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Transgenic Animals

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TRANSGENIC ANIMALS

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

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August 24, 2012

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ABSTRACT

In this project, research was conducted on the topic of transgenic animals in an effort to examine this technology and its role in society. Each chapter focused on a different aspect of the technology, including describing how they are made (transgenesis), investigating how they are used, and discussing the ethics of whether such animals should be made. After examining these facets of this interesting technology, conclusions were made by the authors concerning which types of experiments should be continued, and what types of oversight should be enforced to ensure animal welfare.

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PROJECT OBJECTIVES

The objective of this project was to investigate the topic of transgenic animals, and more specifically to describe the effect of this interesting technology on society. Our analysis was divided into three sections: transgenic technology, applications of transgenic animals and their benefits to society, and ethical decisions in the deployment of this technology. The first two sections provide a thorough scientific background from which to examine the third section and the ethics behind producing transgenic animals. Then the authors provide their own conclusions based on their research.

Chapter-1: Transgenic Animal Technology

Andrew Leverone

Transgenic animals have foreign genes inserted into their genomes for the purpose of giving the animals new properties to benefit society. The animal's genome contains its normal DNA combined with an additional gene or set of genes that the animal would not normally have. The technology that allows scientists to alter an animal's genetic makeup is a scientific breakthrough that has advanced much over the last few decades. Transgenic animals have many uses in society, but they also have ethical and legal issues. The purpose of this chapter is to describe the main techniques used to create transgenic animals, and how potential positives are screened.

DNA Manipulation

The process of making a transgenic animal has its history in the breakthroughs of molecular biology in the 1950's, 1960's, and 1970's that allow DNA to be manipulated. The genome of an animal consists of all of its hereditary information. This information is stored as a DNA macromolecule in the nucleus of a cell. DNA only leaves the nucleus when cells divide, but every aspect of the organism is dictated by this hereditary code. The transgene is the selected hereditary information that will be added to this macromolecule to create a transgenic animal. This transgene can be cloned and prepared in numerous ways that allow it to be combined with the animal's genome. It would be far too complicated to alter DNA in all cells of a full-grown animal because the transgene would need to be inserted into billions of cells. It is far easier to alter a few cells in an animal that is still developing.

The DNA Molecule

Transgenic animal technology is focused around manipulating DNA, and therefore it is important to understand exactly what DNA is. Deoxyribonucleic acid is a macromolecule that encodes genetic information for most living organisms. DNA was first isolated in 1869 by Swiss physician/biologist Johann Friedrich Miescher, who isolated what he termed "nuclein" from white blood cell nuclei showing it was a new type of organic molecule (Dahm, 2005). Although Miescher isolated the molecule chemically, he was unsure of its structure or purpose, which would come 84 years later in 1953 when Francis Crick and James Watson proposed an elegant double-helical structure for DNA (Crick and Watson, 1953). On February twenty-eighth 1953, Crick and his American co-researcher James Watson discovered that DNA usually exists in the shape of a double-helix. After the discovery, Watson and Crick went to a local pub to celebrate, and announced that they had just discovered the secret of life (Crick, 1958). The discovery of DNA structure would later earn them the 1962 Nobel Prize in Physiology or Medicine.

The DNA molecule takes the shape of a double-helix composed of two polymer chains connected by hydrogen bonds (Crick and Watson, 1953). These polymer chains contain four distinct nucleotides, which are connected by a ribose sugar and phosphate backbone. The repeating units of these polymers are the nucleotides Guanosine, Cytosine, Adenosine, and Thymine. When the two polymer strands connect to form the double helix structure, only certain nucleotides can form bonds between each other. Guanosine usually bonds with cytosine, and Adenosine usually bonds with Thymine. This phenomenon is known as complementary base pairing, and it ensures that the information stored in the DNA is not lost when the two polymer strands separate from their combined double helix structure to replicate, and each paternal strand serves as a template to synthesize two daughter strands. The sequence of nucleotides represents

genetic information that dictates the properties of the organism. Making alterations to the DNA sequence alters the organism's properties.

The Flow of Genetic Information

After his breakthrough relating to the structure of DNA, Francis Crick proposed his central dogma of molecular biology (Crick, 1958) describing the general flow of genetic information occurring from DNA to RNA to protein. The main depository of genetic information in most species is DNA. RNA is synthesized from DNA, and protein is synthesized from RNA. Crick's theory finally made it clear how the properties of an organism were directly related to the sequence of base pairs in DNA. Even though some exceptions have been discovered to the central dogma, it is still the accepted normal flow of information in molecular biology (Crick, 1958).

Ribonucleic acid (RNA) is a macromolecule similar to DNA, but is composed of nucleotides linked together by a ribose sugar instead of a deoxyribose sugar. This alteration gives RNA properties different than DNA. While DNA is more stable chemically and physically, RNA is more labile and is more appropriate for temporarily expressing genetic information in a cell. Another telltale difference between RNA and DNA is the presence of the base uracil, which takes of place of thymine. Both uracil and thymine bond to adenine, so no information is lost when DNA serves as a template for synthesizing RNA. An enzyme called RNA polymerase begins the transcription process. The DNA double helix is separated, and one chain (the positive strand) is used as a template. Even though the resulting RNA will have the opposite sequence of bases, it will still have the same genetic information because the order of complementary base pairs is preserved (Cooper, 2009).

There are three main forms of RNA: mRNA, rRNA, and tRNA. Messenger RNA (mRNA), receives information directly from DNA that is to be made into a protein. Messenger RNA uses an organelle abundantly found within cells, ribosomes, to construct a protein from the information stored in the RNA molecule. The mRNA nucleotides are read as triplets, three at a time, which dictate specific amino acids in the protein. Transfer RNA (tRNA) uses the triplets of nucleotides on mRNA to arrange the amino acids in the correct order of the protein being made. Ribosomal RNA (rRNA) is part of the ribosome and interacts with the tRNA when proteins are being translated from mRNA (Cooper, 2009).

Crick's central dogma ends with protein, the final step in the flow of biological information. In most cases, Crick's theory holds true to this day. It is true that genetic information normally flows from DNA to RNA and ends with proteins, but retroviruses use RNA as their genomes, which are converted to DNA by reverse transcriptase in a step that violates Crick's dogma (Cooper, 2009).

Cloning DNA

Before inserting a transgene into an animal's genome, it must first be cloned. Cloning DNA makes copies of it. To clone a gene, it is usually inserted into a cloning vector such as a plasmid or a virus which helps replicate the DNA. These vectors cannot replicate on their own outside a cell, so they must be inserted inside a host cell for the copying to occur (Cooper, 2009). First, the transgene must be amplified and its promoter selected. The transgene encodes the new protein to be expressed in the transgenic animal. The promoter, a short stretch of DNA usually upstream from the transgene, dictates in which tissue the transgene is expressed. The promoter regulates the expression of the gene, and it can be used to activate and deactivate the gene.

Amplification of the transgene is usually performed by polymerase chain reaction (PCR), a technique that can be used to make millions of copies of a DNA template *in vitro*. PCR was invented in 1986 by Kary Mullis (Mullis et al., 1986), a chemist who developed a technique for amplifying DNA *in vitro,* which won the Nobel Prize in Chemistry in 1993 (NobelPrize.org). Using this process, a lab can make thousands of DNA copies in a matter of hours (Rice, 2006). PCR is a well-known process now, which can be performed in a lab using an automated device called a Thermocycler. The DNA to be amplified is mixed in a vial with nucleotides, DNA primers flanking the transgene sequence upstream and downstream, and a special DNA polymerase called Taq polymerase. Taq polymerase is an enzyme discovered in aquatic bacteria that live in an extremely hot environment. It can withstand the high temperature needed to denature the DNA double helix, but it works to rebuild these DNA strands at a lower temperature similar to the environment it was discovered in. The Thermocycler begins by heating the vial to 90 degrees Celsius for 30 seconds. This makes the strands of DNA separate from their double helical state into single strands. Next, the vial is cooled to 55 degrees Celsius to allow the transgene primers to hybridize upstream and downstream to the transgene sequence. In the third and final step of PCR, the Thermocycler reheats the sample to 72-75 degrees Celsius to allow the Taq polymerase to synthesize new strands of DNA using each original strand as templates beginning at the primer sites. This step rebuilds the DNA double helix using the free nucleotides in the vial, but the amplification focuses on the transgene (Nobel Prize, 2006). This whole process can be repeated a number of times to create exponential copies of the DNA sample (Access Excellence, 1992). When enough DNA has been amplified, it is ready for cloning.

To clone DNA, the transgene amplified by PCR is inserted into a plasmid or virus cloning vector. Plasmids are small closed circular loops of DNA that are replicated in the

cytoplasm of bacteria to high copy numbers. The amplified transgene is cut with restriction nucleases to make sticky ends that are compatible with the plasmid cut with the same enzyme. Then the cut transgene and plasmid DNA are ligated together and transformed into bacteria like *E. coli* for making high copy numbers (Cohen et al., 1973). Alternatively, the cut transgene can be ligated into a DNA virus cut with the same restriction enzyme to make compatible ends, and the virus is then used to infect a cell for making high copy numbers (Cooper, 2009). As previously mentioned in this chapter, these vectors must be inside an organism like bacteria to replicate.

Making a Transgenic Animal By Pronuclear Microinjection

Once the selected transgene has been cloned, it is ready for insertion into the genome of a transgenic animal. The most common technique for inserting foreign DNA into an animal is pronuclear manipulation. In this process, the animal sperm and egg are united by *in vitro* fertilization (IVF). Prior to the fusion of the male and female nuclei in the egg cytoplasm, both nuclei exist as pro-nuclei. At this stage, the male pronucleus is microinjected with a solution containing the cloned transgene. Usually the male pronucleus is injected because it is slightly larger than the female pronucleus, and it is located near the periphery of the egg where it is more easily injected (**Figure-1**). The fertilized zygote (diagram center) is usually held in place using a small suction pipette (diagram left), and the transgene is injected using an injection capillary (diagram right).

Figure-1: Photograph of DNA Microinjection into a Pronucleus to Create a Transgenic Animal. The photograph shows a newly fertilized egg (diagram center) held in place by a suction pipette (diagram left), while a very fine needle is used to inject a solution containing DNA for insertion into the animal (diagram right). (Mullin, 2010).

The injected embryos are grown *in vitro* for about 5 days, until they are in the blastocyst stage of development. A blastocyst is a hollow sphere made of an outer trophoblast and an inner cell mass. The blastocyst is then implanted into the uterus of a pseudopregnant female where is grows to maturity (Brinster et al., 1985). Female mice are induced into a pseudopregnant state by mating them with a sterile male. This causes a hormonal change that allows the uterus to accept the implanted blastocyst. It is estimated that less than one third of the implanted blastocysts will mature into healthy offspring. The main advantage of the pronuclear injection technique for making a transgenic animal is it is somewhat reliable and applicable to many animal species. Its main disadvantage, discussed extensively in Chapter-3 on ethics, is the transgene inserts randomly into the animal's DNA, which can destroy a useful host gene or activate an oncogene causing cancer.

Making a Transgenic Animal by ES Cell Manipulation

Transgenic animals can also be produced by manipulating embryonic stem (ES) cells. ES cells are harvested from the inner cell mass of animal blastocysts prepared by *in vitro* fertilization. These cells can divide to make ES cell lines which can be manipulated in a variety of ways to incorporate foreign DNA. While inserting DNA into pronuclei usually requires microinjection, a variety of techniques can be used to introduce DNA solutions into ES cells, including: electroporation (using electric current to deliver the charged DNA molecules across the cell membrane), chemical transfection of plasmids, or infection with viruses. In addition, after the stem cells have been treated with the DNA solution, they can be screened to select for positives that indeed took up the DNA. This is usually accomplished by inserting a gene encoding antibiotic resistance (for example, G-418^r), and then growing the DNA-treated cells in a medium containing the antibiotic that kills cells not containing the added DNA. After positive ES cells are selected containing the transgene, those cells are injected into a blastocyst which is then implanted into the uterus of a pseudopregnant female recipient as described before. Not all of the ES cells in the blastocyst are transgenic (the embryo had its own untreated ES cells), so as the blastocyst continues to develop, only some cells in the animal become transgenic, and the animal is referred to as a *chimera* (Bronson and Smithies, 1994). Chimeric animals are usually bred with other chimerics to eventually select for pure transgenics (Bradley et al., 1984). This is a key difference with transgenics made by pronuclear manipulation, which are pure transgenics from the outset.

In addition to allowing for the screening of positive ES cells that have taken up the transgene, the main advantage of the ES cell technique over pronuclear manipulation is it allows the transgene to be targeted to a specific site in the animal's genome. The random DNA

incorporation of pronuclear manipulation can allow the transgene to *inactivate* an important gene in the animal's genome, or can *activate* an oncogene that can cause cancer. Targeting the desired destination of the transgene not only leads to more ethical treatment of animals, but it can also save a lab time and money otherwise wasted on failed experiments. With ES cells, the DNA can be targeted to a specific location by homologous recombination (**Figure-2**). In this process, large segments of DNA from the selected target site in the animal's DNA are inserted at both ends of the transgene to flank it (shown in the blue boxes on the left and right sides of the lower diagram). When the cloned transgene (and the flanking DNA) are inserted into the ES cell, during homologous recombination the flanking DNA replaces the equivalent DNA in the animal's genome (large blue X's in the diagram), targeting the transgene to the site. The benefits of homologous recombination are undeniable because not only does it ensure the correct insertion of the transgene, but it also reduces the number of lab animals that will suffer from an accidentally inactivated gene or possibly contracting cancer. This gene targeting technique will be discussed more in Chapter-3 when we consider transgenic ethics, as it reduces the possible harmful side effects to the animal of the random insertion technique.

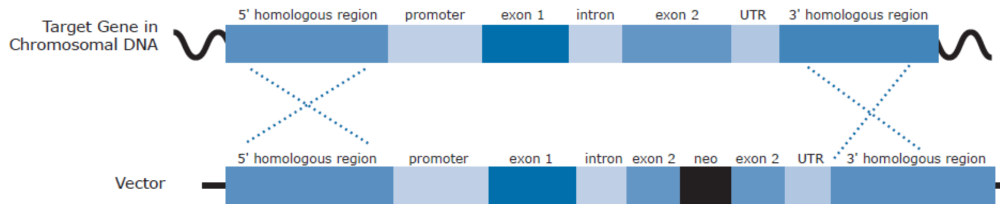

Figure-2: Diagram of Targeted DNA Insertion by Homologous Recombination. This diagram illustrates the process of targeted (not random) DNA insertion into an animal's genome, which reduces harmful potential side effects to the animal of random insertion. The selected transgene (dark blue exons 1 and 2, lower diagram center) is flanked by long stretches of the host animal's DNA (left and right boxes, lower diagram), which is inserted into a cloning vector such as a plasmid DNA. After introducing the cloned DNA into the ES cells, during homologous recombination the long stretches of the cloned animal's DNA recombine with the equivalent regions in the animal's DNA (denoted by large blue X's in the diagram) replacing the host segment of DNA with the cloned DNA. The inserted DNA usually contains a selection marker (black box) that encodes resistance to antibiotics. (Charles River, 2005).

Screening Transgenic Positives

Each of the procedures discussed above for creating a transgenic animal have their own set of problems. Pronuclear microinjection does not easily allow embryo prescreening to determine whether a given embryo took up the transgene. In theory, this could be achieved by PCR assay on a single cell extracted from the embryo, but this is too difficult to perform hundreds of times. And this technique does not allow gene targeting. For the ES cell technique, as discussed above, using ES cells creates chimeras that require subsequent breeding to produce pure transgenics. Creating a line of transgenic ES cells and the subsequent breeding of chimeras takes much time to accomplish. And when using either technique, manipulated embryos are frequently aborted, lowering the process efficiency. And even if the pups are born, the transgene does not always insert in the animal's genome, so positives have to be screened.

There are two main ways for determining whether a particular animal has taken up the transgene: Southern blots and PCR. When screening potentially transgenic mice, typically a very short section of tail tissue is used. It is good practice to take the biopsy from an area of the animal that, if removed, will not harm the animal. The DNA can then be extracted from the tissue sample using organic phenolic solutions and ethanol precipitation. Sometimes PCR can be run on crude alkaline tissue lysates without having to purify the DNA. Once the DNA has been isolated from the cells, PCR or Southern blots can be used, depending on the quantity of DNA available for analysis.

Southern blotting is a technique developed in 1975 by biologist Edwin Southern for whom the technique is named (Southern, 1975). In this assay, restriction enzymes are used to cut DNA strands into smaller pieces. In the case of testing for the presence of a transgene, the assay can be simpler to interpret if an enzyme is chosen that cuts within the transgene but nowhere else in the genome (McGraw-Hill, 2010). After cutting the DNA, the fragments are separated by size using gel electrophoresis. In this process, the DNA solution is layered onto one end of an agarose gel, and an electric current is placed across the gel, with the positive anode facing away from the DNA. The negatively charged DNA moves through the gel towards the positive anode with the smaller fragments moving faster (and farther) than larger fragments. Larger molecules cannot move through the gel as easily, and the end up closer to the sample well they were loaded into. Most genomic DNA electrophoresis applications use agarose gels, but other types of gels can also be used. Generally one sample well is loaded with a size marker and the rest are used for DNA samples; the size marker contains DNA fragments of known sizes to allow the sizing of unknown bands. After electrophoresis has been performed, the pattern of DNA fragments is transferred from the gel to a white membrane that retains the pattern while allowing the DNA to

be hybridized to a labeled single-stranded DNA probe complementary to the transgene. If a band on the membrane contains the transgene, it hybridizes to the labeled probe, and its position is determined by placing a piece of x-ray film over the membrane (Cooper, 2009). Successful transgenesis occurred for a particular sample if one of its bands “lites up” with the probe, or if the pattern of DNA fragments is different than control DNA. If the animal is positive, the Southern technique can be further applied to determine the site of integration. Southern blots are typically performed when the sample DNA is of sufficient quantity (several micrograms per sample), or when it is important to avoid the potential contamination problems associated with PCR.

PCR is the most common technique used to screen for potential transgenics, because it is sensitive and rapid compared to Southern blots. Its only drawback is it is prone to contamination, so false positives often occur. One reason PCR is prone to contamination is that the technique is so sensitive, not only will the original DNA sample be amplified, but also any foreign DNA that contaminates it. PCR was discussed earlier in the chapter. It is a technique that within hours can amplify millions of copies of DNA located between two primers (sense and antisense). If the primers are carefully chosen to hybridize to different segments of a transgene, getting an amplified band of the expected size (between the two primers) is scored as a positive. In the case where transgenesis did not occur, the DNA sample undergoing PCR will not hybridize to the primers representing the transgene, and no amplification will occur.

Since the construction of the world’s first transgenic animal in 1974 (Jaenisch and Mintz, 1974), scientists have revised transgenic technology to become more efficient and rapid, and have expanded the transgenic species from mice to primates (Chan et al., 2001). Despite the advances, the process remains very inefficient and much research remains to be done. And once

the transgenic animal is made, important discussions still remain about whether it *should* have been made.

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Chapter-2: Transgenic Applications

Michael Heard

The transgenic technology discussed in Chapter-1 has been applied to the creation of many different types of animals that benefit society. Their benefit to society strongly factors into their ethics, and the discussion we will have in Chapter-3 of whether such animals *should* be created. The purpose of this chapter is to discuss the various transgenic animals that have been created, how they are categorized, and provide examples within each category.

There are five major categories of transgenic animals: Disease Models, Transpharmers, Xenotransplanters, Food Sources, and Scientific Models. While all the animals created used similar technology for knocking out endogenous genes, and/or inserting new foreign genes, each animal has a very different purpose. These animals have been used to model human diseases, produce protein-based medicines in their milk, provide a pathway for organ transplantation, or produce food more efficiently. The benefits of transgenic animals are endless and still growing.

Disease Models

Transgenic disease models provide a faster and easier way of testing cures and uncovering potential causes of human diseases. Mice have become a medical research favorite because they are easy to work with, produce large litters, and have a short life span. Most animals though, including mice, do not naturally develop these human diseases, so DNA implantation is necessary to give the animals new properties that mimic a human disease. By introducing a foreign gene, scientists are able to create animals that initiate tumor formation or

mimic the early stages of Alzheimer’s disease. Several of these models have led to significant scientific breakthroughs and are discussed below.

Alzheimer's Mouse

Alzheimer's mice are geared towards uncovering the mystery of the disease for which they are named. Alzheimer's disease (AD) is the fourth leading cause of death in the developed world, and was discovered by German neurologist Alois Alzheimer almost 100 years ago (Alzheimer’s Association, 2012). AD is the most common form of dementia, accounting for fifty to eighty percent of all cases. Alzheimer’s normally presents around age 65, and worsens over time. Some FDA-approved treatments are available; however there is no known cure (Alzheimer’s Association, 2012). Both sporadic and genetic versions of AD exist. For the genetic type, researchers have linked AD with several different mutations in the gene that encodes β-amyloid precursor protein (APP) (Goate et al., 1991; Murrell et al., 1991; Chartier-Harlan et al., 1991; Mullan et al., 1992). These mutations are expressed into a mutated APP present in the cerebral cortex and hippocampus, which are areas of the brain responsible for memory and cognitive thought.

Animals do not get AD, except for primates which are extremely expensive models to work with. And most primate species are not available as research models. To create a mouse model for this disease, researchers from WPI and the former Transgenic Sciences Inc. inserted the gene for mutant APP into mouse embryos (Games et al., 1995). The version of the gene inserted mimicked the mutation identified in an early-onset pedigree in Indiana (the Indiana mutation) (Murrell et al., 1991). These mice appear normal for the first six months of life, but within six to nine months, many of the symptoms seen in human Alzheimer’s patients begin to

appear, including an increase in the size of the APP senile plaque deposits and neuro-degeneration in the same areas affected in AD patients (Games et al., 1995). In 1999, this same mouse model was tested by Elan Pharmaceuticals with a vaccine to remove senile plaques and amyloid-β (Schenk et al., 1999). The mouse showed improved cognitive function when vaccinated, which prompted Elan Pharmaceuticals to begin human trials (Schenk et al., 1999).

Oncomouse

The Oncomouse was one of the earlier transgenic animals created (**Figure-1**). In the 1980s, Harvard University created a mouse that was highly vulnerable to cancer by introducing a *myc* oncogene that triggered the growth of tumors (Stewart et al., 1984; Bioethics and Patent Law, 2006). The *myc* oncogene was placed under the control of a mouse mammary tumor promoter to express the oncogene in mammary tissues. So this initial version of oncomouse served as a model for mammary tumors. This cancer-prone mouse allowed for greater research into the initiation of cancer, and a model for screening potential anti-cancer drugs. Other later versions of the mouse carry the *ras* oncogene (Anderson, 1988).

Figure-1: Photograph of Oncomouse. This figure shows one of the later versions of the cancer-prone mouse, in this case the mouse contains the *ras* oncogene which makes it prone to developing cancer. (Bioethics and Patent Law, 2006)

AIDS Mouse

The human immunodeficiency virus (HIV) was discovered in 1983 as the cause of AIDS (Barré-Sinoussi et al., 1983), and to this day AIDS has no cure. HIV normally infects only humans and chimpanzees. Testing of the virus in the most popular experimental model, mice, was thought to be impossible because mice lack the CD4 and CCR-5 co-receptors on their cell surfaces that bind with HIV allowing it to enter the cell. At the National Institutes of Health, Malcolm A. Martin, Abner L. Notkins, Jan W. Abramczuk and others overcame this problem by injecting multiple copies of the HIV genetic code directly into fertilized eggs. These eggs were then implanted into mice to allow the infected embryos to develop (Science News, 1988). The first generation of mice failed to show any symptoms, but their offspring developed some signs of infection, including psoriasis, a skin disease seen in one-quarter of AIDS patients, and pneumonia, before dying.

Since the original HIV mouse experiment, other HIV mice have been created. One strain was created by injecting human lymph tissue into a SCID mouse lacking an immune system. The lack of immune response enabled the human lymph tissue to survive, which could then become infected with HIV (Namikawa et al., 1988). In 2001, Robert Gallo's virology group created a transgenic rat containing the human HIV genome with gag and pol mutations (Reid et al., 2001). The mutations helped ensure the rat does not produce any infectious HIV virions, which makes it a safer model to work with, while the rat contains cellular proteins that allow viral replication.

Parkinson's Fly

Mice are not the only transgenic models fit for disease testing; *Drosophila* (fruit flies) proved to be a great resource for researching Parkinson's disease. Parkinson's patients produce a protein called alpha-synuclein that accumulates into fibrous clumps (Lewy bodies) in the brain area referred to as the *substantia nigra*. This area of the brain contains neurons that secrete the neurotransmitter dopamine. When the Lewy bodies form in the *substantia*, it deteriorates, so patients secrete less dopamine. Dopamine helps regulate neuro-muscular control, so patients show loss of muscle control. Alpha-synuclein has been shown to cause nerve damage in mice; however, only in the fly model do Lewy bodies form (Vatalaro, 2000).

Transpharmers

Transpharmer animals produce pharmaceuticals for humans in their milk, blood, or urine by introducing a foreign DNA under the control of a promoter that ensures the production of the foreign protein expressed from the transgene solely in that specific location. Milk is now the preferred site for production, because producing foreign proteins there has no effect on the animal's physiology, unlike producing the proteins in blood. The goal of these animals is to produce lifesaving drugs at high levels without endangering the animals, have the drug be easily accessible (in the milk), and ensure the transgene is passed along to their offspring (Biotechnology Information, 1995).

Transpharming human therapeutics provides a real solution to the very limited supply of many protein-based drugs. For example, insulin for diabetes patients was previously taken from the pancreas of slaughtered pigs, but now insulin can be produced in animal bioreactors. Several transgenic species, including cows, mice, sheep, goats, chicken, rabbits, and pigs have been

modified to produce human drugs. The scientific breakthrough behind this type of transgenic animal is the combination of successfully cloning the drug's gene and placing it under the control of a mammary gland promoter. By doing this, the gene that is present in every cell of the animal only functions in the mammary gland. Since the drug is only produced in the milk, there is very little danger to the animal (Biotechnology Information, 1995; Ziomek, 1998).

The world's first transpharmer was constructed in Massachusetts at GTC Biotherapeutics in 1987, and was a mouse designed to produce the clot dissolver drug tPA in its milk (Gordon et al., 1987). The first FDA-approved transpharmed drug approved for use in humans was ATryn®, a blood thinning anti-thrombin protein produced in goats (Atryn, 2008). This medicine is used in surgery for people with a condition that causes their blood to clot too easily, which can hinder long surgeries. The condition is present in approximately 1 person in every 3,000, and is due to a missing gene that produces the protein anti-thrombin which delays the clotting of blood. Usually, patients lacking this gene were kept on blood thinners unless they were undergoing surgery; then surgeons would perfuse anti-thrombin protein as necessary. The replacement anti-thrombin was only available by extraction from human blood, which is expensive and can possibly lead to viral transmission. This spurred scientists at GTC Biotherapeutics to produce human anti-thrombin in animals. Their studies have shown that the amount of protein produced by one goat is equal to 90,000 human blood collections. This transgenic breakthrough will make surgery for those with congenital anti-thrombin deficiency much safer (BBC News, 2006).

Herman the Bull was the world's first transgenic cow. Engineered in Europe in 1989 (Hendolin et al., 1989), Herman's purpose was to produce female offspring with lactoferrin in their milk. Lactoferrin is an iron-based protein that is very important for infant growth which helps to fight infections. This protein is found in mother's milk but not traditional cow's milk.

The goal of transpharming lactoferrin milk was to help the growth of children in developing countries who may be lacking breast milk (Herman the Bull….2004). In 1994, Herman became a father to at least eight calves, all inheriting the lactoferrin producing gene (Biotech Notes, 1994).

Pharmaceuticals are not the only item scientists are producing in transgenic animals; goats have been engineered to produce spider silk in their milk (Gillespie, 2010). Tests on spider silk have shown it to be five times stronger than steel and twice as strong as Kevlar, which has created a large interest in harnessing its super strength. After failed attempts of farming spiders for their silk, the so-called "Spidergoat" was created. This goat produced silk proteins in its milk that could be spun into a thread with spider silk properties. This silk can be used to produce strong fiber constructs from bulletproof vests to surgical thread (Gillespie, 2010).

Xenotransplanters

Xenotransplanters are a different kind of transgenic animal; they are animals engineered to produce transplantation organs for humans. The anatomy of pigs is very similar to our own, which makes them a good model for testing tissue survival capabilities (bones, tissue, organs) to transplantation organs. Xenotransplantation is becoming increasingly important as the demand for organ transplants rises. There are more than 100,000 people in the U.S. awaiting organs to save their lives, and every 10 minutes another name is added to that list. In 2011, 18 people died every day because a lack of available organs (Donate Life, 2011). When the process is perfected, successful organ transplants from these transgenic animals will save countless lives.

But pig donors have two important problems: the fear of new viruses being passed to humans via the animal organs, and human rejection of the organs. With respect to potential viral transmission, scientists could screen potential pig donors for known animal viruses, and reject

any contaminated donor, which should help lower the incidence of infection. So, problems would only occur if a new virus not detectable in the screen were present.

With respect to the organ rejection, scientists have traced the rejection to a sugar-producing enzyme in the pigs. Pigs produce a sugar on the surface of their cells, α1,3-galactose, that is recognized as foreign by the human body. The human immune system sees the sugar as foreign and attacks it, leading to rejection and failure of the organ (Kaiser, 2002). So in 2002, scientists at the University of Missouri knocked out the porcine gene encoding the α1,3-galactosyltransferase enzyme responsible for placing α1,3-galactose on the surface of cells (Lai et al., 2002). The University of Missouri team produced four cloned piglets all lacking one of the two copies of this gene. Unfortunately, these four surviving "knockout piglets" still produced α1,3-galactose with their remaining normal copy of the galactosyltransferase gene, so scientists are working on removing the remaining copy (Kaiser, 2002).

In 2003, researchers from the Massachusetts General Hospital transplanted kidneys from transgenic miniature pigs into eight baboons. The organs survived for a maximum of 81 days, surpassing the 30 days seen with normal pig kidneys (Pearson, 2003). The Massachusetts team found that these genetically altered kidneys did not produce the same rapid rejection as seen in previous transplants. However, this new kidney was still not as successful as same-species baboon-to-baboon transplants (Pearson, 2003).

Although the transplantation of organs from pigs to humans is not yet fully successful, the technology continues to advance. In 1997, a transgenic pig liver developed by Nextran was hooked up to a patient suffering from acute liver failure. For three days the pig liver "bridge" was attached to the patient outside of his body to filter his blood until a human donor was

available. Six patients in all underwent this treatment which proved successful (Organ Farm, 2001).

In addition to organs, scientists have also experimented with implanting pig cells into humans. In the mid-late 1990s, the biotech company Diacrin conducted a clinical trial on five stroke victims suffering from paralysis of an entire side of their body. They had thirty million fetal pig cells implanted in their brains. Four of the five patients showed incredible improvement in motor skills, speech, and demeanor; one even went on to complete a half-marathon. Since this trial, Diacrin has gone on to test the effects of fetal pig cells on patients suffering from Parkinson's disease, Huntington's disease, and epilepsy (Organ Farm, 2001).

Food Sources

Transgenic animals engineered for food sources are created in an effort to produce food more quickly and effectively. These new food sources will create easier and more accessible food which will prove especially important for people in developing nations.

Superpig

Superpig was the first animal created in this transgenic group, having the goal of producing more meat from each pig. In 1989, scientists injected pigs with genes encoding both human growth hormone and bovine growth hormone to rapidly increase their size (Miller et al., 1989). The transgenic pigs grew an average of 12% faster than the control group from the same litter, and converted food to weight 18% more efficiently. The initial results showed that these pigs not only grew bigger but were engineered to gain weight more effectively with the same amount of food. These advances, however, were met with a series of health problems. The pigs

with overexpressed hormones were extremely lethargic and had gastric ulcers (Miller et al., 1989), and later the animals developed problems with all major organ systems, so they had to be euthanized (Rollin, 1996).

In 1997, a second set of superpig tests were performed using ovine growth hormone instead of bovine and human (Pursel et al., 1997). Unlike the first tests, these transgenic pigs did not grow any faster than their littermates, but they did have a much higher muscular protein percentage than the control group (Pursel et al., 1997).

Over 20 years after the initial testing, transgenic superpigs are still not in agricultural production. Testing was voluntarily halted when it was agreed that the long list of side-effects (including kidney and liver problems, thick skin, gastric ulcers, joint disease and heart disease) outweighed the potential benefits.

Superfish

Superfish are a more recent biological endeavor with far fewer disadvantages than the superpigs. The greatest benefits with superfish have been seen in the testing of salmon and trout, both members of the *salmonid* family. The original fish were engineered to produce fish growth hormone in elevated amounts by using a strong promoter (Devlin et al., 1997). By 2001, the group had created super-fast growing salmon by introducing an additional growth hormone gene (Devlin et al., 2001). These fish grew four to six times faster than ordinary salmon (**Figure-2**), creating large interest by aquaculture companies to sell them as a food source. However, environmental groups were against the rearing of these superfish, saying that more thorough testing needed to be done first in case of an escape from the aquaculture farms; the effect of

introducing these massive salmon into the ecosystem is still unknown (Stokstad, 2002; Clarren, 2003).

Figure-2: Superfish. Photograph of transgenic fish that grow twice as fast as wild type Salmon. (Marris, 2010)

In 2010, the FDA began reviewing the case for the aquaculture of supersalmon from Aqua Bounty Technologies. This Massachusetts-based company created salmon that grow twice as fast as normal salmon because of the addition of two foreign genes; the first gene controls a growth hormone similar to the earlier models, while the second gene allows growth in cold weather (Aquabounty…2012). This second gene is the more impressive advance in these superfish; normally salmon only grow seasonally in warm weather, but with the addition of this promoter causing growth hormone production year-round, the growth hormone is produced year round. Unlike the previous fish, these salmon do not grow larger than normal salmon they just reach their full size in half the time. This alteration presents a much smaller danger to the natural salmon population than the giant salmon. In addition, these engineered salmon are sterile and cannot breed in the wild in the event of an accidental escape (Gitig, 2010).

Transgenic Scientific Models

Scientific transgenic models are similar to disease models, but provide insight into the function of newly discovered proteins. By knocking out the gene encoding a newly discovered protein, or by over-expressing it, scientists can study the effects *in vivo*. This field's most

prominent undertaking to date is a project to capture all of these transgenic findings into a library for future research.

ANDi the Monkey

ANDi was the world's first transgenic monkey, his name coming from inserted DNA spelled backwards. He was created as a preliminary test to determine whether transgenic primates could be created, with the long term goal of having primate models of human diseases that are more accurate than mice. In this experiment, a primate fertilized egg was injected with the gene encoding green fluorescent protein (GFP) from jellyfish (Chan et al., 2001). Expression of the gene is easy to assay by a green glow under blue light. The group, led by Anthony Chan, cloned copies of the GFP gene into viruses, and used the viruses to infect 224 fertilized eggs. From these eggs, three monkeys were born, and only one ANDi carried the GFP gene. However, he did not express the GFP gene. The world's first *expressing* transgenic monkey came in 2009 (Sasaki et al., 2009). Together, these transgenic monkeys provide hope that primates might become more accurate disease models than mice for diseases like Parkinson's. Or they could help test vaccines more quickly than with human clinical trials (Begley, 2001).

Smart Mouse

In 1999, scientists at MIT and Washington University created a strain of mice that were smarter (Tang et al., 1999). The mice, together named *Doogie*, were created with extra copies of the NR2B gene. NR2B is a subunit of the glutamate receptor which functions in learning and memory. The NR2B subunit predominates when mammals are young, so scientists hypothesized that increasing its production might create more efficient neuronal firing. The transgenic mice,

when tested against a control group, showed a huge increase in both memory duration and quickness in learning (**Figure-3**). The expression of this gene could prove valuable in human memory and its dwindling nature with age (Harmon, 1999).

Figure-3: Photograph of Doogie the Smartmouse. This mouse shows better memory than normal mice. (Orca, 2009)

Youth Mouse

At Harvard Medical School, scientists created a mouse that not only met, but exceeded their expectations. They were testing the effects of the enzyme telomerase on the physiological aging of mice. The tests focused on older mice, equivalent to 80 year old humans, which would normally be close to dying (Hastings, 2010). Mice naturally produce telomerase throughout their life, but when the levels were increased the mice showed less ageing. Not only did deterioration of the body halt, it reversed; organs recovered and fertility returned. These findings could prove to be a key in slowing the human aging process, treating ailments such as organ failure or dementia. The prospects of human testing are not so clear-cut though. Unlike mice, humans stop

producing telomerase in their adult stage which stops the uncontrollable division and growth of cells. The Harvard scientists noted that if the enzyme was reintroduced into older humans it might increase the possibility of cancer cells spreading (Hastings, 2010).

Mouse Consortium Project

A more recent transgenic undertaking may be the biggest yet; a project to create a library of transgenetic data (Mouse Phenotyping, 2011). In 2009, an international project arose aimed at creating and testing 5,000 knockout mouse genes for eventual stem cell research. This has been called The International Mouse Phenotyping Consortium (IMPC), and it will give scientists a great resource for easily obtaining knockouts for specific genes, and studying their effects in human diseases. The findings of the IMPC will be used by pharmaceutical companies to develop new drugs more efficiently (Mouse Phenotyping, 2011).

Transgenic animals have countless benefits, but also create an ethical boundary that will be explored in the following chapter. From transplanting animal organs, to creating more abundant food sources, transgenic animals can solve many of our problems. The technology involved is advancing rapidly and is proving that these models are not just wild dreams, but very real solutions.

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Chapter-3: Transgenic Ethics

Devin Churchman

Transgenic animals are a great advancement in the scientific world. As discussed in the previous chapter, the applications for these animals and the potential research that can be performed with them seem almost endless. The benefit that human beings can reap from this technology can greatly advance our knowledge and help us treat diseases. But even with all the promise that transgenic animals show, there is still a number of ethical problems with this type of research. The purpose of this chapter is to discuss some of the ethical issues associated with the creation and use of transgenic animals.

Transgenic Animal Ethical Fundamentals

One problem with creating transgenic animals lies in the relationship that humans have with the animals they create. While different species have always interacted with each other in the past, this interaction becomes entirely different when humans change the animal's genetic makeup on purpose. In this case, scientists are changing part of the very substance of what gives each species its own natural identity. Whether society can determine what role humans have in nature, and whether it is ethical to interfere with the work that evolution has naturally done over the ages, are fundamental questions. In addition, the creation of transgenic animals toes a fine line between scientific research and its benefits to society, and potential animal cruelty. While many transgenic experiments show great promise for results that have and will benefit society, some types are very harmful to the transgenic animal subjects they use. This raises the ethical issue of how much animal suffering is worth saving human lives. This issue becomes more complicated, as the animals have neither asked nor consented to being test subjects. While a

researcher could not perform any experiment on an unwilling human subject, one must wonder whether forcing animals into these experiments violates their rights as living beings.

For consideration in all transgenic cases, when the transgene is implanted into the fertilized egg to produce a transgenic species, unless using the embryonic stem cell method for producing the animal combined with homologous recombination (discussed in Chapter-1), the DNA incorporates randomly into the animal's genome. Thus, the transgene could *activate* the wrong genes, or *inactivate* a required host gene. Either case can cause problems like birth defects, cancer, or other health problems (Gillespie 2010). And the process of creating a transgenic animal is not efficient; the typical yield of a successful transgenic species is low, with only 1% of eggs injected with the transgene expressing correctly (Gillespie, 2010). This means that the offspring must be carefully screened for the best producers and to determine the site of integration. Because using homologous recombination to insert the transgene allows its integration site to be controlled, this process should be used whenever possible as an alternative to microinjection into newly fertilized eggs.

In addition to the ethical questions surrounding the creation and use of transgenic animals, the vast amount of transgenic applications makes it difficult to regulate their use with one simple law. The imposed experience to the animal and the desired result vary greatly from one case to the next. For this reason, transgenic approvals and use should be monitored on a case by case basis. Each case should be analyzed thoroughly, considering the experience undergone by the animal and the expected benefit of the study, weighing the two against each other, before any experiment is allowed to proceed.

In the following sections, different transgenic applications will be discussed, describing the ethical issues for each specific application. The author of this chapter takes the position that

humans have the right to perform such research and to interfere with animal genetics for acceptable research subjects. Below, the ethical aspect of each experiment will be based on the health and safety of the animal versus the overall benefit of the research to society.

Disease Models

One large category of transgenic research is the study of animals that have been modified to serve as disease models. In this process, animals are given a new gene to allow them, for example, to become infected with a human virus or to begin the neurodegenerative process of Parkinson's disease, which that particular species cannot normally contract. This allows researchers to study the disease and test potential treatments.

AIDS Mouse Ethics

One example of a transgenic disease model is AIDS mouse. AIDS is caused by the HIV virus, discovered in 1983 (Barré-Sinoussi et al., 1983). Animals are not normally infected by HIV, except for for chimpanzees which are expensive and difficult to obtain research models. Mice lack the CD4 and CCR-5 co-receptors necessary for HIV to enter cells. The first AIDS mouse was created by injecting the HIV genome directly into fertilized mouse eggs, which incorporated into the mouse genome and expressed some of the HIV genes (Science News, 1988). The mice do not develop full AIDS symptoms, but they die early, and the animals allow scientists to study viral gene expression. Other researchers achieved a similar result by introducing HIV particles into the rectums of mice that had developed human immune systems as a result of transplantation of human lymphocytic cells (Ambrose, 2007). The development of these mice is considered a great advancement in AIDS research, as they allow us to determine

the *in vivo* effects of specific viral genes and the body's immune response against the foreign proteins. Although these mice may prove an important part in the treatment of AIDS in humans, the mice themselves suffer some symptoms. In one study, the mice developed a skin disease resembling psoriasis, pneumonia, and enlarged spleens, and ultimately died within less than a month (Science News, 1988). This study poses an ethical problem, as the subjects seem to experience a very short lifespan. Still, these mice could be studied more to find an effective treatment for AIDS, although much more research will be necessary to achieve this goal.

In examining the case of the AIDS mouse, it seems that this study should not be continued for two reasons. First, the quality of life of the test subjects is greatly diminished. Their lifespan is shortened by a significant amount, and the health problems that accompany the disease cause a lot of pain. Chimpanzees can naturally contract HIV, and several vaccines encoding HIV proteins have already been tested in these animals, so perhaps we would learn more from these latter experiments than the AIDS mouse study, considering the similarities of chimpanzees and humans. Scientists have also studied simian immunodeficiency virus (SIV) in rhesus monkeys, a disease very similar to AIDS (Science News, 1988). Both of these options should be considered before performing more research on AIDS mice.

Alzheimer's Mouse Ethics

Another prevalent example of transgenic animals aiding human disease research is the case of Alzheimer's mouse. Aside from elderly orangutans, animals do not get Alzheimer's disease (AD), so scientists had no experimental model for screening potential drugs or studying the disease initiation. In 1995, transgenic mice were developed that initiate AD (Games et al., 1995). Once this was accomplished, studies could be performed on the mice to learn about the

disease. Although this research seems very similar to the AIDS mouse, the Alzheimer's mouse is more ethically acceptable that the AIDS mouse due to their experience. The mice initiate the disease, and show some signed of neuro-degeneration and memory loss, but there are few other changes in their behavior. Other than learning at a slower rate than the non-transgenic control group, AD mice were found to be no different than controls (Nalbantoglu et al., 1997). Since in this case the quality of life for the transgenic subjects is only altered slightly, and the animal does not appear to suffer in any measurable way, it is believed that this experiment is ethically sound, and experimentation should be continued due to the great promise that this research shows.

Oncomouse Ethics

The Oncomouse may possibly be one of the most difficult cases to analyze within the disease model category. This research model was engineered to develop tumors so that scientists could study tumor formation and have a model for screening anti-cancer drugs (Bioethics and Patent Law, 2006). While giving an animal cancer may seem like a very unethical practice, one should not be so quick to condemn the study. Cancer causes a great deal of pain and suffering in all parts of the world, and research was limited due to the lack on an animal model. Although animals can naturally get cancer, the natural tumor formation was not predictable as is needed in a good experimental model. Based on the desperate need for a good cancer model, this type of research should be funded, but in an ethical manner. The health and safety of the mice must always be a top priority, and their pain must be controlled by the use of pain killers or any other similar method. Although it may not be humane to inflict mice with cancerous tumors, in this case the promised benefit to society far outweighs the harm to the animal subjects. As long as the

research follows proper ethical codes to minimize mouse suffering, research in this area should be continued in an effort to find effective cancer treatments.

These three cases are just a small sample of transgenic animals in this category, and it is clear that a simple blanket policy would not effectively cover all of them. Therefore, one must consider some kind of guidelines when deciding whether a disease model transgenic study is ethical. First, if the subject does not suffer in any measurable or observable sense, such as the Alzheimer's mouse, the study is ethical and should be continued, so long as the desired outcome is to help alleviate human suffering. In the case of the Oncomouse, while the subjects do suffer a great deal, the promise of cancer treatments could possibly help millions who suffer from various cancers. While one could not easily justify such an experiment for a very rare disease, cancer is extremely common. Also, if there is a natural alternative to the transgenic study, that study should be performed first before moving to a transgenic study. For example, it does not seem ethical to develop a mouse infectable with HIV while other more accurate models such as primates develop the disease naturally. If primate studies proved to be unsuccessful, perhaps studies using mice subjects could then be performed.

Transpharmer Ethics

In terms of ethics, transpharming models of transgenic research are a fairly simple and straightforward category. Through genetic engineering, transgenic species can be created to produce lifesaving pharmaceuticals in animal milk, which the species does not normally produce in nature (Biotechnology Information Series, 1995). This process allows large amounts of therapeutic proteins to be made with little production cost (after the original investment) (Perzigian, 2003). By producing the therapeutic protein in milk instead of the blood, the animal

experiences little to no changes to its physiology. Transpharmers do not appear to suffer in any measurable way.

Once a transpharmer has been developed and begins to provide milk, the pharmaceutical can be extracted from the milk, or if the pharmaceutical is actively absorbed in the gut the milk can be consumed. As an example, a goat was developed to produce the blood thinner anti-thrombin (ATryn®). This drug is administered to patients whose blood clots too easily. The alternate source of anti-thrombin involves its extraction from human blood plasma, which requires a large supply of blood, and increases the risk of viral contamination from the donor supply. The production of ATryn® in the milk of one goat has been shown to be comparable to 90,000 blood plasma extractions (BBC, 2006). Thus, not only is the transpharming method safer from viruses, it produces a staggering amount of the drug compared to the traditional method. In addition, once a transpharmer has been developed, the animal can pass on its transgene through natural breeding. For example, Herman the Bull, a transgenic bull containing the gene for human lactoferrin, fathered countless calves. Each calf inherited the transgene for lactoferrin production, and his female offspring transpharmed the drug (Biotech Notes, 1994). So, although the construction of an original transgenic animal may not be efficient, once created, the transgene can pass to offspring by simple breeding, expanding drug production in a completely harmless and ethical manner.

Based on the tremendous benefits to society and the minimal risk posed to the animal, transpharming should be continued to its fullest extent. While it may cause some initial animal harm if any embryos are lost, the benefits of a successful transpharmer species is just too great for it to be discontinued. Since transpharmer species do not suffer in any measureable way, the key to keeping this process ethical is to use homologous recombination in their creation to target

the transgene to a specific site to minimize damage to the genome, and the proper care of any born animals that were not transgenic. Any animals born with birth defects or health problems as a result of the experiment should be aided in any way possible to ensure a normal life for the animal, whether by medication or operation, or if no such treatment is possible, the animal should be humanely euthanized.

Xenotransplanter Ethics

Xenotransplantation is a category of transgenic animals which are engineered to produce organs compatible with human recipients. Such a procedure has the potential to save countless human lives awaiting transplants with no apparent donor in sight. Several human illnesses and conditions can be treated with a transplant, but the procedures are limited due to the shortage of organ donors (Correa 2001). In fact, eighteen people die every day in the United States awaiting an organ transplant (Donate Life America 2011). More than 100,000 people in the United States are currently on the transplant waiting list, and another name is added to the list every ten minutes (Donate Life America, 2011). Even with the large population of the United States, there are simply not enough organs available for transplant. There were 28,535 organ transplants performed in 2011, a far lower number than the staggering amount of people waiting for transplants (Donate Life America, 2011). By using animal tissues and organs, there is a possibility that these numbers can be decreased dramatically.

But there are a number of concerns with xenotransplantation, including the issues brought up previously about controlling random transgene insertion, and controlling the possibility of animal-to-human virus transmission. Transgene insertion can be controlled by homologous recombination. With respect to viruses, this has been a problem in the past as evidenced by the

spread of the influenza virus (Carnell, 2000). While previous infections have been caused by the consumption of animals, the risk of disease from xenotransplantation is much greater because the organ is directly implanted into the body without going through the digestive system (Correa, 2001). However, known viruses could be screened in potential animal organ donors. Viral problems would then arise only for a new virus not identified in the initial screen, but as new viruses are discovered, they could also be screened against. These issues must be carefully considered before any type of xenotransplantation takes place, otherwise the results could pose a serious risk to society. In general, the benefits to society from xenotransplantation far outweigh the death of the animal to provide the organ. The use of animals as a source of organs is not drastically different from the use of animals as a food source. But the animal must not undergo any suffering and the euthanasia must be performed properly. However, research into animal viruses capable of causing disease in humans should continue. If a cost-effective method for screening animal donors for known viruses can be devised, then xenotransplantation should be performed. Until then, only research to make the process safer should be performed.

Food Sources Ethics

While the majority of these transgenic engineering applications have appeared fairly recently, the concept of "genetic engineering" has long been practiced in the food production industry. Farmers have used selective breeding to create better crops and better livestock in an effort to produce more food of a higher quality at a lower price. Although selective breeding did not involve inserting a foreign gene into the animal's genome, it did involve changing the animal's genome naturally to create new strains. It logically follows that the food industry would move to transgenic animals in a further effort to lower costs and increase food production.

Much research has been conducted in an effort to create transgenic animals available for possible human consumption. Some argue that the practice of raising animals for human consumption is unethical in itself. That issue goes far beyond the purpose of this paper, and is a complex issue in its own right. That being said, this chapter will focus solely on the transgenic aspect of food sources.

The practice of raising animals as a food source has been a part of human society for a long time. A part of this process has been the use of selective breeding to produce the finest animals possible. This has usually been considered an ethical practice, and needed for man's survival. The use of transgenic animals in food sources can be thought of as another method of selective breeding, although the technology is more advanced. Transgenic breeding is a much more efficient method than selective breeding with respect to producing a species with the desired trait; this enables genetic improvement of traits to be achieved at a greatly accelerated rate (Harper et al., 2006). By carefully selecting the exact gene(s) needed to add a desired trait, and by eliminating the time required to randomly screen for a desired trait produced by breeding, prime specimens can be produced faster.

The main issue with transgenics use as a food source is how they would be raised. Normally, food sources are raised on farms in open areas. With transgenic animals, they cannot be bred openly, as the animal could escape into the wild and inter-breed with natural populations and have a potentially disastrous effect on the natural ecosystem (Matheson, 2004). The intricacies of any large ecosystem are so complex, that it is almost impossible to predict the results of such an instance (Perzigian, 2003). Thus, if transgenic animals are to be raised for food, very careful concerns should be made to prevent their possible escape. An example of an acceptable measure taken to account for this danger is seen in Aquabounty's superfish, which

have been bred to be sterile. This eliminates the ecological danger if they escape, for they can produce no offspring, which minimizes the effect on the ecosystem.

In this case, with the exception of superpig that suffered so badly he had to be euthanized, transgenic superfish are not worse off than they would be if they were not genetically engineered (Rollin, 1996). Whether transgenic animals are used in the food production process, animals are still going to be raised as a source of food. The fact that they are being genetically altered does not change their fate. This process can be considered to be ethical, so long as any failures like superpig are immediately euthanized to minimize suffering, and attention is paid to preventing possible escapes. While this may be an expensive task, it is necessary to protect wild species from any kind of harm that could be caused by exposure to transgenic animals. As with the other categories, the welfare of the transgenic animals must be paid attention to in great detail.

Scientific Model Ethics

While some transgenic animals are created with a specific purpose in mind, for example the study of a disease such as Alzheimer's disease or for xenotransplantation, other transgenic animals are created for the purpose of determining the function of a newly discovered gene or protein. These animals have specific genes knocked-out to block their expression, or over-expressed to enhance their expression, for the purpose of determining the effects in vivo. These scientific models do not directly produce a new pharmaceutical, organ, or food, but they provide knowledge. While there may not be an immediate reward from such a transgenic experiment, the ultimate benefits must be considered when determining whether such an experiment is ethical.

Many experiments of this kind attempt to lay the foundation for future transgenic studies. One such example is the creation of the smart mouse. In this case, the introduction of a single gene into the mouse's genome resulted in mice that learned faster, solved tasked more quickly, and maintained accelerated brain function well into adulthood (Harmon, 1999). This discovery allows the potential development of a gene therapy treatment for humans who suffer from brain deteriorating diseases such as dementia, and is a significant achievement in memory and learning research (Harmon, 1999). Another experiment in this category is supermouse. In this experiment, a human growth hormone gene was inserted into a mouse, and as a result the mouse's offspring expressed the gene and grew to an abnormally large size. While nothing was directly gained from this study, it did help researchers understand how transgenic genes are expressed, which in turn led to further studies for immediate benefits such as Alzheimer's mouse or oncomouse (Klein, 1995). In this category, one of the most recent transgenic scientific models is ANDi the monkey. ANDi was the first genetically modified monkey, a very important step in transgenic research as primates might provide more accurate models relative to humans. Although ANDi did not express his green fluorescent protein transgene (Chan et al., 2001), a subsequent monkey did (Sasaki et al., 2009), proving that the entire transgenic process can work in primates. The creation of ANDi might lead to the creation of primate disease models, for example for Alzheimer's, diabetes, or heart disease (Trivedi 2001). While various mouse models have been used to study these diseases in the past, primates likely will model human diseases better than mouse models.

While none of these experiments resulted in a direct benefit for society, the overall benefit of these studies is so great that they must be considered ethical, especially as there appeared to be minimal suffering on the part of the animal subjects. These experiments set the

foundation for future transgenic studies, and scientists have already learned much from them. They have helped scientists determine how to best express a transgene in each species, how to more effectively create a transgenic animal, and how to control the site of integration. If not for these experiments, transgenic research would not have reached the point where it stands today, and we would have no successful models to even begin our discussion of ethics.

While these biological models are an ethical part of transgenic research, it is important that ethical standards are followed with them as in all transgenic categories, to ensure that animal suffering is minimized, transgene integration is controlled, and the animals cannot be created merely for the sake of creating them, they must be part of a larger plan. Animals must always be cared for and treated humanely, which includes proper pain management and health care.

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PROJECT CONCLUSIONS

The engineering of an animal's DNA to contain a foreign gene is termed transgenesis, and creates a transgenic animal. This additional genetic information, called the transgene, acts to give the animal new properties it would not normally have in nature, which makes them very useful in the fields of biology, medicine, and food production.

The first step used in creating a transgenic animal is to clone the transgene of interest. To clone a gene means to make copies of it. Copies of the transgene are usually created using the process of polymerase chain reaction (PCR), which uses a thermocycler to repeatedly alter the temperature of a reaction tube in a series of programmed stages. The outcome of PCR is to amplify millions of copies of the selected transgene. The amplified transgene is then inserted into a cloning vector, such as a plasmid DNA or viral DNA, which helps further amplify and purify the transgene. The transgene is placed under the control of a promoter that controls in which tissue the transgene is expressed. Following transgene cloning, two main techniques are usually used when creating transgenic animals: 1) microinjection into a pronucleus, or 2) manipulating embryonic stem (ES) cells. During the first technique, small amounts of the DNA solution containing the cloned transgene are microinjected into the male pronucleus of a newly fertilized zygote prepared by *in vitro* fertilization (IVF). The IVF embryo is cultured to the blastocyst stage, and then implanted into the uterus of a foster mother for birth. If the pronuclear host DNA incorporates the foreign transgene, all cells derived from the zygote will contain it. During the second method, the solution containing the cloned transgene is inserted into embryonic stem cells using chemicals or viruses. The ES cells are then screened for transgenic positives, and then implanted into blastocyst as described above. The main advantage of the ES

technique is it allows gene targeting using homologous recombination, but not all cells of the animal are positive (the animals are mosaics), so these animals must be further bred to other mosaics to make pure transgenics. No matter which technique was used, the process is inefficient, so all offspring must be screened for positives. This is usually done by PCR or by Southern blot to detect the presence and location of the added gene.

Transgenic animals have many potential uses in society, and can be grouped into 5 main categories. Disease models are transgenic animals engineered to model specific aspects of human diseases in lab animals. These animals can be used to test new drugs and study disease formation, but these animals can suffer as the disease progresses. Transpharmers are animals modified to create pharmaceuticals in their milk or blood. In most cases these animals do not suffer, and they are a very valuable benefit for the drugs they produce. Another use of transgenic animals is to use them as a food source. Various "super animals" have been created that contain growth hormone genes that allow them to grow more rapidly and have more muscle mass. Although some of the mammals in this category have been unhealthy and suffered due to the transgene, the superfish appear to be a success. Xenotransplanters are engineered to provide tissues or organs for a human recipient. These animals would lose their lives to attempt to save human lives. The last category of transgenic animals is scientific models which are used to determine the effects of under-expressing or over-expressing specific genes *in vivo* to help determine their functions.

Transgenic ethics weighs the benefits to society for a particular animal versus the potential for animal suffering. Because the benefits and potential suffering varies considerably between the different categories, these cases should be considered on a case by case basis. Based on the research performed for this project, the authors believe that transgenic animals should be

further pursued, but we unanimously conclude that strong oversight should be taken to assure the health and welfare of these animals. We applaud the use of Institutional Animal Care and Use Committees (IACUC), whose job is to oversee animal research at universities and within companies. In particular, the authors believe the area of Xenotransplanters needs more research before being used large-scale, as these animals could contain animal viruses that transmit to human recipients. So, more research should be performed to identify potential viral threats, and to ensure they are screened in the donors. The authors strongly favor the use of transgenic animals as a food source, especially in view of the increasingly starving planet, as long as the animals do not suffer prior to sacrifice, and pose no threat to the eco system if they escape. Regardless of the category of transgenic animal, if it is determined that the animal is suffering or the experiment did not go as expected, the animal should be immediately euthanized to prevent unnecessary suffering.