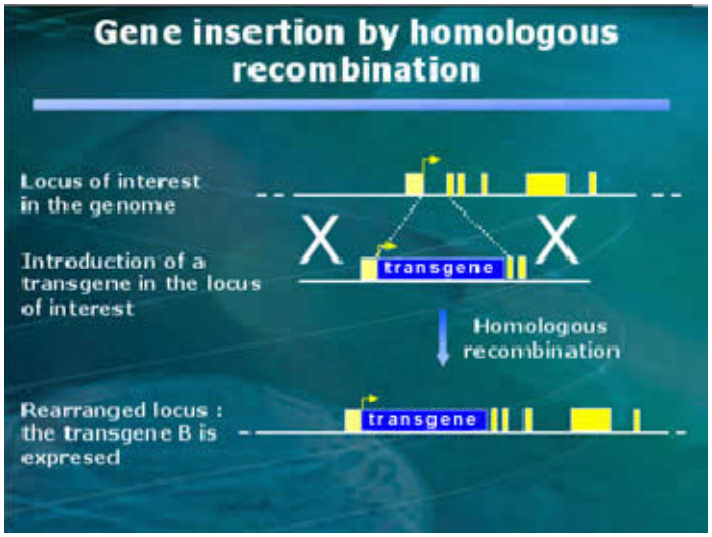

Figure-5: Summary of the Homologous Recombination Method for Making Transgenic Animals.
The transgene (blue) is inserted within a cloned host gene (middle). When the DNA is introduced into a host ES cell, the DNA flanking the transgene recombines with host cell DNA to insert the transgene into a specific chromosomal location (bottom). (Genoway, 2003)

DNA Viral Delivery

Viruses can also be used as a method for inserting foreign DNA into the ES cells. A retrovirus can be modified so that it carries the desired DNA sequence within the virus' DNA. The disease causing genes in the virus are removed so they are not introduced into the transgenic animal. This allows the virus to infect the ES cells with a specific gene, but not cause disease. The germ line cells must be infected for this method to be successful.

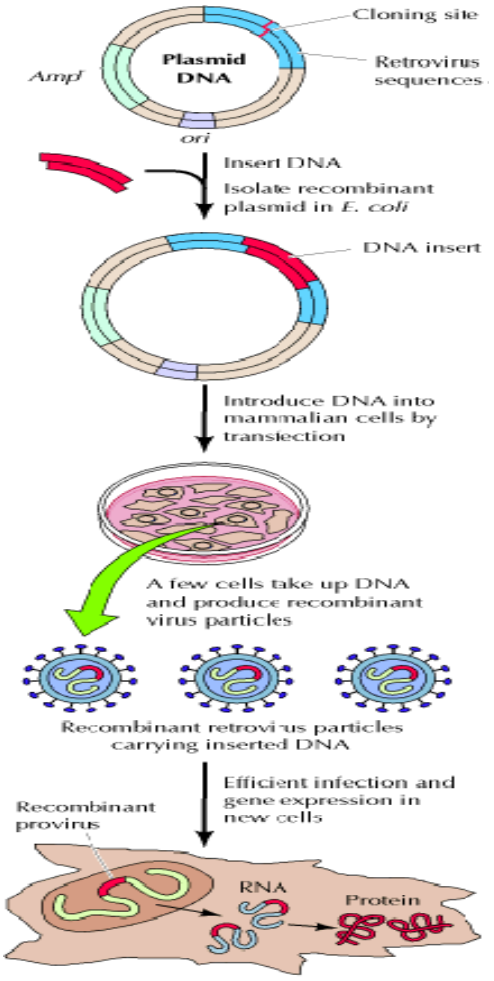

Figure-6: DNA Viral Delivery Method. Viruses can be used to deliver the transgene, and the genes causing the disease can be removed so the transgenic animals will not be introduced to them. (Cooper et al, 2000).

The method of DNA viral delivery is effective, but not as efficient as desired. The size of the transgene sequence that can be added to the viral genome is limited. This method may yield mosaic animals, or interference with the expression of the transgene may occur. Animals created through this method often do not pass the transgene to their offspring. The offspring will receive a copy of the transgene only if the germ cells receive a copy of the transgene.

Somatic Cell Nuclear Transfer

The Somatic cell nuclear transfer (SCNT) is considered more efficient and safe when compared to the large number of embryos that are saved. During the process of SCNT, a nucleus is taken from a somatic cell and inserted with a transgene by use of microinjection. The nucleus then becomes reimplanted in an enucleated egg. Several days later the egg develops into a blastocyst, and is then implanted into a foster mother. Because the newly implanted nucleus already has the transgene in its genome, the offspring is certain to be a transgenic animal.

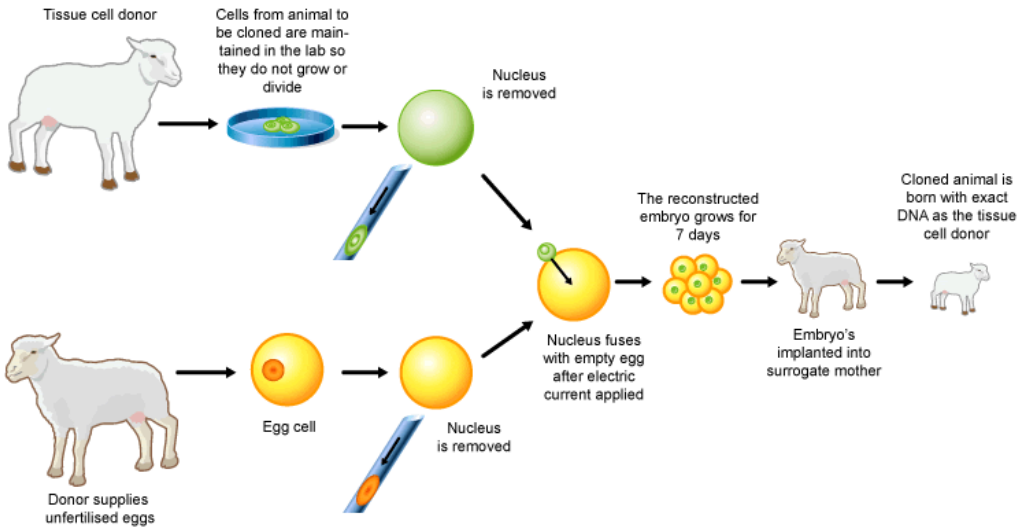

Figure-7: Summary of the SCNT Method for Making a Transgenic Animal. Skin cells are removed from donor animal (upper left). The nucleus extracted (upper center) and injected with transgene. The nucleus is injected into an enucleated egg (center) and that is implanted into a surrogate mother (Kae, 2003). (Picture from http://www.biotechnologyonline.gov.au/images/contentpages/scnt.gif)

ASSAYS FOR SCREENING TRANSGENIC ANIMALS

Southern Blot Test

The Southern Blot test is a way to analyze the genetic patterns within DNA. This technique is utilized in the transgenic offspring before it passes six weeks of age. This test can be used in several ways such as to determine the number of copies of the transgene integrated, the number of chromosomal sites the transgene was inserted into, to validate the status of the transgene, and to determine if the transgene was intact. When scientists select which transgenic animals are to be used for breeding, they usually look for the transgenic animals that have at least five to ten copies of an intact transgene in a single insertion site (Brinton and Lieberman, 2007).

Transgenes usually insert in a head-to-tail position. Due to this, scientists carefully choose a restriction enzyme that will cut once in a transgene to release DNA fragments that are the same in size as the transgenes from the multicopy concatemer. Through electrophoresis, these DNA fragments are sorted by size. The DNA is then loaded on a gel (agarose) and an electrical charge is then passed through the gel. The DNA is attracted toward the positive electrode since it is slightly negative. The smaller fragments move quicker to the bottom, leaving the larger fragments toward the top. The DNA is brought to single stranded form by heating or

chemically treating the DNA in the gel. The DNA is now free to be hybridized with a probe DNA. The DNA in the gel is blotted to a membrane that retains the original DNA size pattern, while still allowing the hybridization probe to illuminate the transgene. The presence of a band hybridizing to the transgene probe indicates the transgene incorporated into the host DNA (Brinton and Lieberman, 2007).

Western Blot Test

Another method for screening transgenic animals is the Western Blot test. Proteins made from the transgene are detected using antibodies directed against the transgene protein. This is similar to the Southern Blot test except protein extracts are electrophoresed and blotted to the membrane instead of DNA, and then the antibody detects the trans-protein on the blot. When the trans-protein is being made in the cells from which the lysate was made, then it will be represented by a band on the gel (Western Blot Activity, 1998).

Enzyme Linked Immunoabsorbent Assay (ELISA)

ELISA is another technique for detecting the presence of transgenic proteins. The ELISA technique and Western Blot test both measure the cellular levels of a specific trans-protein. The Elisa technique is more quantitative. This technique is used to measure the amount of trans-protein found in sample of blood, urine, and animal serums. The first step uses a plastic tray with wells. These wells are coated with a particular antibody that binds to transprotein present in the added lysate. After washing un-bound protein out of the well, a detecting antibody is then added

to detect the captured trans-protein. The greater the concentration of transprotein present in the lysate, the greater the colored signal in the well (ELISA Activity, 1998).

Real Time RT-PCR

Real Time RT(Reverse Transcriptase)-PCR is used to screen for the expression of the transgene. This technique detects mRNA encoding the trans-protein. A tissue (brain, pancreas, liver) is removed and the mRNA in the cytoplasm is extracted. Reverse transcription is performed to synthesize DNA from the mRNA. The DNA is then amplified using a specific set of primers designed to amplify the trans-gene using fluorescent primers. The mRNA that was converted into cDNA is visualized by increased fluorescence into polymer. The Real Time PCR method is useful in proving the expression of the transgene and proves that the DNA sequence of the transgene is being transcribed and expressed (Hunt, 2006).

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Chapter 2: Transgenic Animal Classifications and Examples

This chapter will show examples of each class of transgenic animal, focusing on their benefit to society as an introduction to a discussion of transgenic ethics in Chapter-3. Before we can discuss the balance between benefit to society versus detriment to the animal, we need to have an understanding as to why these animals were made. These animals were created as a way to provide better medicine and science, better sources of medicines, alternative source of transplant organs, or alternative food sources. Transgenic animals can roughly be divided into five classes: Disease Models, Transpharmers, Xenoplanters, Food Sources, and Scientific Models.

Disease Models

Transgenic disease models are animals that have been genetically altered and manipulated to acquire a particular human disease, or to acquire a small portion of a human disease. This is done so that scientists may study the disease without jeopardizing human life. The study of these animals can lead to a better understanding of how each condition develops and progresses and, through testing, can possibly lead to a cure. Since animals and humans are not identical, it is necessary to alter the subjects through the use of a transplanted gene that stimulates the onset of a human disease. This manipulation allows the disease in the animal to progress similarly to the way it would in humans. Once this process is completed, the animal can be observed and tested under conditions that could be harmful to humans. Once a successful therapeutic agent has been identified, that drug is then tested in human cells *in vitro*, then in human clinical trials. However, there are still many ethical dilemmas that go along with creating

diseases in transgenic animals. Examples of transgenic disease models are Huntington's Monkey, AIDS mouse, Alzheimer's mouse, and Parkinson's fly

Huntington's Monkey

The most recent breakthrough in transgenic disease models is the Huntington's monkey. Huntington's disease is an inherited defective gene that can trigger certain nerve cells in the brain to die. It affects five to ten people in every 100,000, and it is fatal with death normally occurring 15 to 20 years after onset. The research team which initially developed the macaque monkey model did so by introducing altered forms of the Huntington gene into macaque eggs with a viral vector. The altered eggs were then fertilized and the embryos were implanted into a surrogate primate mother. The result was the birth of 5 live monkeys, providing the world's first "rhesus macaque model of a specific human disease using transgenic technologies, and providing a marked improvement over the previous mouse models" (Researchers Develop…, 2008). Because the rhesus macaque monkeys come much closer to resembling human make up than mice, these models will help scientists to understand more about this disease in humans.

AIDS Mouse

While monkeys are sometimes used as disease models due to their close physiology with humans, they are rather expensive to purchase and maintain. Mice are very popular for transgenic experiments due to their ease of experimental manipulation, and short generation times, and in 2004 researchers developed a very useful mouse called AIDS mouse. Like the Huntington's monkey, researchers injected newly fertilized mouse eggs with the AIDS provirus DNA. The manipulated eggs were then removed and implanted into a surrogate female mouse (Bunce and Hunt, 2004). AIDS is considered to be the greatest epidemic the world has ever seen and these mice are helping to find a cure for the disease. HIV, Human Immunodeficiency Virus, attacks human cells presenting CD4 and chemokine receptors on their

surfaces. The virus maneuvers itself inside these cells at which point it begins to replicate and, when CD4-T-cell levels drop dramatically, the disease becomes AIDS (Bunce and Hunt, 2004). AIDS disease is characterized by malignancies, problems in lymph node tissues, skin lesions, and cellular and immune irregularities (Kohn, 2001). There is no current cure for those with HIV/AIDS, however, with proper treatment, infected persons can slow progression of the virus.

Alzheimer's Mouse

Created in part here, at WPI (Games et al., 1995), this transgenic animal mimics the symptoms of an Alzheimer's patient. Alzheimer's disease (AD) is a neurological disorder affecting memory. AD is associated with highly neurotoxic β-amyloid synthesis and deposits in the hippocampus and cerebral cortex. For most patients, the onset of AD occurs late in life, in the mid-70's. However, some people have DNA mutations in the amyloid precursor protein (APP) gene which accelerate the production of toxic β-amyloid, so families with this mutation often get early onset AD in their mid-40's. Over time, these β-amyloid proteins aggregate senile plaques, one of the pathological hallmarks of AD.

The world's first Alzheimer's mouse (Games et al., 1995) was engineered to contain an APP mutation that mimics an Indiana family with early onset AD. The production of toxic β-amyloid occurred in the same areas of their brains as with AD patients, and its production caused neurodegeneration (Duff et al, 1996). This mouse model has since been used by Elan Pharmaceuticals to create the first AD vaccine, an antibody against β-amyloid (Schenk et al., 1999). The vaccine in mice proved very effective, virtually eliminating the creation of newly formed amyloid plaques in young mice and reducing mature plaques in older mice (Schenk et al, 1999). However,when Elan moved on to human clinical trials, the vaccine was effective at

removing β-amyloid, but "autopsies on seven patients who died of Alzheimer's during the study showed that nearly all of the sticky β-amyloid protein…had been removed, but all patients still had severe dementia" (Alzheimer's vaccine…2008). Elan has recently initiated a second clinical trial with a slightly different vaccine that, through clinical Phase-II, appears safe.

Parkinson's Fly

Parkinson's disease (PD) is a neurodegenerative disorder that affects the central nervous system, especially the substantia nigra which is an area of the brain that produces dopamine. Symptoms of the disease usually include tremor, muscular rigidity, and slowed physical movement. In 1997, scientists found the first genetic link to the disease, a mutation in the α-synuclein gene which may lead to a lack of dopamine (Vogel, 2000). The ability to monitor the α-synuclein gene in fruit flies has given scientists the ability to monitor the disease throughout its course, whereas in humans symptoms are not usually visible until approximately 60-80 percent of dopamine nerve cells have become damaged or destroyed (Vatalaro, 2000).

Scientists at Harvard Medical School have created three types of transgenic flies that express different versions of the human α-synuclein gene. "In all three strains, the dopamine-producing neurons--the same ones that die in human Parkinson's disease--die off in adult flies... In addition, the neurons form… abnormal accumulations of protein that include high levels of the α-synuclein protein" (Vogel, 2000). The death of the dopamine neurons leads to the flies' inability to climb walls, whereas their normal counterparts have no problem with this feat.

The ability to reproduce the symptoms of Parkinson's disease in flies is not only a great way to study the disease, but it is also extremely cost effective. This model, although seemingly

very different from mankind, could be a very good way of finding a cure for this degenerative disease in humans.

Transpharmers

Transpharmers are genetically engineered animals that produce specidic compounds in their milk, saliva, urine, or blood. While expression in any of these four locations can be achieved, it is most commonly engineered for expression in milk. The idea is to specifically mutate the DNA of the animal so that it secretes a pharmaceutical drug "naturally" into the milk. This type of transgenic animal is probably the least debated genetic altering done to animals because there is no harm done to the animal, its milk is simply harvested to obtain the drug. Normally, scientists first test the method on mice before moving to larger farm animals, since the process is technically more difficult in larger animals. This is to ensure that the process is successful before moving on to the more expensive, more difficult livestock. Transpharming has already been successful, yielding several useful pharmaceutical compounds and proteins in cows, pigs, sheep, and chickens (Gillespie, 2005).

One such example is named Herman the Bull. This bull was created by microinjecting cells with the gene encoding lactoferrin during the early embryo stage. Once cultured, these manipulated embryos were transplanted into recipient cows. The only transgenic calf was Herman, a male. Herman was subsequently bred and his female offspring transpharmed lactoferrin in their milk (Biotech Notes, 1994). This process could result in the production and sale of an alternative version of milk. Lactoferrin is an iron-containing protein that is necessary for the growth of infants. By adding this to milk, it is now possible to create a milk that is suitable for infant growth. It was thought that the new protein-enhanced milk could help to feed

infants in developing nations. Unfortunately, Herman's offspring, while producing lactoferrin in their milk, did not produce enough of it for the milk to be commercially worthwhile, and the product never made it to market. While this lactoferrin example did not pan out, the same technology can be used to transpharm other proteins that can be purified and possibly marketed.

Another example of a transpharmer came in 1997 when scientists at the Roslin Institute generated six transpharmer sheep. Each of these sheep secreted a blood clotting agent in their milk that is found in humans (Schneike et al, 1997). In 1999, scientists created another form of tranpharmer goat which expressed high levels of antithrobin III, a chemical that acts as an anticoagulant in humans. These goats were created using a new process known as SCNT, somatic cell nuclear transfer (Figure-1). In this process, a nucleus of an adult somatic cell is removed and transplanted into an enucleated egg. The egg containing the somatic cell is then stimulated to divide, and it is grown to the blastocyst stage then implanted into a surrogate mother ("Somatic cell…", 2002).

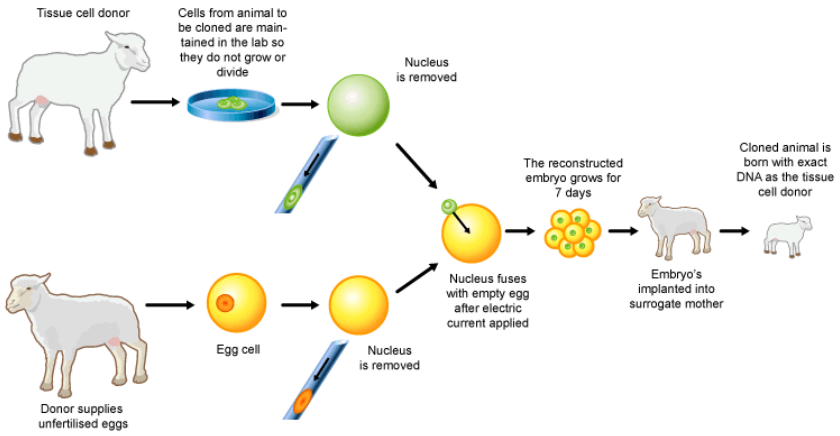

Figure 1: Diagram of Somatic Cell Nuclear Transfer. Tissue is donated from an adult (upper left). The nucleus is extracted and injected (center) into an enucleated egg (lower center). The embryo is grown to the blastocyst stage, then implanted (Diagram right). From: www.biotechnologyonline.gov.au/.../img_scnt.cfm

While this SCNT procedure provides a way of creating transgenic animals from adult nuclei, the process has a low success rate. Regardless, creating transpharmers is a non-painful way to create a desired pharmaceutical in an animal that can continue to produce it at low cost.

Xenoplanters

Xenoplanters, or Xenotransplanters, are animals that have been genetically modified so their organs may be successfully transplanted into humans. The act of harvesting an organ from one species and implanting it into a different species is called a xenotransplant. Although this process normally causes immunorejection of the organ, xenoplanters are engineered so they do not express key foreign antigens to avoid causing immunorejection. Due to our current lack of organ donors, xenotransplantation could develop into a great way for humans to receive donor organs to last them until human organs become available.

However, not all of the problems have been worked out, and most xenotransplants have failed. Yet there is still much hope surrounding this technology. Several xenotransplants have been attempted with varying response, mostly negative. In 1984, doctors at the Loma Linda University Medical Center in California attempted a xenotransplant that involved transplanting the heart of a baboon into an infant child. Baby Fae, as she became known (Figure-2), was born with a fatal heart defect known as hypoplastic left heart. At first the transplant looked to be a success, and doctors were pleased with the progress she was making, but two weeks after the implant, heart complications arose and the infant's body began to reject the heart. Baby Fae died three weeks after receiving the heart (Wallis, 1984).

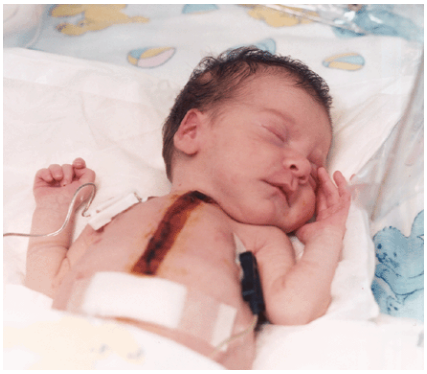

Figure 2: Picture of Baby Fae After Receiving her Xenotransplanted Heart from a Female Baboon. Credit: Loma Linda University Medical Center. From: http://www.sciencemag.org/cgi/content/full/295/5557/1008/F1

Other examples involving the transplant of primate organs into humans have occurred, but no person receiving a xenotransplant has lived for more than 9 months. Although these results do not have the type of outcomes scientists and doctors strive for, they are huge steps in the right direction for the process of xenotransplantation.

More recently, scientists have turned to pigs as the main source of xenotransplants. While primates have very similar genomes to humans, size differences limit the number of patients who can receive the organs. Because a pig's physiology is quite similar to that of our own, researchers have attempted to figure ways to engineer them so successful xenotransplants can occur. The problem that exists with pig organs is the hyperacute rejection that occurs when human antibodies come in contact with the surface of the porcine organ cells (Couzin, 2002). A gene in pigs codes for an enzyme known as alpha-1, 3-galactosyltransferase which is an enzyme that adds the sugar alpha1, 3-galactose to the cell surface. When human antibodies come in contact with these sugars, which riddle the surface of pigs' cells, it is viewed as foreign, leading to hyperacute rejection (Pearson, 2003). In order to avoid this, scientists have experimented with avoiding the entire transplant of the organ and attempted to use individual porcine cells to treat

the patient intravenously. At several hospitals in countries around the world, doctors have taken patients with acute liver disease and hooked them up a machine that treats their blood with porcine liver cells. Circe Biomedical of Lexington, Massachusetts conducted a study of 171 of these patients. While the procedure did not result in a cure, it did prolong the survival of a majority of patients, giving each more time to find a human donor (Couzin, 2002).

Although physical xenotransplantation of animal organs has not been nearly perfected, there is hope that it may one day be a viable option to patients in need. The use of porcine cells, however, does seem to be a procedure that could help to save lives. With time we will determine whether or not xenotransplantation of organs can be successful, but until then, animal cell therapy seems to be the best option when a human donor cannot be found.

Food Sources

This classification of transgenic animal is used, as its name implies, as a food source for humans. These animals are genetically altered to increase the food supply by maturing in less time and using fewer resources to achieve larger growth. An example of this is when a growth hormone is applied to a particular animal's genome. This results in a "super" animal that has several advantages over the same animal without the applied hormone. Examples of this are "Superfish" and "Flavr Savr".

Superfish

In Vancouver, British Columbia, scientists at the federal Fisheries and Oceans Canada research lab have been experimenting with salmon. They have taken normal coho salmon and inserted an extra growth hormone gene from the sockeye salmon. These coho salmon (Figure-3)

grow approximately four times faster than coho salmon without the inserted gene. By microinjection of the sockeye growth hormone gene into a newly fertilized coho salmon egg, a coho fish containing the combined genes is produced. In this case, the result is a faster growing, larger fish (Clarren, 2003).

Figure 3: Photo of wild coho salmon (left) compared to transgenic coho salmon (right). From http://www.hcn.org/issues/253/14058

First attempted in the late 1980's and early 1990's, by A/F Protein Inc. of Massachusetts, the superfish technique was used to create fast-growing fish to counteract the dwindling number of wild cod and salmon on the East Coast. Since then, the practice has become rather commonplace, but not without controversy. Because wild salmon have natural instinct that are lacking in the pen-raised, transgenic salmon, there are issues with the problem of breeding between the two. In a computer model created at Purdue University, researchers found that 60 transgenic fish released into a population of 60,000 wild fish, would take just 40 fish generations for the the wild species to become extinct (Clarren, 2003). This could be a very grave outcome but, if properly monitored, these transgenic fish could become a staple of every person's diet, also helping to feed those who lack food elsewhere in the world.

Flavr Savr

This transgenic food source, known as the Flavr Savr was one of the first transgenic foods to be FDA approved. Flavr Savr is aptly named because it is a tomato that ripens slower than conventional tomatoes to stay on the vine longer. It was created through a process known as antisense technology, in which RNA complementary to the mRNA that encodes a specific gene is expressed in a cell. The complementary RNA binds to the mRNA inactivating it, thus reducing the amount of protein it encodes. This procedure was used to knock down expression of the enzyme polygalacturonase which is necessary for the synthesis of ethylene. Ethylene breaks down pectin to initiate ripening in normal tomatoes. Producing less polygalacturonase leads to a slower ripening. In the case of the Flavr Savr, it can be picked from the vine red and remain that way for several weeks until it starts to go bad (Krimsky and Murphy, 2002). The Flavr Savr tomato was the first genetically modified whole food to be marketed to consumers in the United States.

Biological Models

Transgenic biological models are genetically engineered to teach us something about the function of a particular protein *in vivo*. Specific proteins can either be over-expressed or under-expressed (knocked out), and its effects observed on multiple systems in the body. Having already taught us a great deal about genetics and biology, the goal of these models is to continue this expansion of knowledge through the use of transgenic scientific models. Two such examples are Smart mouse and ANDi.

Smart Mouse

In 1999, researchers at Princeton University genetically engineered a mouse to over-express gene NR2B. This gene is essential to the brain's ability to associate one event to another, a basic feature of learning, especially in young animals. When studying mice that lacked the NR2B gene, researchers found that these mice had learning impairments and poor memory. NR2B has proved to be so essential because it is a key subunit of the NMDA receptor. NMDA acts as a receptor in the brain and is an excellent tool for creating memory. While experimenting, researchers not only gave mice extra NR2B genes but they engineered them to increase NR2B activity with age. This resulted in mice with a far greater learning response than those without the extra gene, so they were called Smart Mice. Moreover, the mice with the NR2B gene retained brain activity normally specific to adolescent mice even after the Smart Mice aged (Harmon, 1999). This finding helps prove the hypothesis that NR2B protein functions to provide a more efficient firing of the NMDA receptor, as occurs naturally in young animals. "The finding also shows that genetic improvement of intelligence and memory in mammals is now feasible, thus offering a striking example of how genetic technology may affect mankind and society in the next century" (Harmon, 1999). To know that genetic improvement is possible in animals is a huge breakthrough and it is certain that much will be done in an attempt to convey similar results in humans.

ANDi

In an attempt to create the first transgenic primate, researchers at the Oregon Regional Primate Research Center injected a genetically modified virus into an unfertilized rhesus monkey egg. The egg was then fertilized and implanted into a surrogate mother. Although this was done

to several eggs at the same time, many of which failed, it did result in one success. ANDi, which stands for inserted DNA spelled backwards, was born with the foreign gene encoding green fluorescent protein (GFP). While gene transfer is rather commonplace in other species, this is the first demonstration that the manipulation of a primate egg can result in a successful birth. The GFP, extracted from jelly fish, is present in ANDi cells, but does not have the same affect that it does on jelly fish (Vogel, 2001).

Figure 4: ANDi, the first transgenic primate contains the green fluorescent protein. From: http://www.sciencemag.org/cgi/content/full/291/5502/226a/F1

While the idea of a green monkey is rather useless, the fact that scientist have been able to create a genetically altered primate is fantastic, as it opens the door for testing the function of other specific proteins in a model similar to humans. Although smaller subjects with less compatible physiology such as mice and rats are useful, primates, unlike mice, are able to fit in magnetic resonance imaging machines. This could lead to researchers using such machines to track organ development without hurting any primate. Ideally, transgenic primates, with their similar physiology to humans, could do great things for the advancement of science and medicine.

Chapter Conclusion

This chapter attempts to provide a background on the success of transgenic research to allow subsequent comparisons of their benefit to society versus detriment to the animal in Chapter-3. Although it does not go into every aspect of transgenic animal research, it is meant to form a basic understanding on the topic of transgenics and the main categories of animals formed to date. While still hotly debated, transgenic animals have led to a far greater understanding of diseases and medical conditions. The medical wonders that have developed from xenoplanters, disease models, and transpharmers could lead to cures for countless diseases. Food source transgenic animals could help feed those in need. And biological transgenic models are helping us learn more about the functions of specific proteins *in vivo*. If practiced humanly, transgenic animals could have an even more profound effect on human life.

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Chapter-3: Transgenic Ethics

In today's world, as discussed in Chapter-2, transgenic animals have the ability to contribute to scientific and medical advancement in a big way. However, their benefit to society is not the only thing to consider with this new technology; ethical and moral issues arise with the creation and modification of life. In an age where scientists' capabilities to manipulate life seem endless, it is important to decide what boundaries will be set. In order to do this, we must carefully weigh all the advantages and disadvantages of this technology. We must take into account the concerns of many different public sources, including different cultural and religious backgrounds. We must also take into account which types of these new animals would be acceptable with regards to ethics, requiring a discussion of the amount the animal suffers versus the medical benefits gained. In some cases there is barely any animal suffering with great benefit to society, while in others the suffering is severe. So where do we draw the line?

Concerns of Transgenics

Probably the biggest concern would be the treatment of the animals. How much do the animals suffer during these experiments? Are scientists making sure that they suffer as little as is needed to carry out the research, or are they treating them as objects and not drawing the line at any particular point? Then there come the cries that scientists have no right to do the research in the first place. Many animal rights groups and environmentalists believe that it is wrong to alter and potentially harm any animal's genetics. Then there is also the concern of where transgenics stops. Will transgenics carry over to humans? Will we begin to alter the genes of humans and

ultimately end up playing God? Some would say that it's not about playing God but creating better life for people. Humans have always made engineering advancements with the intention of improving life. Isn't this just another one of those instances?

Why all of a sudden is there an out cry from the public that transgenics is unethical; that it is a way for scientists to play God. But, hasn't similar events been taking place for many years now. Take the farming industry for example. Farmers have been using breeding techniques for generations in order to grow stronger animals faster than ever before. The faster these animals are grown, the more food there is to feed the world. Yet, this is not considered wrong and transgenics is. Isn't transgenics just picking up where selective breeding let off? If this is true, then why aren't both considered unethical?

As previously stated, the largest concern from the public is the treatment of the animals. Many are against any kind of suffering that may occur, but not everyone has the same ethical views. Most animal rights groups tend to have a Kantian point of view, in which they believe that an action can only be right or wrong in and of itself. In this case, our actions of making an animal suffer, by itself as an action is considered wrong. On the other hand, scientists and researchers adopt a utilitarian point of view in which the action can be considered good or bad based on the results which it produces. Therefore, it is not necessarily wrong for an animal to suffer, as long as the amount of human suffering the research stops outweighs the amount of suffering which occurred during the research. A perfect example of this would be AIDS mouse. The research done on these mice has helped scientist learn more about a disease that has killed countless people. How can the sacrifice of these mice have been considered immoral?

Benefits of Transgenics

People can scream immoral all they want, but they must take into account all the societal benefits of transgenics. These benefits are seen in the food industries, as well as the medical world. Medically, transgenics is working wonders. Animals such as xenotransplanters, AIDs mouse, Alzheimer's mouse, smart mouse, youth mouse, and oncomouse all make huge contributions to the medical field. Xenotransplanters are helping bring about the technology for transplanting animal organs into humans. The disease mice are helping to find a cure for AIDs, Alzheimer's, and certain cancers. The smart and youth mice are helping scientists make human improvements in the areas of memory and heart disease.

In the world of agriculture, benefits are seen with the creation of transgenic plants that are disease resistant, and some transgenic animal food sources (especially superfish) that can be grown larger and faster on less food, making more food readily available for the world. All of these advantages of transgenics must be talent into account before condemning it.

Oncomouse

Oncomouse is a perfect example of how close to the ethical line a transgenic animal can come. This is one of the most widely disputed cases. As discussed in Chapter-2, this mouse was created in the early 1980's when Harvard medical researchers genetically modified a mouse to include a human oncogene, which can cause the growth of tumors. This mouse was going to allow them to greatly increase their knowledge of cancer, so Harvard and Dupont decided to get a patent for their new creation in the U.S. and many other countries.

This case was met with many ethical dilemmas. Of the questions raised, the two most important questions were as: should animals be able to be patented? And what will the new

moral limits be in this case? Think of it what it would mean to have a patent on an animal. That would mean it was your property and would totally objectify it.

Although the patent was eventually awarded in the U.S., when Harvard applied for the patents in different countries, they were met with different attitudes. The European Patent Office also granted Harvard a patent, but using different reasoning. They decided that, while it would not be alright to patent an animal variety, oncomouse was not an animal variety and was therefore permissable. They further applied a utilitarian test, they took a look at how much the mice would suffer and weighed it against the benefits it would produce for mankind, and decided the the benefits exceeded the harm to the mice.

Years later, the European Patent office came across a very similar case. Upjohn Pharmaceutical company created a transgenic mouse that would lose it's hair. The purpose was find a cure for human baldness. The EPO again applied the utilitarian test and ruled the opposite of oncomouse. They again weighed the suffering of the mice against the beneifts of the research and decided that benefits would not be great enough to outweigh the suffering. They thus decided it would be immoral to patent such a creature.

Canada had some mixed feelings when it came to oncomouse. At first they used the definition of patentable materials as a reason for rejecting Harvards new creation. Under their patent laws, the object being patented must be a "manufacture or composition of matter." They realized that while the oncogene injected into the mouse was a mixture of ingredients and therefore patentable, the body of the mouse was not. However, later rulings found that the invention that went into the mouse altered it, so Oncomouse represents an animal no longer found in nature, and it was thus a "composition" of matter and therefore patentable. But later

Canadian Supreme Court rulings denied the patent. The legal aspects of this case will be discussed in Chapter-4.

Superpig

Another interesting case to look at is Superpig. Superpig was created by injecting a pig with a transgene for growth hormone. This boost in growth hormone was supposed to produce leaner meat and allow the pig to grow much faster. This would mean there would be an increase in pork production and more people would have food. However, the problem with Superpig is the amount that it suffers because of this growth hormone; there were many health problems, including arthritis, and eventually multiple systems failure including heart complications, pneumonia, and kidney disease. Even the scientists felt that the creation of such an animal was wrong, so they euthanized the pigs and placed a voluntary moratorium on any other further research that involved animals and growth hormone.

Alzheimer's Mouse

Unlike the previous examples, Alzheimer's mouse is a type of a transgenic animal that does not appear to suffer. Created in part here at WPI, by injecting a mouse with β-amyloid gene, the mouse develops some of the symptoms of Alzheimer's disease (Games et al., 1995). By researching this mouse, scientists will better understand the pathology of the disease. In fact one of the major societal benefits of this mouse was the development of the world's first Alzheimer's vaccine that clears out β-amyloid and senile plaques (Schenk et al., 1998). One of

the major questions these mice answered was whether β-amyloid and the senile plaques it eventually forms, are the cause of Alzheimer's disease, or a side effect. This mouse proved that β-amyloid synthesis by itself is sufficient to initiate the disease (Games et al., 1995). As stated before, the mice do not suffer during the research which makes this a wonderful technology. The only problems they have are with memory and learning, but this does not cause the any pain.

Transgenic Fish

Transgenic fish are yet another species in which scientists make genetic alterations. By injecting the fish with genes that boost growth hormone, as in the superpig case, the fish are able to grow larger and faster than domestic fish. Unlike superpig though, these fish do not show the same adverse side effects. So in this case they do not appear to suffer, but problems could arise if they escape into the environment and out compete native fish.

Transgenic fish have to be kept isolated from natural fish. They could not be allowed into nature because at the speed at which they can grow and reproduce, they would totally disrupt the natural balance, consuming all resources. So these fish must be carefully regulated.

Religious Views of Transgenic Animals

When it comes to transgenic animals, religion appears to have mixed feelings on the subject. The problem is there are not clear guidelines regarding the manipulation of animals. It all depends on how you interpret each religion. Take Christianity for instance. There are many arguments against transgenic animals. Some say that God created man and animals in a certain way and to tamper with that would be a sin. On the other hand, there are others who

say that human beings have been given “dominion” over all animals, and they are entitled to do with them as they please.

Other religions such as Buddhism, and especially Hinduism, hold animals sacred. Hindus believe that all living creatures are sacred and have many animal representations for their gods. On the other end of the spectrum there are the Muslims. The Muslim religion believes in animal sacrifice and is all for transgenic animals, as long as it benefits mankind.

Chapter-3 Conclusions

Judging from the material presented in this chapter, one can see that this topic presents quite an ethical dilemma. The chapter presented many of the benefits to society of these animals, including larger amounts of food, better knowledge of diseases, and possible organ donors for patients awaiting transplants. But these benefits must be weighed against the potential for animal suffering, and the tampering with nature. Transgenic animals such as superpig and oncomouse undergo huge amounts of suffering in order to produce those benefits, while others such as transgenic fish and Alzheimer’s mouse do not. So we conclude that transgenics as a whole must be broken down into specific situations, and each experiment weighed by itself. Even looking at the major religions which have been around for centuries one can see that it is hard to arrive at one clear cut answer.

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Chapter-4: Patentability Issues with Transgenics

The United States patenting process in itself is very difficult, and when applied to the issue of patenting life, things become even more complicated. After all, the purpose of a patent as described in the case of *Warner Jenkinson v. Hilton Davis Company*, is as follows " The patent law is directed to the public purposes of fostering technological progress, investment in research and development, capital formation, entrepreneurship, innovation, natural strength and international competitiveness." ("*Warner Jenkinson Co...", 1997*). The goal of patenting, and the guidelines within it, allow the introduction of new technologies that will benefit society. The main question in the patentability of transgenics is who, if anyone, has the right to patent life. In order to patent an invention, according to the United States Patent and Trade Office, the invention must meet three requirements. A product must have proven novelty, utility and non-obviousness. But if one can apply all three of these criteria to a patent on life, should it still be granted? In this chapter these questions will be explored through several historical cases, and by taking a look at the benefits and drawbacks of patenting life.

Diamond Vs. Chakrabarty (1980)

In 1793, Thomas Jefferson wrote that a patentable object was "…any new and useful art, machine, manufacture, or composition of matter, and any new and useful improvement on any art, machine, manufacture, or composition of matter"(*"A Brief History of the Patent Law…", 2003*). The phrase that has caused the most controversy in the quote above is "composition of matter" and what exactly does it constitute. Everything in our world is a composition of matter,

from oceans, to mountains, and even life-forms. Biotechnology, which can be defined as the study and "…use of microorganisms to perform specific industrial processes" (*WordNet® 3.0, 2008*), constantly deals with the composition of matter, which some consider living breathing organisms. Initially composition of matter was not considered "life", but one of the first cases to challenge that interpretation was *Diamond Vs. Chakrabarty.*

In 1972, Dr. Ananda M. Chakrabarty discovered a new way to break down crude oil with a created bacterium. By introducing two plasmid DNA's into the gene structure of a *pseudomonas* strain of bacteria, Chakrabarty invented not only a new organism, but a new technology that could be used to benefit man and the environment. Its main benefit was found in its ability to assist in the clean up of catastrophic oil spills.

Figure-1: Photo of Dr. Ananda Chakrabarty.

Chakrabarty's first attempt at a U.S. patent with the General Electric Company had all but one of three claims filed accepted. The two claims that passed were for a method of producing bacteria, the second was for a carrier which existed in a material floating on water. The third claim was rejected because it was considered a claim for patenting "a product of

nature", was for the bacteria itself. This was no surprise to Chakrabarty, for this was an attempt to patent a living organism, which had never been done before.

Under the U.S. Patent and Trade Office, Chakrabarty's filed patent did meet their three required criteria under section 101. Its novelty was that at the time there was no organism in nature that could break down crude oil. Its usefulness was its ability to assist in the clean up of oil spills, and its non-obviousness was actually quite obvious. Dr. Chakrabarty was not going to give up so easily, with such a great invention and strong case to back it up. Almost as soon as his patent was shot down, he appealed to the Supreme Court using *The Plant act of 1930*. The Plant act gave a patent to asexual reproductive plants, living organisms. This was just not enough of a case to get his patent approved. The Patent Office Board of Appeals stated 35 U.S.C. 101 was not originally intended to apply to living organisms. The case was also outdated, and if he was to win the rights to his patent he needed a more recent case to support him.

Figure-2: Drawing of Dr. Ananda Chakrabarty.

In a different case, in 1977, a patent for a microorganism was finally granted to Malcolm E. Bergy. In the case of *Malcolm Bergy v. Lutrelle Parker,* the C.C.P.A. accepted Bergy's application for a patent of a microorganism that helped create an antibiotic. The Acting Commissioner of Patents and Trademarks stated that "the fact that the microorganisms are alive is without legal significance".

Using this 1977 case as a precedent, this time around Chakrabarty had a better case, but it was still rejected based on pertinence. Once more Chakrabarty battled back and asked for a remand, followed up by a *writ of certiorari* which allowed him to have his case reviewed by a lower court and have his full case record reviewed. Considering past court cases and interpretations, the Supreme Court in 1987 finally granted Chakrabarty his patent rights, to the method, the inoculum, and the patent to the micro-bacteria itself. This created a new gateway in the field of transgenics with living organisms.

On April 21, 1987, seven years after the landmark case of *Diamond Vs. Chakrabarty* the Patent and Trade Office released this statement: "The Patent and Trademark Office now considers non-naturally occurring non-human multicellular living organisms, including animals, to be patentable subject matter within the scope of 35 U.S.C. 101"(*Patent and Trademark Office Notice, 1987*). Thus, Biotechnology was given its first patented life, opening a new door to the world of transgenic research, which would continue to cause controversy even to this day.

Events Leading to the First Animal Patents

Days before the 1987 ruling of *Diamond vs. Chakraburty*, on the 3rd of April, the courts had denied the case of *Ex Parte Allen*, which involved an attempt to patent a process to create edible oysters by exposing them to pressure. It was struck down on the basis that the "multicellular animal involved was not a bar to patentability" (*Woessner, 1999*). After the ruling on the 21st, the court needed to once again revise their findings to comply with their previous decision. The Patent and Trade Office stated that they would accept "nonnaturally occurring

nonhuman multicellular living organisms, including animals." Through this statement they reiterated the fact that the obviousness of the patent was their main reason behind striking it down the first time, not because it was a multicellular organism. With a firmer definition on patentable life, it wouldn't be long before first animal patent was accepted.

Harvard & DuPont's Oncomouse

Oncomouse was originally produced to further cancer research by inserting an Oncogene that promoted tumor growth in the mouse. With a tumor present, scientists could start to study, learn, and develop new ways to treat them. The invention of the Oncomouse raised two issues; one being that this new patent dealt with a high-order animal, should patents be granted to mammals, and the second was the moral implications of the suffering caused to the animal versus the medical benefits.

April 12th, 1988 Patent NO. 4,736,866 was awarded to Harvard University geneticist Philip Leder and University of California's Timothy Stewart. For the first time in United States history an Institution gained rights to a living animal. Their claim included: A transgenic non-human mammal all of whose germ cells and somatic cells contain recombinant activated oncogene sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic stage (*Woessner, 1999*). Although the claim explicitly excludes human patenting, it still caused much controversy. Many believed that this was too broad of a claim; it encompassed not only the rights of the animal and its Oncogene, but also its ancestry as well.

The Oncomouse's claim met all three of the Patent and Trade Offices Requirements. Since animals and genetic sequences are created naturally, some said it wasn't possible for living matter to be novel, others argued that transgenic biotechnology drastically changed the organism. So much so, that it differs greatly from its original form, making it a novelty, like Oncomouse. The utility of that patent was found in its medical benefits. It had great potential in assisting the field of medicine, pharmaceuticals, and disease models. However, the realistic aspect of the product can be misperceived. The amount of animal suffering compared to the actual medical benefit from it needs to be accounted for when reviewing a patent.

The Patent and Trade Office is now required to examine the reasons behind a rejection of an invention. The non-obviousness requirement in Biotechnology can be at times misleading, therefore in 1995, amendment 35 U.S.C. 103 was reviewed and changed to include that " in order to ascertain the obviousness of an invention, the invention must viewed in light of other inventions in the prior art," (*Walter, 1997*). Controversy arose where others believed that even though the patent met all three requirements, it was a little too much of a stretch, which fueled the opinion of them having such a wide claim on the organism.

On January 19th, 2000, DuPont reached an agreement with Harvard University & the National Institute of Health for an exclusive license of Oncomouse in exchange for further

funding Harvard. DuPont increased business by distributing the Oncomouse through a license agreement to Taconic.

Founded in 1952, Taconic has evolved into a major international supplier of pathogen-free lab animals. Although this opened up the mouse to be used by a broader spectrum of scientists for various studies, there were still guidelines associated with patent. Taconic requires companies and scientists to comply with a contract which includes submission of annual reports of their studies, which many find a hassle and unnecessary. Also, by having to pay for a commercial licensing fee, many believed that research would be restricted.

Due to the broad claims on the U.S. Oncomouse patent, the Patent and Trade Office made adjustments to narrow down terms of agreements on Transgenic Patents. This way no single company can control a patent, which allows them to set competition for other companies. To deny technology's advancement would go against the definition of a patent itself. Needless to say, the Oncomouse caused quite a stir in the world, and is now the precident for attempts at animal and living organism patents today.

Oncomouse Abroad

Much of the same debate and controversy that surrounded the Oncomouse decision in the United States was seen abroad, in Europe, where their final ruling on the issue wasn't concluded until 2004. The European Patent Office decided this very complex case by applying the European Patent Convention standards to the Oncomouse Patent. They used two important provisions to conclude their findings. One being Article 53(A) which states and excludes inventions that "the publication or exploitation of which would be contrary to ordre public or morality".

The second key article was Article 53 (b) which excludes patents on "animal varieties or essentially biological processes for the production of …animals"(*Bioethics and patent law.com*). They also applied the utilitarian balancing test to address the case. The test looked at the suffering of the mouse versus the potential medical benefits. The European Patent Office concluded that the benefits in cancer research to be seen from allowing this patent outweighed the moral implication of testing on animals. They did revise their original application to apply specifically to mice, and not animals in general. In a similar case of the Upjohn Mouse, the utilitarian approach was used once again. The Upjohn patent was for a mouse that was introduced with a hair loss gene, and would be used for the treatment of balding and wool

production. The European Patent Office ruled against the patent, stating that the medical benefit didn't outweigh the animal suffering that would be caused.

In 2002, The Supreme Court of Canada made a final ruling over the Oncomouse which had initially been rejected under its first examination. The initial rejection was based on the fact that transgenic animals didn't fall under the definition of an invention. The final ruling concluded that because Oncomouse was a high-order animal, it could not be patentable. They based their findings off the interpretation of "manufacture or composition of matter", which has constantly been debated. They said that "Composition of Matter" was interpreted as "ingredients or substances that had been combined or mixed together by a person, and that the word "Manufacture" was understood as a "non living mechanistic product or process"(*Bioethics and Patent Law*). Basically, multicellular microorganisms in the initial stage (as a human created mixture used to make the mouse) was patentable, but the actual creation and body of the mouse was not patentable.

Positives of Animal Patents

The ability to create patents and give entitlement rights allows for continuing advancements science and technology in general, but allowing Intellectual Property Rights to

apply to life forms specifically enhances the quality of Transgenic research and Biotechnology. The United States has been a leading country in bioethics and transgenic research, and has discussed not just animal patenting, but the issue of human gene patenting as well.

The issue of Animal Patents allowed for the next step in biotechnological research of the human genome. Although a sure conclusion on the topic of human gene patents is still up in the air to this day, it is clear to see the potential benefits. Disease research, food production, and the medicines that can address the worlds health issues all fall under the umbrella of transgenic research today. Morality and ethics will always linger in the discussion of the issue, which is often complex. Methods such as the Utilitarian Test used by the European Patent Office will assist in the decision making process of this controversial topic.

Negatives of Animal Patents

Probably the most obvious drawback to Animal Patents is the suffering caused to the animal during the research. PETA, CELA, and BUAV are some of the animal activist groups that are challenging the Trangenic Research used today. A constant source of support for the groups is found in the Animal Welfare Act. This piece of legislation provides protection of "…any live or dead dog, cat nonhuman primate, guinea pig, hamster, rabbit or other warm-blooded animal, which is being used , or is intended for use, in research, teaching, testing, experimentation, or exhibition purposes, or as a pet"(*Perzigian, 2003*). Notice that this Act specifically excludes "birds, rat…mice..bred for use in research, and horses not used for research purposes, and other farm animals such as, but not limited to livestock or poultry." Where there's a push, there will always be a resistance and compromise is the only way to continue looking after the well being of animals while still advancing in the care of humans. Although one could say, as stated above, that animal patenting cracked the door open to the consideration of human gene patenting, and this could be a great benefit to society and technology, others disagree. Experimenting with human genes leads to studies and applications of the technologies on humans themselves, which is the last phase of testing on any pharmaceutical today. Much of the world is not ready to fully open that door to human gene patenting and testing.

Animal Patents Since Oncomouse

Currently there are around 670 animal patents in existence world wide, used for biotechnology and medical research. Now there are several patented mice used for the research of the major health issues including HIV and Alzheimer's. Transgenic cows, pigs, and fish have all been patented including a wide variety of others. There are a staggering number of court cases that continue to run through the legal system today and will continue as advancements continue to improve society. How far will transgenic research go? No one can truly say, but currently there's much debate over animal-human chimeras, and who knows much farther research could truly go from there.

CONCLUSIONS

This project investigated various aspects of transgenic animals such as what they are, how they are made, the five main catagories of transgenic animals created to date, and the legal and ethical battles that surround the topic. With the large growth of the industry, transgenic research has gone from a hit or miss experiment to a highly efficient business, teaching us vast amounts of information regarding human disease, pharmaceutical production, organ transplantation, and the functions of biological molecules. While the process of creating transgenic animals is nowhere near efficient, further research will not only allow greater benefits to be discovered, but it could also lead to a more humane way of treating those animals. Regarding legalities, we believe that legislation should be passed to ensure that transgenic animals are used responsibly (transgenic fish should not be released into the wild), ensure animal suffering is minized, and ensure their safety. With intelligent guidelines in place, transgenic technology should be allowed to prosper, as the medical benefits are too great to ignore, continuing to improve their contributions to humanity.