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TRANSGENIC ANIMALS

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ABSTRACT

In this IQP we examine the social issues surrounding transgenic animals in terms of the biology, ethics, and legality of transgenics. After evaluating these issues and their societal impact, we feel the medical and social benefits outweigh any animal suffering and that transgenic animals can greatly benefit society.

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EXECUTIVE SUMMARY

The Purpose of this IQP is to examine the social issues surrounding transgenic animals and evaluate their impact on society. With the recent advances in genetic engineering and biotechnology, the creation of animals with foreign genes is now taking place. Several methods have shown success to date, and some are advantageous for specific purposes. Retroviral insertion consists of modifying a retrovirus to make it harmless, engineering it with a gene of interest, and allowing it to infect target cells. Pronuclear microinjection involves directly injecting the gene of interest into the pronucleus of a fertilized zygote. Homologous recombination of embryonic stem cells allows targeted insertions to be performed in mice (unlike the methods mentioned above); unfortunately ES cell lines have not been successfully used in larger mammals. Nuclear transfer, however, makes use of homologous recombination technology in larger mammals, allowing targeted gene insertion into mammals for disease model or pharming purposes. These methods continue to be elucidated at an astounding rate.

With these methods of transgenic animal creation come many potential uses of transgenic animals. Mice and other mammals can be used as disease models through targeted deletion or modification of a gene or genes. Farm animals could potentially be engineered to produce more meat or genetically modified meat. Proteins can be 'pharmed' from the milk of large mammals for pharmaceutical use; and in the future, the potential even exists for raising pigs as a source of human organs. Unfortunately some of the largest problems with transgenic technology lie with ethical and legal considerations.

Three things to consider when evaluating the ethics of transgenic animals are man's relationship with nature, responsibility of scientists and public's attitude. A person's view of how man is related to nature affects whether or not they approve of developing transgenic animals. Scientists have certain responsibilities to the public such as educating them about their research, and keeping the public updated on developments. The more educated the public is the more they understand what the scientists are doing and the more they support it. There are many concerns that have been voiced about developing transgenic animals such as animal rights, environmental concerns, and their effect on humans. Simple regulations can keep all of these issues in check.

Accomplishing the task of making a transgenic animal is not easy or cheap. In order to encourage scientists to explore this field of biotechnology some type of intellectual property protection should be in place. The first transgenic animal to ever be patented in the US was a mouse known as Oncomouse, which was created to develop mammary tumors mimicking breast cancer in humans. Harvard was granted a patent for the mouse in the US, Europe, and Japan. The decision to grant a patent for Oncomouse in Canada is still under debate. Issues that are debated include whether or not animals can be considered patentable subject matter under current patent laws and whether or not current patent laws should even apply to such biotechnological advances because these laws were made long before the creation of transgenic animals and do not consider any moral or ethical issues. Many environmental and animal rights organizations feel that granting a patent for an animal could lead to dangerous environmental damages and promote animal suffering. However, these organizations seem to fail to realize that even if transgenic animals could not be patented they would still be made. Instead of opposing

patents, those organizations should focus on lobbying for laws that regulate the release of transgenic animals into the wild and the use of these animals' labs. In countries where transgenic animals are patentable issues such as the enforcement of these patent laws have also been a concern. Companies that own the property rights to certain transgenic animals have debated with researchers over fair agreements of use and are slowly coming to agreements that try to restrict research as little as possible. With organizations in place to help provide better guidelines for future patents, patents on transgenic animals will help promote biotechnological advancements by increasing disclosure of new developments and by providing an incentive to take on 'risky' and expensive research by providing intellectual property protection.

The development of transgenic animals holds many hopes for the future, and this IQP team has concluded that the benefits of this technology outweigh public concerns so long as tight restrictions are placed on their production. Ethical review boards composed of a variety of experts and laymen should be set up to establish how strong the medical benefits are likely to be for given experiments, as well as the likelihood of animal suffering. Hopefully such a committee will be able to assess what will be affected by transgenics, and be able to qualm the fears of the public.

PROJECT OBJECTIVE

The purpose of this IQP was to investigate the social issues surrounding transgenic animals. These issues include moral and legal concerns. Through investigation of these issues we examined the impact of transgenic animals on society.

CHAPTER 1

TRANSGENIC ANIMALS: DESCRIPTION AND CONSTRUCTION

Molecular Genetics and Transgenics

Introduction to Molecular Genetics

For hundreds of millions, perhaps even billions of years, DNA has been the integral molecule by which genetic information is transferred from generation to generation. It exists ubiquitously in viruses, bacteria, plants, and even the most complex mammals. The sum of DNA from which a specific organism receives its traits is termed the *genome*, and is unique between every individual in every species on the planet. The term *gene* refers to a sequence of DNA which codes for a single polypeptide chain (a building block of a protein) or, in few cases, an RNA molecule. These DNA products are the functional molecules of the organism; DNA is relatively inert, serving primarily as a blueprint for the creation of these molecules.

Genomic DNA in organisms is stored in chromosomes, which are self-replicating structures, each of which contain a defined linear string of genes. The number of chromosomes between species differs; bacteria usually bear one circular chromosome called a *plasmid*, which codes for all of the bacterial proteins necessary for survival and replication. In contrast, human somatic cells (non germ-line cells) contain two unique copies of each of 23 chromosomes, for a diploid total of 46 (meaning that each person carries two copies of each gene, often in different forms).

These different forms of the same gene often exist between individuals in a species; this phenomenon explains different hair colors, heights, and thousands of other traits not shared between two individuals. The different forms of a gene are called *alleles*, and are the basis for the genetic diversity shown between all organisms. Each allele contains a slightly different DNA sequence, which in turn can alter its product in many different ways; some of which can be harmful or fatal. The location of all the alleles of a single gene is called a locus, and describes the chromosomal location of the gene.

In every organism, DNA is composed of 4 nitrogenous bases – adenine, thymine, guanine, and cytosine; the linear sequence of these bases determines the amino acid sequence of the polypeptide chain, which is the product of a single gene. Each amino acid in this sequence is determined by a 3-base sequence, or codon, in the DNA sequence. For example, three adjacent cytosines in DNA (in the correct reading frame) code for the amino acid Glycine. Because codons consist of three bases, each of which can be A, T, C, or G; 64 total codons exist, coding for 20 different amino acids and a stop codon which signals the 'end' of the protein. Because more than a single codon may code for the same amino acid, the code is termed *degenerate* (Cooper).

Equally as stunning as the universal code is the mechanism by which DNA replicates, discovered by James Watson and Francis Crick in 1953. As they deduced, DNA bears a double-helical structure; that is, it consists of two complementary strands 'woven' together to form a stable, double-stranded molecule. The two strands are bound together via interactions between opposing base pairs, which follow a specific base pairing rule integral to function: adenine always binds opposite thymine, and guanine binds opposite cytosine. In effect, each strand of a DNA molecule contains the

information to synthesize a complementary strand, which is exactly what it does in the proper conditions with the help of enzymes: the double-stranded molecule splits apart, and new bases are added to form complementary strands. The result is two identical, double-stranded molecules. The science of transgenics is made possible by these two important features of DNA: a universal mechanism of protein translation, and the ability of DNA (and cells) to replicate where each daughter molecule or cell retains the same genetic information.

Introduction to Transgenics

Transgenics is the science of altering an organism's indigenous genome by the addition, deletion, or modification of a DNA sequence or sequences. The applications and possibilities of such a science are still being realized, and even today seem endless. Technically, transgenic techniques have been practiced since bacteria were first used to produce proteins coded for by foreign DNA; this was achieved in the 1970s. While its application is very useful in producing some proteins for pharmaceutical use and many other research uses, bacteria lack the ability to make many post-translational modifications of proteins; that is, bacteria cannot add modifications such as sugars which may be necessary for full protein function. Plants and animals, however, have this important ability, and are very useful systems in the high-scale production of protein.

Another important function of transgenic animals (specifically mammals) is that they can be used to study diseases *in vivo* by altering the genome to produce a human genetic disease, which can then be studied in the context of an entire organism. A few applications that could come to fruition in the near future also exist; for example,

mammals that are engineered to produce more meat, or animals that are raised for organ farming (Xeno). Though these methods have not been perfected, much progress has been made. Each of these types of transgenic animals is produced best by a distinct method, each of which bears several advantages or disadvantages.

Development of such novel processes took a long time to develop; prior to the creation of the first truly transgenic animals were chimeric mice, first constructed in 1974 (Trans. Anim. Sci.). These mice were produced by the introduction of cultured cells of a specific strain into an embryo of a different strain. The resulting mice exhibited characteristics of each strain (Bradley *et al.* 1984). Although not transgenic in the sense that their genomes had been specifically altered with foreign DNA, these mice represented great advances in gene transfer technology. The first animals whose genomes had been altered intentionally with the introduction of foreign DNA were created with the use of retroviruses (Stuhlmann *et al.* 1984).

Transgene Introduction via a Retroviral Vector

Retroviruses are a specific class of viruses including HIV, which are characterized by an RNA genome as opposed to the standard DNA genome of other viruses. These viruses infect cells by creating a DNA transcript of their RNA genome, which is subsequently integrated into the host cell's genome. These new genes are then transcribed by the cell's own machinery. One RNA transcript serves as the genome of a new viral particle, while others are translated into proteins needed for structure, function, and cell transformation of a new virus. Essentially, the virus makes use of the cell's machinery to

replicate copies of itself, which then infect other cells and repeat the same process (Cooper).

The retroviral vector to be used for transgenesis is first genetically altered so that it is unable to replicate and cause infection in the target cells, but the genes that allow integration of the genome into the host cell are left intact (Harper). Then, using recombinant DNA techniques, the virus is altered to incorporate the transgene into its genome; therefore, subsequent infections of cells by the modified virus introduce the transgene. The retrovirus is then used to infect an early-stage cultured embryo, and some of the cells usually take up the transgene.

There are a few distinct advantages and disadvantages to this method of utilizing a retroviral vector to insert a transgene. One advantage of this is that only a single copy of the transgene is integrated into the genome; this is significant because multiple copies of the transgene are often transcriptionally silenced by the cell; they are simply turned off and do not affect the organism(Lois *et al.* 2002). Transgenes integrated by retroviral insertion are expressed more often than by several other methods. A specific type of retrovirus known as a lentivirus has recently been shown to yield the highest levels of cellular expression (Lois *et al.* 2002). This technique is also inexpensive relative to other methods of gene transfer.

Unfortunately, several disadvantages have been associated with this method; the most significant of which has been the high proportion of chimeric animals produced. Since a multiple-cell embryo is infected, the retrovirus will rarely infect every cell, and there is always high probability that germ-line cells will not be infected (Trans. Anim. Sci.). The result is an adult animal with many cells that do not carry the transgene, or an

adult unable to pass the transgene to offspring (Proc. For Trans.). This problem has recently been overcome by techniques that allow direct injection of the retrovirus before fertilization into oocytes (eggs) (Chan *et al.* 2001) or spermatogonial stem cells (Sci. Daily 10/01), which give rise to sperm. Other limiting factors include a limited transgene size (Trans. Anim. Sci.) and the possibility of recombination with other viruses, which can be disastrous (Monastersky, Gulezian 1997).

Pronuclear Microinjection of a Transgene Construct

Currently, the most widely used technique of creating a transgenic animal is direct injection of the transgene construct into the male pronucleus of a fertilized oocyte, immediately following fertilization. The pronucleus is the membrane and contained DNA initially carried by the sperm or egg; a pronucleus exists for each for several hours, until they fuse together to form a single diploid zygote. This is accomplished by harvesting the fertilized egg from a donor female, injecting the DNA of interest, and transferring to a recipient female. If the animal develops properly and expresses the transgene in the germ-line, it is known as the *founder* of a transgenic lineage (Trans. Anim. Sci.).

This technique was first successful in mammals in 1981 and is still the primary method of transgenesis of larger farm animals (Buy); more productive techniques are usually chosen for mice, which have been very vigorously studied in transgenics. Because of this, pronuclear microinjection is usually used in the pharmaceutical industry to produce large quantities of protein from animals such as goats and sheep (Trans. Anim. Sci.), which secrete large quantities of milk. Normally the protein is targeted to the milk of the animal for easy isolation (GTC Biother.). This high freedom of transgene

customization makes microinjection a very attractive method of creating transgenic animals.

Transgene Construct Creation

The first and most labor intensive step in the microinjection process is meticulous creation of a transgene construct, which will be injected into the pronucleus in its entirety. Several modifications and additions are made to the gene of interest to increase the rate of expression and integration into the genome. First, the gene of interest must be isolated from its original source, which may be a bacterium, plant, or even another mammal. The DNA is either isolated in genomic form or cDNA form; the genomic form of a DNA sequence is the sequence that occurs naturally in the endogenous genome. A cDNA gene is a modified gene in which all the non-coding regions are removed. The result is a smaller gene which consists of all coding DNA. For unknown reasons, genomic DNA has been shown to yield better expression; it is possible that the non-coding DNA somehow regulates or enhances expression (Rexroad). Once the cDNA or genomic gene of interest has been found, it is then cut out of the surrounding DNA by use of restriction enzymes, which act as scissors to cut DNA at a specific DNA sequence of a few base pairs (Betsch 1995). The isolated sequence can then be enjoined to other sequences with an enzyme called DNA ligase if the ends to be ligated match; that is, if the ends have been cut by the same restriction enzyme. This allows creation of various combinations of DNA from different sources.

Following isolation of the cDNA or genomic DNA of interest, many modifications and additions are required, the final product of which is called the

transgene construct. Perhaps the most important of these modifications is the addition of a promoter sequence upstream of the gene (transcribed prior to the gene). Promoter sequences serve to 'target' the protein product to a specific tissue or location (Trans. Anim. Sci.). In larger mammals, this sequence is normally used to target the mammary gland of the animal, so the protein will be directed to the milk. One other interesting potential use of promoters, which is still being perfected, is precise gene regulation; that is, control of when the gene is active. This could be achieved by insertion of a promoter which responds to a specific substance; when this substance is injected or ingested, the promoters would respond, causing transcription of the gene, and ultimately protein expression. Unfortunately, this can also be a hindrance if a natural diet contains substances which activate promoters; the gene could be over-expressed, potentially leading to health problems (Rexroad).

In addition to the promoter, untranscribed enhancer sequences upstream of the promoter and gene seem to help increase gene expression. Signal sequences located immediately upstream or downstream from the gene serve to target the protein's excretion or intracellular target – this is integral for greater ease in isolating the protein. At the terminal end of the construct, downstream of the gene, is a poly-A tail, which serves to assist accurate transcription of the gene (Trans. Anim. Sci.). Once these regulatory sequences have been added and joined via restriction digests and ligase, the transgene construct is complete. It is immediately sequenced to ensure that each piece was ligated correctly.

Before injection, though, it is necessary to produce a large quantity of the transgene construct for injection. This is usually accomplished by introducing the

construct into a bacterial plasmid which includes a resistance gene to an antibiotic such as ampicillin. The bacteria are then plated on media containing the antibiotic, and resultant colonies most likely contain the construct. Several colonies are then chosen and grown up in a large bottle of liquid media, and the reproducing bacteria all contain the identical transgene. The plasmid is then isolated from the bacteria, and the construct is once again removed using restriction enzymes. The final product is a large quantity of the construct for injection, some of which is used to test the transgene efficiency by transfection *in vitro*.

Preparation of Embryos and the Surrogate Mother for Microinjection

Following the transgene test, a line of mammals must be identified that bear advantageous traits such as large pronucleus size (for greater ease in injection), high frequency of embryo survival, and good response to super-ovulation. Once this strain is identified, the female is hormonally super-ovulated to produce more eggs during ovulation (www.buav.org 2000) - this is necessary to maximize the total number of embryos which incorporate the gene and survive to adulthood expressing the transgene. Immediately after super-ovulation, the donor female is mated with a fertile male as the recipient female is simultaneously mated with vasectomized (infertile) males to begin the reproductive cycle while avoiding pregnancy (Trans. Anim. Sci). Several hours after breeding of the donor (usually the morning after), the oviducts containing the embryos are removed, and the donor is euthanized. It is essential that the embryos are isolated prior to the first replication of DNA, otherwise the resulting animal will be chimeric, and is less likely to have incorporated the transgene into germ-line cells

(www.brinkmann.com). The embryos are then isolated from the oviducts by dissection or flushing, and the clumps of embryos are separated with a series of solutions. The separated embryos are plated in sterile media, placed under a microscope, and are now prepared for microinjection (Trans. Anim. Sci.).

Pronuclear Microinjection Method

Once the embryos are located under the microscope, a small pipette tip is used to hold each embryo in place while the microinjection takes place. The pipette used for the microinjection must be precisely crafted as not to damage the membrane of the oocyte. A small volume of the transgene solution is then picked up by the pipette, and the pipette is placed under the microscope in the same field as the target embryo. Once the embryo is focused in such a way that the pronucleii are visible, the pipette is carefully thrust through the membrane, cytoplasm, and finally the male pronucleus. Although injection into either pronucleus is feasible, the male pronucleus is normally larger and easier to inject. This technique is more difficult in larger mammals due to their opaque embryos, which make the pronucleus harder to view. If the injection is successful, the pronucleus should show visible swelling from the injected solution. Once the DNA is injected, the pipette is carefully removed. The embryo is moved to the far end of the plate, and the process is repeated for the next embryo (Trans. Anim. Sci.). Once the process is complete, the injected embryos are then transferred to the recipient female oviduct by pipette and brought to term. After birth, the genome of the animal can be screened for the presence of the transgene by a DNA amplification technique called Polymerase Chain Reaction, or PCR.

Unfortunately, the success rate in creating a founder which passes the transgene to its offspring is very low (as low as 1%) for many reasons (Rexroad). Even if the transgene is injected prior to the first replication of DNA, the time of integration is random; if it does not integrate by this time, it will most likely be present in a fraction of cells in the animal. The transgene has even been known to integrate at later developmental stages (Rexroad). Additionally, even if the transgene integrates at the correct time, the insertion site is random (Monastersky, Gulezian 1997); very often it can insert at a transcriptionally silent location in a chromosome, and will not be expressed (www.nii.res.in). Transcriptional silence can also occur when several copies of the transgene join together to form a linear array called a *concatemer*, which is often silenced by the cell after integration (Monastersky, Gulezian 1997). Another consequence of random insertion is interruption of an endogenous sequence, which would usually be required for survival or normal function of the animal. Unfortunately, it is also extremely expensive to maintain embryos though birth that may not even possess a transgene; since genetic screening is not possible until birth, the developing animals must be maintained regardless of their genetic states (Schnieke *et al.*). Even when animals are screened at birth, some cells will test positive for the transgene when they haven't incorporated it into a chromosome; the transgene can remain free-floating in the cell for an indefinite period of time, further complicating the screening process.

Despite these shortcomings, direct microinjection is still the most viable method for larger farm animals. As low as 1% success sounds, other methods of creating transgenic farm animals have met with even less success; targeted transgene insertion, which will be described below, succeeds an average of less than one cell per million.

Consequently, a success rate of 1% is often very desirable because of the lack of other successful techniques. Another important advantage is that if the transgene is incorporated into the genome at the pronuclear stage, the resulting animal will be 100% transgenic, meaning all germ-line cells are also guaranteed to carry the new gene (Transgenic Animals, www.brinkmann.com). Other techniques are often much more random, resulting in chimeric animals whose germ-line genetic profile is unknown. The relative ease of the microinjection process also makes it an attractive procedure since it consists only of creating a construct, cloning it, injecting it into a single-cell embryo, and transplanting the embryo; the rest is done by the surrogate mother as the pup is brought to term. For these reasons, pronuclear microinjection remains the method of choice for creating transgenic animals for pharmaceutical purposes.

Targeted Gene Modification via Homologous Recombination

With the techniques described above, scientists hold a powerful tool with which to modify organisms. By adding foreign genes to an organism, scientists can create farm animals which produce valuable proteins in their milk, test genes that may be factors in human disease, and test theoretical gene therapy procedures among countless other things. Unfortunately, a common disadvantage of the aforementioned techniques is a lack of certainty as to where the gene will integrate, if at all. Although retroviruses exist for the purpose of integration, the outcome is often an interrupted gene or transcriptional silencing, each of which is undesirable. An ideal system for the creation of a high rate of transgenic animals would clearly be one in which a specific gene at a specific locus is modified, added, or interrupted.

Fortunately, a novel system of this gene swapping exists in nature during the production of gametes, and is ultimately the mechanism by which genetic diversity exists. As mentioned above, each human somatic cell contains two copies each of 23 chromosomes. The two chromosomes of each pair are similar in the sense that they contain the same sequence of genes at the same locations, but it is important to note that they are not identical, because many different forms of a single gene may exist in nature. During the production of gametes in meiosis, the two homologous chromosomes can exchange information by a mechanism called *crossing over*. The result is two genetically unique chromosomes, each of which is passed on to a different gamete (each gamete receives only a single copy of each chromosome; at fertilization, the genomes of the egg and sperm combine to form a complete, 46 chromosome zygote). This ensures that each gamete contains genetic material distinct from all others, and explains why individuals born of the same parents are genetically unique.

Scientists have recently met with success in mice (more recently even goats and cows) using this method; in short, they created a transgene construct whose sequence closely matches the sequence of the endogenous chromosome. This is achieved by synthesizing a large, completely homologous stretch of DNA and replacing the endogenous gene to be removed with the transgene, which until recently was usually a selectable marker such as the neomycin resistance gene (this allowed for selection of transgenic cells by subjecting them to the antibiotic neomycin); obviously the exact sequence of this large homologous region must be known to accomplish the synthesis of an identical fragment. Unfortunately, even small genetic variations between members of the same species can severely decrease the efficiency of this method; consequently, the

construction of a suitable transgene construct is extremely time-consuming (Trans. Tech.).

Despite the time-intensive transgene construction, gene replacement via homologous recombination is still extremely useful in many ways; with the ability to target genes, scientists are now able to create more stable, accurate models for human disease by selectively targeting and 'knocking out' specific genes (www.nii.res.in). The first knockout mouse was created in 1987 (Intro.), and endless advancements have taken place since by defining the roles of many genes simply by inactivating them (Trans. Mice 1997). Even homozygous recessive disorders can be studied with this method; when two transgenic mice knocked out at the same allele are mated, a large fraction of the offspring will carry the mutated gene in each chromosome. This is a very useful technique for studying non-lethal loss-of-function of genes (Majzoub, Muglia 1996). The most notable example of this technology is the p53 knockout mouse developed at Baylor College of Medicine in Houston, TX. After the gene was inactivated, the mouse developed spontaneous tumors, which supported the importance of the p53 gene in tumor suppression (Phelan 2001).

While homologous recombination was initially restricted to knocking out or inactivating a gene, a mechanism for gene replacement has recently been discovered and is now becoming widely used. The Cre-loxP system, referred to as a 'site-directed recombination system,' (Majzoub, Muglia 1996) allows specific excision of sequences in genomic DNA. First recognized in the bacteriophage P1 (Az-Zubair, Kabir Banu), the loxP site is a sequence of DNA targeted by the enzyme Cre recombinase. When Cre encounters a pair of loxP sites, it cuts the intervening DNA in much the same way as a

restriction enzyme (Trans. Mice). These loxP sites must be in the genome at the site of transgene insertion (Az-Zubair, Kabir Banu), as well as flanking the gene to be inserted. When in the presence of Cre recombinase, both intervening sequences are excised and the transgene is incorporated between the genome loxP sites, which have been cut with Cre (Trans. Mice). The result is a chromosome with a transgene inserted at a specific site.

The Cre-loxP system also provides advantages to disease model mice by allowing tissue-specific gene expression to be regulated. It has often been a problem in the study of loss-of-function mutants that the organism is unable to survive without at least partial expression in some tissues. Cre-loxP allows loss-of-function to be studied in specific tissues, often resulting in an otherwise healthy animal. This feat is accomplished by creation of a transgenic mouse line with a tissue-specific Cre gene and promoter, and a transgenic mouse line with the gene of interest flanked by loxP sites. The result is a transgenic Cre mouse in which the only cells showing loss-of-function are those which express Cre (Cre/LoxP).

With this brief background in homologous recombination and the Cre-loxP system, it is also important to understand the method by which homologous recombination results in a complete transgenic animal. Unfortunately, this method is currently extremely inefficient; on average, only one of a million cells in culture take up the transgene by homologous recombination. More often, the entire construct is randomly integrated into the genome, completely defeating the purpose of targeted transgene insertion (S. Pati *et al*). Because of this, it is important that positive and negative selectable markers are present in the construct. These additional genes allow selection of cells that took up the transgene only by homologous recombination.

The positive selection gene used for this purpose conveys resistance to neomycin, which is normally an antibiotic lethal to cells. The cells which take up the construct also take up the neomycin resistance gene; therefore, any cells which take up the construct by any method will survive neomycin. Unfortunately this selection is not sufficient to identify the cells that have taken up the DNA by homologous recombination; cells which randomly integrate the construct will also survive the neomycin selection. Therefore a negative selection is subsequently required (Trans. Mice).

This negative selection is accomplished by insertion of a Thymidine kinase gene into a region of the construct distant to the transgene. This gene is responsible for conveying susceptibility to the antibiotic gancyclovir. Normally, cells are immune to the drug's effects, but after taking up the TK gene gancyclovir becomes lethal. This is useful because the many cells that take up the construct by random integration also incorporate the TK gene. Following neomycin selection, the surviving cells are subjected to gancyclovir, and cells that integrated the gene by homologous recombination survive, because the TK gene was not inserted into the genome. After both of these selections, the few surviving cells are those who have integrated the transgene at the proposed site (www.nii.res.in).

This method of homologous recombination in cells is used in two ways which will be described below: the transformation by homologous recombination of embryonic stem cells, and the nuclear transfer of cells transformed by homologous recombination.

Homologous Recombination via Embryonic Stem Cell Transfer

Embryonic stem cells are cells derived from blastocyst stage embryos which have not differentiated; therefore they have the potential to become any type of somatic or germ-line cell (Buy). Upon harvest from the embryo, the cells are grown *in-vitro* in the presence of a growth factor known as Differentiating Inhibiting Activity, which prevents differentiation of the cells. They are also required to grow in the presence of a feeder layer of cells, usually fibroblasts that have been prevented from dividing (www.nii.res.in).Consequently they are able to be grown indefinitely in culture. Like most other mammalian cells, the uptake of foreign DNA in ES cells can be induced through electroporation, in which an electric current is used to disrupt the cell's membrane and thereby allow uptake of DNA (Harper 1999). Although ES cells are ideal targets of homologous recombination, random insertion of a transgene construct is also a viable and less time consuming option, though often less successful. Once genetically altered by either method, these cells can be placed into embryos and contribute to the genetic profile of the resulting animal (Buy). For these reasons, ES cells are ideal targets for homologous recombination. Unfortunately, only a few strains of mice have been found with ES cell lines that have been proven to contribute to the creation of transgenic individuals; ES cell lines have been identified in cows and sheep, but none have given rise to transgenic animals (S. Pati *et al*).

Once the ES cells which have been successfully altered are selected for by positive/negative selection, the target blastocyst is harvested from the donor mother. Several ES cells are then injected into the inner portion of the blastocyst, several of which are then grown in a pseudo-pregnant host which carries the animal to term. The resulting animal is chimeric, containing DNA from the blastocyst as well as the modified ES cells,

and very often bears the transgene in its germ-line, though this is not guaranteed (www.nii.res.in). To verify contribution, the ES cells used often contain a gene for a coat color which differs from the endogenous homologue; therefore, mice with two coat colors are transgenic (Manip. Mouse Gen.). To verify germ-line contribution specifically, PCR or southern blots must be run. If the animal is indeed germ-line transgenic, it is usually mated with another transgenic animal which carries the same gene at the same allele (Trans. Mice). The result is a generation of mice homozygous for the altered allele which can stably transmit the gene to offspring.

Homologous Recombination via Nuclear Transfer

To address the problem of creating targeted mutations in larger mammals such as sheep and cows, the process of nuclear transfer, or cloning, is ideal. Nuclear transfer refers to the method by which the nucleus of a donor cell is transferred to an unfertilized egg whose own nucleus has been removed or *enucleated* (Schnieke *et al.* 1998). The result is an egg containing the exact genetic material of the donor cell. Though the molecular mechanisms which allow the process to work are not well understood, several cell types are known to be ideal donors for nuclear transfer (Schnieke *et al.* 1998). Usually fetal fibroblasts or other embryo-derived cell types are used for this purpose, but recently a cloned sheep named Dolly was created through the nuclear transfer of an adult mammary epithelial cell (Svaren). Dolly was subsequently proven to contain genetic information identical to that of the donor, proving that in some cases even the DNA of differentiated adult cells can be injected into an egg and result in a healthy, genetically identical organism.

The greatest potential of nuclear transfer science lies in the production pharmaceuticals by large mammals. Nuclear transfer is currently the only method by which a large transgenic farm animal can be created through homologous recombination. While pronuclear microinjection is often successful in farm animals, the site of integration cannot be controlled, and injection often affects expression level or interrupts endogenous gene sequences. Likewise, ES cell modification allows for genes to be introduced or knocked out at specific locations, but the technology is not yet available in farm animals. By controlling the locus of integration, the highest expression levels are usually shown, and transcriptional silence and endogenous gene interruption are avoided. Expression levels can also be monitored *in vitro*, whereas expression levels following microinjection can only be monitored in the newborn animal (S. Pati *et al*). This allows scientists to avoid costly procedures when they are not necessary, and to maximize the expression levels experimentally in culture.

With the advent of targeted gene insertion or deletion in larger mammals came the potential to create transgenic mammals for the purpose of *xenotransplantation* – the transplantation of organs from mammals to humans. While human to human transplants are ideal, the demand for organs unfortunately far exceeds the supply. Pigs are the primary target for xenotransplantation studies because of their physiological similarity and size to humans. Unfortunately, pigs bear a surface protein that humans and some other primates have lost. This results in the human immune system rejecting the organ because it recognizes the surface protein as a foreign antigen and subsequently destroys the organ. Using targeted deletion of this gene, scientists are hopeful that pigs lacking the surface protein can be bred and used for human transplants. Currently, two groups have

concurrently succeeded in knocking out a single allele of the gene, resulting in pigs expressing only half the normal protein (Lai *et al.* 2002). Scientists have also attempted to mask the antigenic protein with another protein to alleviate the immune response; this successfully eliminated the hyperacute rejection, but still only allows primates to survive for a few months (Lambrigts *et al.* 1998).

Transgenic Animal Creation via Sperm Injection (ICSI)

During the development of nuclear transfer techniques, scientists also explored the possibility of mixing exogenous DNA fragments with membrane disrupted sperm heads. Scientists had earlier experimented with mixing live sperm heads and exogenous DNA – the result varied drastically from 0-100% for transgenic embryo creation (Brinster *et al.* 1998). In this latest study, scientists disrupted the membrane of mouse sperm using a detergent solution, effectively "killing" the sperm (Kimura *et al.* 1998). Despite this, the sperm heads mixed with DNA show an extremely high frequency (up to 95%) of embryo transgenesis.

The process of injecting transgenic sperm into oocytes is simple relative to other methods. The transgene is first identified and isolated – in this case the transgenes used were the reporter genes GFP and β-galactosidase, which allow expression to be monitored visually. The sperm are then subjected to membrane disruption by a detergent solution, freeze-drying, or freeze-thawing (A.C.F. Perry *et al.* 1999). After membrane disruption is verified, the sperm heads are incubated for 1 minute with the transgene, and immediately injected into the recipient oocyte. Following verification that the embryos contain the transgene, they are then transferred to surrogate mothers and brought to term.

Unfortunately, this method is not yet well understood; scientists are still trying to discern whether the exogenous DNA interacts with the sperm prior to injection, and if so, how this occurs. So far results seem to support this hypothesis; for example, sperm heads washed following incubation with the DNA still support development of transgenic offspring (A.C.F. Perry *et al.* 1999). Before the method can be practically applied, though, it must be thoroughly elucidated and adapted to mammals.

CHAPTER 2

TRANSGENIC ANIMALS: CLASSIFICATION AND EXAMPLES

Transgenic Animals in Xenotransplantation

Because many thousands of people die each year awaiting organ transplants, it has been suggested that using organs from foreign species may be beneficial due to the severe lack of a human organ supply. Even for spinal or neural disorders, it maybe become possible in the near future to *xenograft* foreign cells into a recipient to restore at least some function (Xeno). Putting into practice such an idea is obviously a great undertaking; ironically, the largest obstacle is the immune system of the recipient, which is designed to destroy any foreign cells entering the body. Even if the immune response is somehow overcome, the threat of foreign viruses being carried to humans via organs is a great one. Despite these problems, the potential benefits of xenotransplantation and xenografting are well worth exploring.

Before researchers can explore many of the questions inherent to xeno-transplantation, a suitable donor species must be selected. Several factors must be taken into consideration to identify the most efficient donor. Most important of these is that the donor organs are close in size and physiology to humans' own. Next, production impacting factors such as litter size and reproduction time must be addressed. Unfortunately, many of the animals who fit these criteria must be discounted for ethical reasons; in addition to a slow generation time, breeding other primates for organ farming is considered by many to be ethically intolerable. Most scientists currently support pigs as the prime candidate for organ transplants for the reasons stated above (Lai *et al.* 2002),

and there is less ethical debate since pigs have been raised as a food source for centuries. The next step is to address the problems associated with transplanting foreign tissue or organs to humans, and to discuss the risk of viral infection.

The most significant problem in xenotransplants is the hyperacute immune response, which occurs when the body's immune system recognizes a foreign molecule and mounts an immune response to rapidly destroy it. In this case, many primates have lost a cell surface sugar through the process of evolution which is common to other mammals. When this sugar, alpha 1-3 gal transferase, is detected by a corresponding antibody, the immune system destroys the organ within hours. This problem was first addressed by creating pigs with a human complement-inhibiting protein, which masks the hyperacute response significantly. Unfortunately, the organs are still able to last for only a few months (Kaiser 2002). It is now presumed that the only effective solution is to knock out the gene that codes for alpha 1-3 gal transferase by transgenic means to ensure that all cells exhibit the modification.

One group of researchers has recently succeeded in knocking out a single allele of the gene in pigs (Lai *et al.* 2002). They accomplished this through nuclear transfer of cells genetically modified by homologous recombination at a single allele (Lai *et al.* 2002), as described in Ch.1. Clonal fetal fibroblasts were used as nuclear donors and enucleated pig oocytes were the recipient cells. A reverse transcription polymerase chain reaction assay was performed on the cells to further isolate cells that had successfully been knocked out. Following nuclear transfer, the embryos were transferred to a surrogate mother. The group verified that several of the piglets did contain the modification. Although this is a great step in surpassing the rejection process,

homozygous null pigs are required for xenotransplantation use, which can be acquired simply by breeding two heterozygotes. This has already been accomplished in mice with no ill effects, which indicates that it should not be detrimental to pigs (Thall *et al.* 1995). If the process is successful, it could potentially eliminate all of the rejection stages, and will almost definitely eliminate hyperacute rejection.

Unfortunately, another potential problem with xenotransplantation exists: the spread of pig viruses to humans. Of greatest concern to scientists is a retrovirus known as PERV which is endogenous to pig cells and is passed to offspring through sperm and eggs (Paradis *et al.* 1999). The fear is that the virus could jump the species barrier and cause infection and epidemic in human populations. A research group recently concluded a 12 year study of patients who had been xenografted with living pig tissue (Paradis *et al.* 1999). PCR analysis and immunoblotting of patients' DNA indicated no PERV infection, supporting the idea that it may not be harmful to humans. Even if PERV is subsequently proven to be harmless, there is still a fear that other as yet unknown viruses may surface and cause infection, so scientists must proceed with caution.

If these problems can be overcome, the future for organ transplants is very bright indeed. Because the only barrier to human-human transplants is organ supply, the primary advantage to the use of foreign cells and organs in human transplants would be a ready supply of organs awaiting transplantation. This would greatly reduce the number of deaths attributed to lack of an available and compatible organ, which reaches an estimated 100,000 per year. In addition, many medical costs could be alleviated if organs are readily available. It would eliminate the need for long-term medical treatment for organ failure, such as kidney dialysis machines. In regard to xenografting, a very strong

potential use lies in neurodegenerative diseases such as Parkinson's, where Dopamine producing neurons are affected. Using xenografting techniques, dopamine-producing cells could be grafted into the brain for at least a modest improvement. Physical injuries to the spinal cords could also potentially be repaired in this manner; a xenograft could help to repair nerves that have been damaged. If the problems discussed above can be overcome, xenotransplantation could ultimately save tens of thousands of lives each year.

Transgenic Animals: Food Sources

Altering the genome of an animal has been of interest to the field of agriculture since the technology to do so had first arisen. Throughout time, many breeders have bred the best of their livestock to produce cows that gave the most milk, to make the largest pigs for more meat, and to produce livestock that in general had the most preferable characteristics. All of this had been done using what resources they had to generate the best livestock and the most profit. Applying biotechnology to agriculture would allow one to make animals that produced more meat, and leaner meat, to create animals that were resistant to disease, and to create animals that produced food for human consumption faster (such as more milk from cows and more eggs from chickens). This technology could be used to create a higher rate of food production more cost efficiently and create a surplus of food to feed the entire nation.

Transgenic Pigs for Food:

Attempts to apply transgenic technology to create transgenic animals as a source of better meat, has not been completely successful yet. There was one such attempt in the late 1980s that involved adding the bovine growth hormone gene to pigs to try to increase

the meat yield and produce healthier meat (Pursel et al., 1989). Using a transgene that consisted of bovine growth hormone (bGH) along with a strong constituitive promoter (for metalliothionine, MT), 7000 pig eggs were microinjected. The efficiency of production of transgenic pigs is low in comparison to mice, and only 8% of the 7000 injected eggs developed to birth, and only 7% of those pigs were classified as transgenic. The transgenic-positive pigs were subsequently tested for levels of the foreign bovine growth hormone in their blood. The levels of MTbGH found in the transgenic pigs ranged anywhere from 5-944 ng/mL which yields about a 0.6% integration efficiency of the transgene. The levels of foreign growth hormone were also taken for 30-180 days after the birth of the second generation offspring. The average foreign growth hormone found in the second generation pigs ranged from 23-1600 ng/mL. The differing amounts of MTbGH detected in the plasma of the pigs are probably due to the influence of the chromosomal position of the transgene (meaning that the transgene may be in different positions on each pig's chromosome), and the level of activity of the MT promoter (Pursel et al., 1989).

The pig that resulted from the initial microinjection was called the 'founder' pig of that line, and founder pigs were bred and each generation from the line of the founder pig was studied. The level of MTbGH of each generation was determined and the results indicated that the level of MTbGH that was present in the founder pig remained fairly constant throughout each generation. Tests were also done to determine which organs expressed MTbGH mRNA. It was found that it was mainly expressed in the pig's liver, kidney, adrenal gland, and pancreas. They found some expression in the duodenum, the lung, and the gonads (Pursel et al., 1989).

The main focus of the experiment was to try to create larger pigs to produce more meat. They found that the founder group of pigs did not seem to exhibit a dramatic increase of daily weight gain. However, they seemed to be more efficient at converting their food into bodyweight and muscle than the control pigs that did not have the MTbGH transgene. It was determined that the MTbGH pigs were 16% more efficient than the control group at converting food into bodyweight. It was also determined that they had an increased amount of selective organ weight gain, and an increase in long bone weight and circumference than the control group. The experiment proved to stimulate pig growth, enhance food conversion to protein, and decrease the amount of subcutaneous fat (fat underneath the skin) using the MTbGH transgene (Pursel et al., 1989). However, unforeseen physiological side effects wreaked havoc on the pigs. The pigs eventually developed gastric ulcers, arthritis, dermatitis, and renal disease.

Although some of the first attempts to create transgenic pigs resulted in damaging physiological side effects, there are some preliminary results that indicate transgenic pigs are being created that do not seem to have the same physiological side effects. Using a growth hormone construct with an MT promoter and a cDNA sequence of porcine growth hormone gene, 2.8% of the embryos injected (using the pronuclear microinjection technique) developed into live born transgenic pigs. Through different experiments, researchers determined that the amount of transgene detected in the plasma of the pigs could be regulated through a high zinc diet (Nottle et al., 1999). They hoped that determining how to regulate the level of transgene expression could help them avoid the physiological side effects that other researchers had run into in previous related experiments. Other results indicated that the transgenic pigs had an increased growth

rate, a decrease in food intake, an increase in muscle mass, and a decrease in carcass fat when compared to the control pigs (Nottle et al., 1999). So far, the pigs have not developed any negative physiological side effects and further experiments are being done.

Transgenic Cows for Food:

Improving the yield and quality of meat has not been the only interest in applying transgenic technology to the field of agriculture. The fact that humans rely heavily upon the proteins found in milk has led researchers to focus on developing transgenic cows that will alter the traits of milk to increase the range of dairy manufacturing options and improve the properties of milk, such as making it more disease resistant. By adding transgenes that will be expressed in the mammary glands of animals, one can change the physical and functional properties of the protein system that affects milk production.

For example, experiments have been done that allow antimicrobial human lysozyme to be expressed in the mammary glands of mice. Human lysozyme is a protein "that attacks the polysaccharide coat which makes up the cell wall of bacteria. By weakening the cell wall, it will be unable to resist osmotic pressure, so the build up of pressure will then cause the cell to lyse" (Human Lysozyme, 1998). Creating a transgene to express this protein in the mammary glands, it was hoped that the antimicrobial activity would decrease the levels of bacteria found in milk. It was found that expression of human lysozyme in the milk of transgenic mice significantly decreased the growth of the organism *Pseudomonas fragi*, which is a cold-spoilage organism, *Lactobacillus*

viscous, and a strain of *Staphylococcus aureus* that causes mastitis (Maga et al., 1998). These findings demonstrate that transgenic animals created to express human lysozyme in their mammary glands increase the antimicrobial properties of milk. These findings, when applied to transgenic cows, could possibly decrease udder diseases and produce healthier milk for humans to consume.

Another experiment done with transgenic mice expressing human lysozyme in their mammary glands has also shown that the milk produced has some other altered physical and functional properties of the milk. The milk taken from these transgenic mice showed a 35% decrease in rennet clotting time, smaller micelle size, and a 2.5-3 fold increase in gel strength than control milk (Maga et al., 1995). Smaller micelles result in an increased amount of surface area available for curd to collect which would lead to an increase cheese yield. Mice have shown that altering the physical and functional properties of milk do yield results that are applicable to the dairy industry. Improving the quality of milk and increasing the amount of cheese that one could yield from one cow are one of many possibilities present. This ongoing research with the dairy industry continues, and transgenic dairy cows are expected to be used for producing human consumption milk within the next two decades (Murray et al., 2000).

Transgenic Animals: Disease Models

More than 3000 genetic diseases have been identified, and the rapid advances made in transgenic technology when applied to animals create a variety of new possibilities in the field of medicine. Methods developed to transfer foreign genes into a genome create the possibility for animal disease models. The areas of interest to

researchers include the fundamental causes of diseases and effective treatments. (Mepham et al., 2002). Transferring genes that are known to cause diseases in humans into an animal, such as a mouse, can mimic certain aspects of that human condition to aid further analysis. This creates an incredible research tool that allows one to learn about the causes of a disease, and to test treatments on the animal to cure the disease without having to test on humans.

Cancer Mouse

Cancer has and continues to be one of the leading causes of death throughout America. It comes as no surprise then that as soon as biotechnology made it possible to create animal disease models, animal models of human cancer have been produced in numbers. There are many different triggers to cancer, including smoking, nutrition, chemicals, and radiation. All of those triggers can be classified as external triggers. There are also internal triggers, specific genes termed oncogenes, that have been identified that help trigger breast, colon, brain, and other forms of cancer. Usually some combination of both internal and external triggers results in one developing cancer. The exact causes and potential cures are still being studied. Researchers have created a number of mice that model different types of human cancer.

One such cancer mouse has been created to be highly sensitive to tumor promoters. A transgene was constructed that consisted of a 0.95 kb DNA fragment and an initiation codon fused to an oncogene known as v-Ha-*ras*. The fused gene was then microinjected into 12-hour-old fertilized inbred FVB mouse embryos. Four different lines of transgenic mice carrying 3-10 copies of the transgene were created. The lower

backs of the mice were then shaven and a solution containing 5 μg of phorbol 12-myristate 13-acetate (PMA, a tumor-promoting compound) and 100 μl of acetone was applied to the shaven area three times a week for 4-6 weeks. The mice were then monitored at least twice a week for tumor growth. Within 6-8 weeks, 36 out of the 37 transgenic mice receiving the PMA acetone solution developed papillomas and the relative numbers and sizes of the papillomas seemed to be dependant on the amount of the solution applied to the mice. Two out of 8 transgenic mice treated with acetone alone developed only two papillomas. None of the non-transgenic treated mice developed papillomas. Twelve out of the 37 PMA acetone treated transgenic mice developed malignant skin tumors after 6-12 months of the application process (Leder et al., 1990). Since not all of the mice reached the 12-month mark after they began the PMA acetone applications, the researchers are likely to believe that had the mice been given more time, more of them would have developed malignant tumors.

Tumors usually develop from a two-step event. The first step depends on an irreversible mutant gene that will predispose one to developing cancer and the second stage depends on a reversible event that causes tumor promotion, such as the application of a tumor-promoting agent in the case of the mice. The creation of these tumor-promoter sensitive mice will allow researchers to use this strain to screen different tumor promoters and to test blocking agents to tumor growth. This will serve as a valuable tool in developing potential anti-tumor agents for humans.

Oncomouse

The most famous cancer mouse, as well as the most famous transgenic animal, is called Oncomouse. Created by Timothy Stewart and Phillip Leder of Harvard, Oncomouse was the first transgenic animal ever to be patented. Oncomouse was created by injecting a modified c-myc gene into mouse embryos. The c-myc has been previously shown to cause cancer. By modifying the regulatory function of the gene, they found that when inserted into the mouse embryos, the transgenic mice had a tendency to develop mammary tumors, thus modeling breast cancer in humans. This mouse model can now be used to test potential breast cancer treatments without having to use human testing. Oncomouse became the most famous transgenic animal because of the debate that surrounded the issue of patenting this mouse (which will be discussed in a later chapter).

Alzheimer's Mouse Model

Alzheimer's disease is the nation's fourth biggest killer (King, 1995) and up until recent developments, a transgenic disease model did not exist for this disease. Early attempts to create a mouse model for Alzheimer's disease had failed, and the cause of the disease at that time was still unknown. In 1995, research supported by Exemplar Corp. (Games et al., 1995) created the first pre-clinical model for Alzheimer's disease.

The Alzheimer's mouse was created by taking a mutated gene that is known to cause an early onset version the disease in humans. This gene is a mutated beta-amyloid protein, which is a toxic fragment of the normal protein (Kolata, 1995). This gene was inserted into mouse embryos, and the brains of the mice that developed were studied. It was found that amyloid deposits occurred in certain parts of the brain of these mice after about 3-6 months. These amyloid plaques increased in size and density with age, and

showed that the plaques caused neuronal degeneration and thinning synapses (Games et al., 1995). After about 8 months, the pattern of the size and density of the amyloid plaques resembled that of Alzheimer's disease in humans (Games et al., 1995).

This animal model was a landmark discovery for Alzheimer's research. It showed that the amyloid plaques were not a side effect of the disease, but instead helped initiate the disease. Now, the first pre-clinical testing could begin on potential treatments for removing the plaques.

Research performed in 1999 using this Alzheimer's mouse model has already produced some promising results. Since the disease is caused by amyloid plaques, the research focused on preventing these plaques from being formed. They used the Alzheimer's mouse model to study the effects of immunizing the mice against amyloid-plaque-related proteins (Schenk et al., 1999). If young mice were immunized, they never subsequently formed any plaques. If old mice, already having plaques, were immunized, the plaques were reduced, and the brain showed a decrease in neuronal damage (Schenk et al., 1999). These results are promising and suggest that immunizing humans against amyloid-plaque-related proteins could result in a possible cure or at least treatment for Alzheimer's disease.

Transgenic Animals in Transpharming

Perhaps the greatest economical and medical benefits of transgenic animals lie in the mass production of complex proteins by farm animals. Appropriately termed 'transpharming,' this area of transgenics consists of inserting a gene into an animal, using the animal as a system to produce large amounts of the encoded protein, and harvesting it

for medical or health benefits. With the recent leaps in transgenic technology such as gene addition at a specific locus, it is becoming possible to achieve high rates of protein production.

The idea of using biological systems as protein manufacturers is not a new one; bacteria have been used for this purpose for many years. Although they are more cost efficient to produce and engineer, bacteria are much less complex than mammals and are often unable to make the post-translational changes to proteins that are necessary for function in a mammal; very often the modification involves the addition of a sugar or sugars to the protein. This is a severely limiting factor in the use of bacteria for protein production. Mammalian cells in culture were then experimented with for a brief time since they bear the ability to produce complex proteins (Enserink). Unfortunately mammalian cell culture is much more sensitive and expensive than bacteria, and results in a very small amount of protein relative to cost. Mammals are most ideal for this purpose because of a close genetic similarity to humans, and the ability to target proteins to mammary glands for easy isolation and separation.

For several years, researchers faced a dilemma in the production of transgenic farm animals; no method existed for the targeted insertion of a gene at a specific locus. Mice could be produced in this way using embryonic stem cells, but no ES cell lines have been successfully used in farm animal production. Unfortunately, mice lack the ability to mass produce protein simply because of their size. With the recent advances in nuclear transfer technology, mammalian cells (including large farm animals) can be genetically modified in culture and then transferred to eggs which have had their nuclei removed. Control of the locus of integration usually results in higher protein output as well as

minimizing interruption of endogenous genes, which often leads to severe defects in the resulting animal.

Nuclear transfer is also indispensable in the field of transpharming for economic reasons. With a high increase in efficiency of transgenic animal production (from about 5% for microinjection to 100% for nuclear transfer), costs are significantly lower because all cows raised and brought to term are guaranteed to be transgenic. The other main concern of commercializing this technology is the amount of protein produced. With nuclear transfer the amount of protein produced can reach several grams per liter (Moffat 1998) – in cows that can produce thousands of liters of milk in a lifetime, this advancement is unprecedented. Unfortunately, embryos produced by nuclear transfer are still susceptible to terminal abnormalities, although virtually all live births are viable transgenic animals (Larrick 2001).

The first transgenic farm animal produced for transpharming purposes was a cow called Tracy. This cow was produced in 1988 (History 2002) by the relatively unreliable method of pronuclear microinjection, in which there is no control over the integration site. In this case the process was successful, and Tracy was shown to produce a significant amount of alpha-1-antitrypsin in her milk. This protein is able to inhibit an enzyme called elastase, which is partially responsible for cystic fibrosis and hereditary emphysema (Moffat 1998). The drug, being produced by PPL Therapeutics is currently in human clinical trials, and a commercial release of 2007 is expected (Whitehouse 2002).

Several other transpharmers have also been in development; Genzyme is currently in clinical trials for antithrombin III produced in goats' milk. It is hoped that this protein will reduce blood clotting during surgery (Larrick 2001). Other drugs in production for

blood clot treatment include Human Protein C, Factors VIII and IX, and tPA. If drugs currently in clinical trials prove to be successful, it is certain that a wave of transpharmers will follow.

CHAPTER 3

TRANSGENIC ANIMALS: ETHICS AND SOCIAL ISSUES

Science was developed as people attempted to describe what happened around them. Since then we have progressed from describing to changing our surroundings. Biology's progress over the years has raised many ethical concerns. Since the discovery of DNA, biology has progressed into genetic engineering, the altering of life forms by direct genetic manipulation. Genetic engineering is not just building a machine out of inanimate objects but is altering the structure of living organisms in a way that is much faster than traditional breeding methods. This altering of nature has raised many ethical issues most of which concern human's role within nature.

Nature and Man

Most people's concerns about the creation of transgenic animals are based upon differing views of nature. Depending on how they view man's relationship with nature determines how they will feel about man altering a life form for his use. Daniel Callahan (Shannon, 1979) offers three views of nature: plastic, sacred, and teleological.

There are those people who believe that, like plastic, nature can be molded and shaped by humans to be used as we see fit. The only limits to our use of nature are those we place ourselves, such as lack of knowledge, experience, or technology. (Shannon, 1979) Those supporting this view of nature see nothing wrong with the development of transgenic animals since we are able to do it, we therefore should.

Some religions view nature as being sacred, something that should be held in reverence. Taoism teaches that man should strive to conform to nature so that the individual can become part of the cosmic whole. Bonaventure, a medieval theologian saw nature as a reflection of the glory of God. Such perspectives accompany an attitude of stewardship to nature and conservation of nature. Any intervention by man into nature should be discrete, infrequent and reverent. (Shannon, 1979). Developing transgenic animals is altering nature in an, at most times, unnatural way. Those who view nature as sacred see this type of alteration as stepping over our boundaries into a territory that we don't belong in. According to this view we should maintain a reverence, and respect for nature and not interfere with it.

There are also those who believe that nature is teleological, that nature has its own purpose and logic. It is believed that nature is dynamic, that it is ever changing and evolving. This dynamism is leading nature to certain ends or goals. Any human interference must be respectful of these ends. Nature's dynamism sets a limit on the extent of human intervention. Those who hold this view may very well see developing transgenic animals as tipping the balance of nature, since at most times, transgenic animals that are developed would not naturally occur in nature. As long as the interference in nature is parallel to the goals of nature then the interference is acceptable. Therefore, if the transgenic animal could occur naturally in nature then they would not have a problem with it.

Judeo-Christian Views

The Judeo-Christian creation story, found in Genesis, can be traced as the source of this belief of human's having ruling power over nature. According to Genesis, God created all of nature and placed it in man's care. God's relationship with man differentiates him from God's other creations, making man a "higher" life form. This does not give man domination over nature but a duty to care for the land and animals. (Macer, 1990).

The Christian reverence for their God, who created and cares for them, has led some to fear that scientists are "playing god." They fear scientists are creating and destroying life forms without reverence for God or reference to the opinions of other people. Useful applications of technology are positively advocated in Judeo-Christian tradition as good stewardship. (Macer, 1990) The question then becomes, how useful and necessary is this application, and do the scientists have the necessary knowledge and wisdom to alter this life form.

In 1995, scientists succeeded in creating a mouse model for Alzheimer's disease, one of the nation's biggest killers. Using this model it was discovered that amyloid plaques were the cause of Alzheimer's disease, and are necessary to initiating the onset of the disease. Using this knowledge and the mouse model, an immunization is now being developed for use in humans. Animal disease models such as this are allowing scientists to make ground breaking discoveries in creating cures for previously incurable diseases.

Christian belief system holds that human's have a duty to feed, house, and heal people. These are seen as major justifications for the pursuit of practical knowledge. The application of this knowledge is therefore advocated. If there is a way to improve

upon an old technique to better fulfill this duty, then this new technique should be applied. (Macer, 1990)

Responsibility of Scientists

As the creators of new technology scientists have a certain responsibility to the public. They are the ones who are making the interventions into nature, and so are the ones in control of how far they go. James Gustafson has proposed four models of ways to think of a scientist's responsibility.

The first is the idea of total intervention. Using this model, scientists would have the right to do whatever they were able to. This is justified by the inherent value of knowledge, that the benefits of acquiring this knowledge out weigh any of the consequences of the actions. The only limit placed on scientists by this model is their own technical capacity.

No intervention is the second model proposed by Gustafson. This model mirrors the belief of nature as sacred and asserts that research violates the limit imposed by nature. These are opposing views to the previous model and so provide a strong check.

The third model is that of limited interventions. This is model is related to the teleological view of nature and sees a balance that nature maintains. Interventions into nature are allowed, unlike the second model, but they are checked by human nature, not technical capacity like the first model.

The fourth model is directed interventions. According to this model, interventions into nature have a goal and are working towards improving the quality of

life for humans. This allows for a high level of intervention to control and direct human development. (Shannon, 1990)

Scientists may not follow one model alone, but base their decisions on a mix of these models. Knowing the objections that could be offered by other views allows scientists to defend their position better. After analyzing possible objections to their research, scientists can decide if their new technology is truly better than what is already in use.

For example, scientists developed a transgenic pig, which grew to be larger, required less food, and had less fat under its skin (Pursel, et al., 1989). All are desirable traits in an animal to be used as food. Sadly this pig developed many health problems such as ulcers, and arthritis. Scientists decided that the side effects were not worth the benefits of breeding this pig. After all in order to get more meat you could simply breed more of the pigs we already have, and you can develop a less fatty pig by selective breeding methods.

Public Attitudes

The public will feel consequences of scientific research, therefore it is essential to educate the public about new developments, and consider their opinions when deciding to continue research. The terror campaigns that have been launched against animal experimentation have served as a warning to scientists about educating the public.

Decisions about altering life forms should not be left to the scientists and corporations alone. They can wear blinders and not see many of the issues that the public

may be wary of, leading to much opposition to their research. This opposition can impede their research; it is better to directly address these issues as they arise rather then letting them grow until all research is halted.

Regulatory and advisory committees should have lay members to ensure public participation. These lay members should include those with relevant experience, along with a reasonable representation of society in terms of race and religion. (Macer, 1990) For example, a committee discussing the development of transpharming animals should have physicians, pharmacists, farmers, minorities, and religious leaders such as a catholic priest, protestant minister, rabbi, etc. This combination of lay members provides a well-rounded group, including those who will distribute the medicine developed, those who will raise the animals, and others who will benefit from the development of the transpharming animals. Discussion within such a group will be able to include all aspects from development to distribution, allowing many concerns to be addressed, and any fears to be qualmed.

Discussions about any planned experiments should be in public. This helps to educate the public about new developments. Many terror campaigns against biotechnology have been launched because of the public's lack of understanding about what was being done, and what could be done. In surveys conducted in several countries it has been found that in general the public doesn't understand what biotechnology is or what the knowledge gained from it is used for, yet they support applications of it that would improve their lives. It was also found that as the education of the individual increased, their understanding of the subject also increased (Macer, 1990). These surveys reinforced the idea that in order for the public to support research such as creating

transgenic animals they must understand what is being done, and what will be done. Fully educating the public leaves no room for suspicions to grow, and for terror campaigns to gain support.

Issues surrounding transgenic animals

With an understanding of the basis of differing ethical belief systems we can now begin to analyze specific concerns that have been raised surrounding the development of transgenic animals. Since the practice of animal experimentation became widespread there have been those who have been concerned about the animal's welfare, and advocating that animals have the same rights as humans. With the ability to change an animal's genetics comes specific concerns raised by a belief of changing our environment too rapidly.

Peter Singer wrote a book titled *Animal Rights* in the 1970s. In this book he introduces the idea of "specisim." Specisim is as defined discriminating against animals because they are animals.

> To say that animals should have no rights because they are animals is no more logical than to say that women should not have rights because they are women, or that Blacks should have no rights because they are Blacks. (Kopel, 1998)

Singer identifies the best test for rights as the question of "Can it suffer?" If you tortured a human with a cattle prod, it would be said that you were violating that persons rights. Animals react similarly to how human's act when they are in pain, they scream and try to

avoid the source of the pain. Therefore, if you used a cattle prod on an animal you are violating the animals the same as you would violate a human's rights. In fact it has been shown that animals pain-sensing portion of their nervous system is structurally similar to that of humans. (Kopel, 1998)

The major concern of animal rights activists is the quality of life for an animal used for experimentation. Transgenic animals, who are developed as disease models, are of particular interest to these activists. They question whether it is right to develop and breed an animal who is meant to develop a disease and die from it. How much of a quality life can the animal have?

There are ways to monitor a transgenic animal to determine if they have a quality of life that is comparable to their related non-transgenic species. By monitoring the survival rate, growth, other developmental measures, clinical observations, anatomical and pathological observations, and neurobehavioral tests and comparing results with non-transgenic animals the quality of life of the transgenic animal can be determined. As long as these tests come out as normal, it can be safely determined the disease they are breed to model does not affect that the animal's life. (ANZCCART, 1999)

The impact that a transgenic animal would have on its environment if it was let loose is another concern that has been expressed. Since these animals have been altered in ways that are at times very much not like their natural counterparts it is hard to predict what the impact would be if they were released. In nature if a species changes it happens over time and usually the environment around it is changing as well, so a certain balance is maintained. In the case of transgenic animals this change happens quickly and its environment has no time to adapt as well and so the scales could be tipped.

Animals that are part of the human food chain are of particular interest. These animals must go through rigorous testing by the FDA to be determined as safe for human consumption. If a transgenic animal were to be used as food, FDA testing would be required to ensure that the changes to the animal would not have a deleterious affect on humans who eat it. One example is a transgenic pig who was altered to produce more growth hormone. It must be proven that the growth hormone is broken down in the human gut as any other hormone would be and not have an effect on the humans who consume the meat.

If an animal is produced that is resistant to a pathogen that it is normally a host to, will that result in pathogen mutations? Or if an animal is engineered to serve as organ host for xenotransplantation, perhaps a virus could be spread. Interestingly, these pigs have been altered in such a way that the human immune system will not recognize their organs as foreign to them, so hopefully in the future immunorejection will be minimized. It is plausible that a virus previously only found in pigs could mutate in these animals in such a way that it would now be able to be infect humans. With no human immune defense against such a virus it would be a widespread epidemic. However, there are ways to screen for a variety of known viruses in these animals, and to grow them under sterile conditions, which minimizes their ability to serve as viral bioreactors.

CHAPTER 4

TRANSGENIC ANIMALS: LEGAL ISSUES

Accomplishing the task of making a transgenic animal is not easy or cheap. The risk involved when taking on the project of creating a transgenic animal may be enough to deter scientists from exploring this field of biotechnology. The protection of advances made through patents has been considered one way to 'award' the researcher(s) for taking on this risk. Patents would allow researchers to receive certain benefits for their work and protect the ideas and methods that they have discovered or invented.

Background on Patenting:

In order for something to be patented, it has to meet all the requirements specifically outlined in the patent law. The patent law has three basic tenets. They are novelty, utility, and non-obviousness. The novelty requirement specifies that the invention in question must be something new, and that does not occur naturally. For substances such as gene sequences and chemicals which do occur naturally, they can be patented if extracted and purified and have a greater use in their purified state than in their natural one. The utility requirement specifies that the invention in question must be proven to be useful in some sense. The non-obvious requirement specifies that the invention in question must not be something that could be easily accomplished through general knowledge. If an invention clearly meets all of these requirements, then "whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent thereof,

subject to the conditions and requirements of this title"(Walter,1997) as stated in the US Constitution. The patenting of transgenic animals would clearly fall under the composition of matter.

Diamond vs. Chakrabarty:

The first case of a patent being granted to a genetically modified organism was the case of Diamond v. Chakrabarty in 1980. The case involved a scientist who worked for General Electric Co. who was trying to patent a bacterium that was genetically modified to degrade oil. The US Supreme Court decided that there should be no distinction between patenting a living or non-living thing and granted the patent. This landmark decision opened the door for the idea of patenting higher life forms.

ExParte Allen:

The case of Ex Parte Allen on April 3, 1987, was the first case after the Diamond v. Chakrabarty case to really test the patentability of multicellular organisms. Ex Parte Allen tried to obtain a patent for a process that made more edible oysters by putting them under pressure. The Patent and Trademark Office (PTO) rejected the patent application. Ex Parte Allen appealed and the case was brought in front of the Board of Patent Appeals. The Board rejected it as well claiming it did not meet the non-obviousness requirement. Although the pressure oysters were never granted a patent, the idea that multicellular organisms could be patented was brought up. Later during that same April after the Allen decision had been made, the PTO stated that they "now consider non-naturally occurring non-human and multicellular living organisms, including animals, to

be patentable subject matter within the scope of 35 USC101. An article of manufacture or composition of matter occurring in nature will not be considered patentable unless given a new form, quality, properties or combination not present in the original article (Walter, 1997). It was not long after this statement before a controversy of opinions had already arose concerning the ability to patent life forms. Jeremy Rifkin, president of Economic Trends, along with animal welfare and religious groups tried to gather national support for a legislation, brought about by Charles Rose, that would impose a two year moratorium before the PTO could issue any patents to transgenic animals. Opposing this legislation were biotechnology organizations, along with research and medical associations. Not even a year after the statement and despite the growing controversy about the patenting of living organisms, the PTO granted the first patent to a transgenic animal.

Oncomouse Debate in the US

This animal was created by Philip Leder and Timothy Stewart of Harvard University. They were issued US Patent No. 4,736,866 for Oncomouse on April 12, 1988. Oncomouse is a "genetically engineered mouse that carries multiple copies of a single cancer causing gene, c-Myc" (Patents Relating to Genetically Engineered Animals, 1996). Claim 1 of the Harvard Mouse Patent states that the patent covers: "A transgenic non-human mammal, all of whose germ cells and somatic cells contain a recombinant activated oncogene sequence introduced into said mammal, or an ancestor of said mammal, at an embryonic state."(Woessner, 1999). Claim 1 noticeably has three obvious features. They are that the patent granted for oncomouse covers any mammal, not

specifically mice. It also covers any offspring of animals that have received this oncogene. Lastly, it is not specific to the oncogene c-Myc that is present in oncomouse, however it covers any oncogene introduced into a mammal.

Oncomouse clearly meets all the requirements to be eligible for a patent. It was not simply changed through natural means, like the pressurized oysters, however genetically engineered and created through someone's intellect. It is also obviously something new that is a valuable tool for cancer research. However, the concern of patenting oncomouse was never really focused on how well it fit into the current requirements for eligibility, but was more focused on whether or not it is ethical to patent life forms. When the decision was made to patent oncomouse, Donald J. Quigg, the commissioner of patents and trademarks, commented in a letter to Rep. Rose that he "cannot arbitrarily discriminate against any type of invention. There is no public policy exception contained in the patent laws" (Patents Rel. to Gen. Eng. Animals, 1996). Although this comment is true, many people believe that is the underlying problem with using the current patent laws and applying them to biotechnology, a field that was not considered when the patent laws were determined. They feel that with the growing advances in biotechnology, new laws should be created to govern the patenting of these advances to ensure that the ethical and moral considerations are taken into account.

For those reasons, after oncomouse was granted a US patent, animal welfare groups challenged the PTO's authority to grant a patent for an animal. Brought in front of the district court, the patent to oncomouse was affirmed by deciding that the PTO had the authority to grant the patent.

In July of 1988 the legislation, Rep. Rose had tried to pass to impose a moratorium on patents granted for transgenic animals, was rejected. In September of 1988 the house passed a bill that would have allowed the PTO to grant patents on transgenic animals with a waiver for small farm owners, which would allow them to use 'transgenic farm animals' without paying any fees, on to the Senate. The Senate never specifically acted on it, however once tried to endorse a bill that incorporated the legislation in it but this was never enacted. Senator Mark Hatfield, another opponent to patenting transgenic animals, has tried to get an indefinite moratorium on patenting transgenic animals since 1987 and has introduced his bill in each congress. So far, no law in the US has been approved that would place a moratorium on patenting transgenic animals or take the authority away from the PTO to patent them either.

Oncomouse Debate in Europe

Harvard was not as lucky obtaining patents in other countries as it was in the US. Since they filed an application in Europe in 1985, the growing controversy of 'patenting life' has continued to grow. The European patent system is set up with the European Patent Organization acting as a central body of administration. It is made up of the Administrative Council and European Patent Office, which is governed by the European Patent Convention (EPC). The EPC includes the 15 member states of Europe, Switzerland, Lichetenstein, Monaco, and Cyprus. In a decision made by the Examining Division on July 14, 1989 the application for a patent on oncomouse was rejected. It was rejected on two grounds, Article 53(b) and Article 83 EPC. Article 53(b) states that a patent cannot be granted to plant or animal varieties or to biological processes for the

production of plants or animals. Article 83 states that one "must disclose the invention in a manner sufficiently clear and complete for it to be complete for it to be carried out by a person skilled in the art." (EPO press release, 2001). Not even two months passed before Harvard appealed the decision on September 7, 1989.

The Boards of Appeal decided in 1990 that the application to patent oncomouse did in fact meet the requirements of Article 83. There was not enough evidence that supported the case that this procedure could not be carried out in duplicate. The Board also believed that the interpretation of the Examining Division that Article 53(b) not only excludes animal varieties but animals in general from ever being patented which the Board did not feel was an accurate interpretation. Another question raised was to determine if Article 53(a) EPC would prevent oncomouse from being patented. Article 53(a) is in short a morality law. It states that "European patents should not be granted in respect of inventions the publication or exploitation of which would be contrary to core public or morality, provided that the exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation in some or all of the contracting states'" (Cornish, 2000). Now, unlike the decision in the US to grant a patent for the mouse, the morality of patenting oncomouse was in question. The Board of Appeal handed the case back to the Examining Division to discuss the issues at hand and make a decision regarding the patent.

On May 5, 1992, the Examining Division granted Harvard the European patent for oncomouse. "The patent covers transgenic mice and other animals yet to be developed with an activated oncogene predisposing them to cancer" (Kaiser, 1995). However, the controversy this decision had drawn from the public would not be ignored. Unlike the US

patent system, Europe's patent system is set up so that third parties can challenge any decision made.

Once a patent is granted, the European Patent Office has a set period of nine months where an opposition can be filed. An opposition is a challenge to a decision made to grant a European patent. It allows the opportunity for a group or groups to challenge a patent they feel was granted under false pretenses and provides the chance to fix a mistake. An opposition can be filed to challenge a patent on five grounds "1) The subject matter is not regarded as an invention; 2) The subject matter falls within the exclusions for patentability; 3) The subject matter is not novel; 4) The subject matter is not inventive; and/or 5) The subject matter is not susceptible of industrial application" (Cornish, 2000). After Harvard was granted a patent for oncomouse, seventeen oppositions were filed on the grounds of Article 83 – there was not adequate information disclosed to repeat this experiment, Article 52(2) – patents can not be granted for discoveries or scientific theories, Article 52(4) – patents can not be granted for methods for treatment of animal body by therapy and diagnostic methods, Article 53(a) – it is not moral, Article 53(b) – claiming it is an animal variety which excludes it from being patentable, Article 54 – it is not novel, Article 56 – there was no 'inventive step' involved in the process of making oncomouse, and Article 57 – there is no industrial application. Two of the seventeen groups, including the British Abolition of Vivisection (BUAV) and the Compassion in World Farming, opposed the patent of oncomouse for moral reasons. "Genetically engineering animals to develop a painful, lethal disease is morally unjustifiable,' argued CWF" (Gavaghan, 1998). The BUAV believe that patenting animals is, "fundamentally immoral. [They feel that] animals are not merely 'inventions'

but remarkable sentient creatures that should be treated with respect and compassion" (BUAV). By granting transgenic animals patents, the BUAV feels that the suffering of animals through genetic engineering will be encouraged, and make the practice of developing them more profitable.

Oral proceedings were held November 21-24, 1995 in Munich. Decisions regarding the certain claims were resolved and the opposition Division ruled that the arguments made against oncomouse on Articles 83, 52(2), 52(4), 54, and 56 were not convincing enough to prove that the patent failed to meet these requirements. The EPO also suggested that Harvard make revisions to their patent claims. One of the suggested revisions would limit the patent to only mice with an oncogene instead of all mammals. The patent holders agreed to make revisions and the EPO closed the hearing before any conclusions were made regarding the industrial application of oncomouse, whether or not it should be considered an animal variety, and the morality of the patent. To give all third parties a chance to comment on any revisions made to the patent claim, the EPO announced that any views on the matter should be submitted in writing.

This hearing in Europe was said by Keith McCullagh, chair of the UK BioIndusty Association, to be a "'sideshow'…to a debate on biotech patents in the European Parliament" (Kaiser, 1995). The real debate going on in Europe was not the issue of directly patenting oncomouse; the issue was really about what guidelines should regulate the patentability of transgenic animals. Since oncomouse was the first case of patenting a transgenic animal, Parliament felt that the decision made regarding this case could set a precedent for future animal patents. This is why clear guidelines were needed that directly addressed the patenting of biotechnology and its products. A European Union

Directive, driven initially by the biotech industry's desire to create a patent legislation more like that of Japan and the United States which allows animal patents, had already been discussed, argued over, and in the making for about six years when the oral proceedings were held for the opposition of the oncomouse patent. The EU directive would provide common standards and ground rules for the patentability of genes, cells, and other biological material. However, it has not had an easy ride through the legislative process. "It took more than 5 years of negotiating before the European Parliament and the Council of Ministries agreed on a draft text in December 1993. This was modified February 1994 to address the biotech community concern that the directive would have been more restrictive that the EPC" (O'Brien, 1995). In early March of 1995, the EU directive was rejected by the European Parliament in Brussels. Two reasons given as to why they voted no were that the directive did not clearly ban the patenting of human germ line therapy and that the directive would inhibit research. Environmentalists and animal rights groups considered the rejection of the directive a victory in their fight to ban transgenic animal patenting all together. Scientists were disappointed because now the same guidelines for patenting would still be in place, and those guidelines have no clear rules for patenting transgenic animals. Without proper clarification of the law, the EPO felt that no decision should be made regarding transgenic animal patents, and put on hold any application regarding animals. This meant that the fate of the oncomouse patent would not be decided until a directive was passed that had clear guidelines for the patentability of animals.

The hold was in place for 8 years before the approval for the European Biotechnology Directive was granted by the European Parliament on July 6, 1998, 3yrs after it had been

rejected. The directive made the patenting of plants and animals possible, and at the same time provided very clear guidelines to follow when determining what is considered patentable subject matter. The directive also clarified the issue of animal varieties. The new wording states that an invention concerning an animal can be considered patentable as long as the "'practicability of the invention is not technically confined to a particular...animal variety'" (Abbott, 1997). Now that the legislation for biotech patenting had been clarified, the EPO felt that they could then start making decisions regarding the backlog of applications that they had put on hold. The oncomouse patent appeal case could now be decided.

In the last couple of months of the year 2001, the European Patent Office decided to uphold the patent granted to Harvard for oncomouse. The decision was made based on the claim that the benfit to society that oncomouse poses is greater than the suffering of the animal. The patent, however, was restricted from covering all mammals to only covering mice. Opponents of this decision still feel this decision encourages maltreatment of animals in laboratory research and the spokesman at the time for Greenpeace commented that "The EPO's position lacks logic, both ethically and legally" (Abbott, 2001). Despite the conflicting opinions regarding the patenting of oncomouse, as of November 2001, 20 more animal patents have been granted by the EPO.

Oncomouse Debate in Canada

Harvard has had an even tougher time patenting oncomouse in Canada than in Europe. On June 21, 1985, Harvard applied for a patent in Canada covering oncomouse. A Canadian patent grants the patent holder exclusive rights to invention for 20 years with

a sufficient amount of disclosed information regarding their invention. After the 20 year period has ended, the information becomes free for anyone to use. In 1993, the Canadian patent office ruled that a patent could be granted for the process that was used to create oncomouse, but could not be granted for the mouse itself. Harvard appealed this decision, and the case was then brought before the Commissioner of Patents in 1995. Using the basic criteria to determine patentability as defined in the Patent Act; being novelty, non-obviousness, utility, and uniform reproducibility, the Commissioner of Patents upheld the first rejection for the patent by the Patent Office. They claimed that oncomouse could not be considered a patentable 'manufacture' or composition of matter because it was not produced entirely by the inventor. In this respect, they also claimed it could not be considered an invention. To be considered an invention, they felt that more than one feature of the subject had to be controlled. Therefore, "the mice in their essences were products of nature but for the characteristic of the additional gene sequence" (Kondor, 2000). Harvard argued that the control they possess over the oncogene they inserted, and the certainty that the oncogene is expressed in the mouse's offspring, shows that they have a definite control over uniform reproducibility. Harvard decided to appeal this decision to the Federal Court of Canada.

The Federal Court also ruled against Harvard on April 21, 1998. The main argument against Harvard was that the mouse could not be considered an invention because the characteristics of the end product could not be controlled by the inventor. In this regard Judge Nadon ruled that oncomouse was not patentable and stated that, "On even the broadest interpretation I cannot find that a mouse is 'raw material' which was given new qualities from the inventor…[Harvard] can make no claim to being able to

reproduce the mammal at will by doing anything other than ordinary breeding…the mouse is not truly reproducible, as that term is understood in the Patent Act" (Candadian and Int. Property Office, 2000). He felt that the oncomouse claim could not be considered an invention because too much was left up to chance by nature.

Harvard appealed against this decision and brought the case in front of the Federal Court of Appeal. According to Judge Rothstein, the other court decisions made had erred in accurately applying the patent laws to determine the patentability of oncomouse. Judge Nadon had interpreted 'reproducibility' of the mouse as the actual biological reproduction of the mouse, which is solely dependent on nature. In contrast, Judge Rothstein interpreted 'reproducibility' of the mouse to mean that enough information is disclosed in the patent application that someone else would be able to produce an 'oncomouse.' Judge Rothstein also felt that most inventions rely on the laws of nature and as long as there is a specific act of human intervention, the end product can be considered an invention. In the case of oncomouse, the human intervention was the act of inserting the oncogene into the mouse, "The fact that the inventors do not have control over the colour of the mouse's eye, or the length of its tail is irrelevant…The degree of control must extend to the features that are claimed; in this case, the presence of the gene in the offspring" (Gavelle, Wong, 2000). The majority of the Court of Appeal also felt that the Patent Act had no provisions outlined that made a distinction between the patentability of lower life forms and higher life forms. Since it had already been decided that lower life forms could be patented, the Court of Appeal felt that higher life forms could be patented as well. Thus, on August 3, 2000, the Federal Court of Appeal in Canada made the landmark decision of ruling oncomouse patentable.

This decision inspired animal rights organizations and religious groups to write letters of appeal claiming that the decision to patent oncomouse was wrong. In a letter to Parliament from the Canadian Environmental Law Association (CELA), they asked for an appeal to the decision claiming that the patenting of mammals is, "not within the existing definition of 'invention' under the Patent Act" (Muldon, McClenaghan, Senarchuk, 2000). Their main argument, expressed in the letter, suggests that patenting animals raises moral and ethical concerns that have to be addressed and that a patent granted for the process of creating oncomouse should be sufficient. They believe that, "the extension of the patent to the living mammal is unnecessary, inappropriate, and wrong in law"(Muldon, McClenaghan, Senarchuk, 2000). Organizations such as CELA are not the only ones opposing the decision made in August 2000. The Government of Canada, represented by the Commissioner of Patents, also filed for an appeal, which will be brought in front of the Supreme Court of Canada, and even made the motion to put off enacting the Court of Appeals decision to patent oncomouse until the decision of the Supreme Court has been rendered. The Government of Canada also felt that a public dialogue surrounding the controversy should be heard and advice regarding the patenting of biotechnological products should be taken into account. The Canadian Biotechnology Advisory Committee (CBAC), set up by the government in 1999, launched a program to engage Canadians in a debate regarding the patentability of higher life forms. The conclusions that the CBAC reaches will be passed on to the government and the hope is that the advice will have taken into consideration all sides of the moral and ethical concerns surrounding this issue.

The appeal to the Supreme Court of Canada took place on May 21, 2002. In addition to the arguments made by the Commissioner of Patents and Harvard, four other organizations also made arguments against the patenting of oncomouse. The four other parties included the Canadian Environmental Law Association, the Canadian Council of Churches, the Sierra Club of Canada, and the Animal Alliance of Canada. The last two mentioned had only provided written submissions. The arguments made against oncomouse were the same ones that had been made in the previous courts. The arguments that oncomouse does not meet the definition of 'composition of matter' or 'manufacture' because lifeforms can not be manufactured products and therefore can not be considered an invention. The CELA argued that, "Parliament never intended an invention to include genetically engineered higher life forms" (Ledgely, 2002) and the Patent Act should only be able to be used to patent lower life forms. Lawyers for Harvard argued that the term 'invention' as stated in the Patent Act can have a broad or inclusive interpretation and is not clearly defined to exclude oncomouse. The decision of the Supreme Court of Canada has not yet been made and the date the decision will be made is still unknown.

Current US Issues

During the 15 year unresolved controversy in Canada over the patenting of oncomouse, the US had already begun patenting a number of other transgenic animals and had reached a new legal debate regarding patents. Now that patents had been issued, the debate became focused on the use of these patents in the research field. Research institutions that had patented transgenic animals, such as mice, did not have the desire to

begin mass breeding and distributing the animals themselves. They would end up awarding companies and commercial biotech industries the license to market the animals. This created a problem of commercialism interfering with research. Commercial industries began imposing high prices for an animal, and put restrictions in place outlining how the animal could be used.

Patenting Enforcement Issues and GenPharm:

One such company, GenPharm, was originally charging between $80-150 per transgenic mouse and prohibited researchers from breeding them. This meant that anytime a lab wanted to use more than one mouse they had to pay GenPharm for each mouse they intended to use instead of buying two and using offspring for free. This meant that buying the mice alone for a research project could end up costing thousands of dollars, more than most research facilities can afford to spend on mice. After an uproar from researchers in 1993 against GenPharm's policies, GenPharm decided to allow researchers to buy an initial breeding pair of mice and then breed them as many times as they desired for an annual fee of $1,000. Although this policy was more reasonable, geneticist Tyler Jacks of MIT stated that he's, "still not convinced we've reached the optimal solution" (Anderson, 1993).

Patenting Enforcement Issues and Dupont:

DuPont, a science and technology based institution, holds a patent and license on a method, and the mice created from this method, that manipulates genes in mice. This technology is termed cre-lox P, and the mice created from this technology are termed

simply cre-lox P mice. DuPont was controlling this technology through a no-cost research license. This allows institutions to use this technology and share these mice only with other institutions that also have this research license. However, by agreeing to these terms, any institution that makes a discovery while using this technology will be mandated to pay an unspecified amount of royalty fees to DuPont. Some institutions feel that DuPont has the right to enforce their patents and these institutions are willing to sign the agreement for the no-cost research license. Other institutions feel that DuPont does not have the right to enforce their patents as they see fit, and their unrealistic terms are restricting the use of this technology. Like many other academic research facilities, Harvard ended up licensing oncomouse to DuPont to manufacture and sell. DuPont's pricing policy regarding oncomouse contained what is called a 'reach-through' clause that required anyone who made a discovery or product while using oncomouse, or another strain of it, was required to pay DuPont royalty fees. Many scientists felt that this would inhibit the desire for researchers to use oncomouse and inhibit any potential developments oncomouse could bring about.

In 1998, DuPont and the National Institute of Health (NIH) reached an agreement regarding the restrictive terms of the use of the cre-lox P mice. A letter written by Harold Varmus, the director of NIH at the time, warned DuPont that, "the company's restrictive terms could 'seriously impede further basic research and thwart the development of future technologies that will benefit the public'" (Marshall, 2000). DuPont then agreed to drop the reach-through claims and allow a more 'relaxed' mouse sharing policy for NIH researchers. In 1999 DuPont and NIH reached another agreement, this time about the terms of the use of oncomouse. DuPont decided to allow NIH researchers to freely

exchange the animals and information among each other without consulting DuPont first. Currently, most academic institutions have free access and use of the mouse as long as they only share the mice with others that have obtained this free research license or have a license to use oncomouse. They also must file an annual report regarding any information they have discovered and experiments they have done with oncomouse. These terms seem a lot more realistic; however, some still argue that these terms will impede research. Recently, DuPont has been trying hard to enforce these terms and have not received support yet from MIT or the University of California. When DuPont informed MIT that they did not have the appropriate license to use oncomouse, and listed names of three researchers that were using oncomouse or a strain of it, the reaction they received was not supportive. Tyler Jacks, one of the individuals listed as using a strain of oncomouse, "strongly objects' to DuPont's claim that 'any animal with germ line disruptions that is cancer prone' must be licensed for research use under the oncomouse patent. He's disappointed that institutions seem to 'back down' to such broad patent claims" (Marshall, 2002). DuPont does not understand how a free research license could be a burden, and is still waiting to hear from MIT and the Univ. of California about working out agreements to use oncomouse.

Patent-free Options:

Meanwhile, other companies such as Merck & Co., a pharmaceutical company, are trying to make patent free transgenic mice. Merck & Co. announced an 8 million dollar plan to create 150 patent free transgenic mice. The mice will be made at Lexicon Genetics and available through the Jackson Laboratory. Merck is trying to promote

research with no 'legal-strings' attached and claims that no restrictions on use will be enforced. The NIH has also taken a similar approach by supplying grants to sequence the mouse genome and to create transgenic animals through that knowledge. They are also insisting that any researcher supported by one of those grants should not file for a patent. Although neither institution is exclusively against granting patents, these actions speak more about how these patents have been enforced. As issues surrounding the enforcement of patents persist, these patent free transgenic mice will encourage the use of transgenic animals until optimal patent enforcement agreements are arranged.

Issues Surrounding the Patent Debate

Many issues and concerns of different organizations have arisen throughout the world during the case of oncomouse. These issues encompass a wide range of ideas that may or may not be directly related to the actual patenting of transgenic animals. The real issues at hand do not seem to be directly opposing the patenting of transgenic animals, but opposing biotechnology itself. The broad range of issues brought up include agricultural concerns, environmental concerns, animal rights issues, moral and religious concerns, and research concerns.

Agricultural Concerns:

Many small farmers fear that the growing technology of creating transgenic animals will put them out of business. Their fears focus on the idea that the best crops and livestock will eventually be transgenically produced and patented, and small farmers will no longer be able to breed and sell their best produces because the 'material' will be

patented. Small farmers believe that the price to own a patented transgenic animal will be too expensive, leaving only the large farm corporations to have the monetary ability to breed and sell the products of these 'transgenic farm animals'. This would create a monopoly in the agriculture business placing the large farming corporations at the top, putting the small farmers at the bottom and broke. These fears seem valid and that is why many countries, including the US, have been working on passing legislation that will waive patent fees for small farm owners. However, even with the waivers, small farmers should not be too concerned with the technology of transgenic animals infiltrating too much into the agricultural business. The main interest and use of transgenic animals is in the field of medical research and it does not seem likely that 'transgenic farms' will appear in the near future.

Environmental Concerns:

Environmental organizations, such as the National Wildlife Foundation (NWF), also oppose the patenting of transgenic animal because of the effect that they may bring about on the environment. Not too long ago, there was a concern about what would happen if transgenic catfish were to be let loose in a pond. Many concerns about the damages these creatures could bring about have created a large environmental controversy of patenting transgenic animals. Even though they believe that granting patents to these animals will encourage their production, even if they can not be patented they will still continue to be made. This implies that the environmentalists are approaching the technology of transgenic animal creation in the wrong way. Instead of focusing on opposing them from being patented, they should focus on lobbying for

legislation that would regulate how transgenic animals are released into the wild. The PTO should not be the office to provide environmental legislation regarding biotechnology by refusing patents for those reasons.

Animal Rights Concerns:

Animal rights issues throughout the patenting debate have mainly focused on the cruel treatment of animals. Animal rights organizations feel that 'programming' an animal to die a very painful death is unnecessary and unethical. However, this opinion can also be applied to any type of laboratory research that uses animals, not specifically just the use of transgenic animals. The use of animals in laboratory research has been an area of controversy long before transgenic animals, and denying patents for them will not decrease their suffering. Humans have advanced and produced immeasurable benefits to society through research using animals as test subjects and denying this research all together will impede any future developments in finding cures for AIDS, cancer, cystic fibrosis, and Alzheimer's disease, (just to name a few). Patents that have been granted to transgenic animals have only been granted if the benefit this animal poses to society outweighs the suffering the animal will go through. Researchers do not intentionally make animals just so that they can suffer; they make them to try to advance medical research. Take for example super pig, which would not be able to be patented, and the Alzheimer's mouse, which has a patent. Super pig was genetically altered with a growth hormone to grow to be an enormous size to increase the amount of produce that could be sold. However, unforeseen side effects cause the pig to suffer tremendously, especially from arthritis. The pig was put down and scientists no longer tried to produce another

super pig. They understood that the benefit to society did not outweigh the pig's suffering. On the other hand, Alzheimer's mouse, created to develop Alzheimer's in a similar manner that humans do, does not appear to be suffering and provides a great research tool in the investigation of a treatment for Alzheimer's disease in humans. That is not to say that every transgenic animal has little to no suffering; however denying patents will not regulate any suffering that an animal may go through because transgenic animals will still be made if they can not be patented. Current legislation exists that regulates the use of animals in lab research, however does not regulate the use of transgenic animals in research. Instead of focusing on the patent debate, animal rights organizations should focus on trying to expand the current legislation to include regulations for the use of transgenic animals in research.

Religious Concerns:

Religious concerns about patenting transgenic animals focus on the commodification of living things and the disregard for the sanctity of life. Granting a patent essentially grants ownership over an animal with the mindset that this animal was created by the 'inventor'. Religious organizations feel that by doing this, biotechnology is a field that people are using to 'play God' in a sense. They believe that all of God's creations deserve respect and the idea that a living being can essentially be owned, strips away any dignity the animal has. This argument does not seem to directly oppose the patenting of the animal but more so opposing the 'creation' of these animals. Researchers try to produce the best possible transgenic animals they can to make the best possible living environment for all of humankind. They are not trying to play the role of

a god and do not claim to have created the entire organism. They just claim to have invented the characteristics the animals has, such as being cancer prone, that have required their ingenuity to exist in that animal.

The other argument religious organizations make is that soon the value of human lives will drop when patents are able to be granted for humans or human parts. In Canada, the Evangelical Fellowship of Canada (EFC) states that, "The Patent Act, as it stands, allows for patents on any composition of matter, with no distinction between simple and complex lifeforms, or between human, animal, and plant life…[and] Granting a person or corporation ownership of human life through patenting would violate human dignity"(EFC, 2001). This statement made on May 23, 2001 would seem reasonable, however, in the court ruling in Canada granting oncomouse patentable made on August 3, 2000, (almost a year before the EFC's statement), "the ruling was very clear: patenting does not apply to humans because it is a property-based concept, and humans cannot be considered property" (Brown, 2000). This made it very clear in Canada that human lives could not be patented. Countries that have allowed the patenting of transgenic animals have also made similar rulings as Canada did that humans are not considered patentable subject matter. When patenting an animal, moral considerations should be made in regards to the benefit of humankind as a whole and whether or not the suffering of the animal can be justified. Although religious organizations may not agree with this technology, it is used in the best interest of society.

Research Concerns:

There is also a concern that patenting will impede research. Some scientists believe that the monopoly that biotech industries hold by their restrictive terms on many patented animals will discourage further research. This is the present legal debate that was discussed that is presently taking place in the US. However, with the granting and enforcement of patents for transgenic animals being a relatively new field, companies are bound to make mistakes that will be 'fixed' over time. In the case of oncomouse, in the US the oncomouse patent is very broad and encompasses all mammals predisposed to a cancer gene, whereas other patents granted in the US after oncomouse are much more inclusive. The same trend has taken place in the enforcement of these patents. Early on, the terms of use were quite restrictive and the prices for the animals were quite high. However, as time went on and complaints were made, more adequate terms have been imposed. Although currently not every one appears to be satisfied with the modified terms, time will eventually produce changes as shown in the past. No one wants to prevent further research, therefore research institutions and biotech industries will eventually find the right balance between terms and usage.

Other scientists believe that patents will promote further research by providing 'rewards' for risky research ventures. Also, in order to obtain a patent, the information surrounding the product or technology has to be disclosed. This allows the information to be widely available and prevents secrecy. Without protection, researchers would treat new inventions as "trade secrets" (Brown, 2000). Dr. Rashimikarthary, a scientist in Ottawa, states that, "'without protection, other laboratories could quickly reproduce the work that you did at great expense and effort without having to compensate you. "'With

the possibility of protecting your work that won't happen'"(Brown, 2000). Time is the only way to really know how patenting will affect the field of biotechnology.

Current Patent Information

Currently, Harvard holds three US patents for oncomouse. One for non-human mammals with a predisposition to cancer, one for the, "method for providing cell culture from the transgenic-nonhuman mammal"(US), and one for, "Testing method using transgenic mice expressing an oncogene"(US). Oncomouse was also issued a patent in Austria, Germany, Europe, and Japan. The patent from Canada for oncomouse is pending the Supreme Courts decision that is yet to be made. The US has also granted a patent for a mouse the develops a large prostate gland, a virus resistant mouse, a rabbit infected with HIV-1, and a number of mice that are models for human pathology such as Polio, Parkinson's syndrome, sickle cell anemia, Leukemia, and a mouse susceptible to prion infection. Animals that have been made to produce specific proteins in the milk have also been granted numerous patents. A few of these animals include a sheep that produces blood coagulation factors, a pig that produces human hemoglobin, and a pig, sheep, goat, and cattle that can all produce protein C. All of these transgenic animals are currently being used, along with many others, to further developments that will benefit society.

Future of Patenting

With the increasing number of patents being granted in the US since the patenting of oncomouse, the future of patenting is still to be determined. Currently, Jeremy Rifkin

of the Foundation of Economic Trends in D.C. and author of several books related to genetic engineering techniques, along with Steward Newman, a professor at the New York Medical College, are jointly applying for a patent for the creation of human/animal chimeras in the US. Although they have never actually created a human/animal chimera, they are trying to obtain a patent for a broad method of creating them. If they are granted the patent, they claim that they will use it to restrict any research involving the technology. Their patent application was used to bring about a debate regarding the patentability of life, specifically human life. So far, their application has been rejected by the Patent and Trademark Office in 1999. Rifkin and Newman stated that they will appeal decisions made against their application all the way up to the Supreme Court if they have to in order to raise awareness and promote specific guidelines to be put in place regarding the patenting of transgenic animals and humans. The EPO feels that this is just a publicity stunt. Others feel that the application will be rejected based on the fact that the chimeras have never actually been created, avoiding debate of patenting humans altogether. Either way, Rifkin and Newman do raise legitimate issues that must be considered when deciding on future guidelines and limitations of patenting transgenic animals and where the line should be drawn as to how much human genes can be used before something is considered 'too-human' to be patented.

Future of Patent Laws

Under the current US patent laws transgenic animals are patentable. Whether or not patents should be granted for moral or ethical reasons should not be for the Patent Office to decide. Without any changes, the only guidelines that should be considered

when granting a patent should be the objective application of the novelty, nonobviousness, and utility requirement. Since biotechnology does however raise moral and ethical issues, an evaluation of consequences involving the patenting of transgenic animals should be made and used to create clear patent guidelines that do incorporate these issues. Understanding the importance of these types of guidelines, the US, Europe, and Canada have all set up some sort of organization to review biotechnological issues and advise the government on how to incorporate ethics and morality into patent legislation. Europe encouraged the development of the EU directive, Canada organized the CBAC, and the US created the National Bioethics Advisory Commission. These organizations will be reviewing such issues such as what will be considered too human to patent. Patent laws have been used in every other industry to promote growth and development. Denying patent laws in the field of biotechnology, "will result in…a reduced initial incentive to engage in biotechnological studies"(Walter, 1997). The arguments against patenting transgenic animals were shown to be misguided and less about patenting and more about the technology itself. With organizations in place to help provide better guidelines for future patents, patents on transgenic animals will help promote biotechnological advancements by increasing disclosure of new developments and providing an incentive to take on risky and expensive research by providing intellectual property protection.

CHAPTER 5

TRANSGENIC ANIMALS: CONCLUSION

Since the first transgenic bacteria were produced in the 1970s, transgenic science has progressed in leaps and bounds. We now have the technology to develop transgenic animals who serve as disease models, organ donors, and improved food sources. As techniques continue to improve, the horizon of possibilities broadens. With this increased ability also comes an increased responsibility to the scientist.

As scientists perform more experiments with transgenic animals they are able to refine techniques and gain better control over expression in the animal. As of now creating a transgenic animal can be an inexact science, resulting in the production of many unwanted offspring for the formation of only a few with the desired traits. The desired transgenics are then able to breed to produce more with the same desired traits. New methods such as the Cre-LoxP system are being created to make gene insertion a more exact science.

Transgenic animals are playing a very important role in the future of medicine. Using transgenic animals to provide immuno-compatible organs for donations to humans could save thousands of lives every year. The creation of human disease models in animals allows the discovery of a cure for a genetic disease to progress much faster than if there was no model. Because of the strict regulations for human experimentation, it is hard to obtain enough valid information to develop a cure. With the help of animal models this process will proceed much faster. Using transgenics it is also possible to improve current food sources by making them healthier, such as having less fat, and

reducing bacteria in milk. It is also possible to make animals grow bigger and convert their food better resulting in more meat using less space and food. This would benefit third world countries where there is a shortage of such things.

With the development of this new technology and its promise for the future also comes many public concerns and ethical questions. Scientists have a responsibility to educate the public in new developments in their field. The more educated the common man is the less likely he will fall into the trap of a terror campaign against new science. By being honest with the public a scientist will be able to earn their trust.

The public should also be involved in discussions over the applications of the science surrounding transgenics. This will aid in the education of the public as well as keep a balance between science and society. The public will feel more involved and more informed about the developments, as well as being able to voice their concern and question motives. It is possible for a scientist to get wrapped up in his work and not think of the consequences it will have on the environment and society. Having committees to monitor such things will help keep this in check.

There are many issues surrounding transgenic animals such as animal rights, environmental concerns, and effects on humans. Animal rights activists advocate that animals have the same rights as humans do and so should be treated equally. They question whether animals are able to enjoy a quality life when being altered to die from a disease. But it is the conclusion of this IQP team that experiments having strong medical benefits with little animal suffering should precede forward. In many instances, transgenic animals can enjoy the same quality of life as non-transgenic animals. The effect of a transgenic animal would have on the environment is also questioned, but as of

yet transgenic animals have been held in controlled environments and have not had a negative effect. Thus this is not a major concern of this IQP team.

Concerns about deleterious affects that transgenic animals could have on humans have also been raised. Transgenic animals that are made as food sources are of particular concern, but their development falls under control of the FDA and their strict regulations. The possibility of new mutant viruses spreading to humans during xenotransplantation experiments we believe is a concern, but should not prohibit the technology from being further developed since these animals are held in sterile environments and are screened for known viruses.

Scientists who create transgenic animals and wish to profit from it file for patents. The ability to patent a life form has been under debate. In several cases the US Supreme Court has up held its decision to allow the patenting of transgenic animals, so it has moved from being a legal battle to one of ethics. Many groups are bringing their concerns to the forefront, and attempting to stop the patenting of life.

Currently, there are no specific restrictions placed upon the creation or treatment of transgenic animals. What is controlled is their use. Government orgaizations such as the FDA, and the EPA control their release as a food source, pharmacutical manufacturer, and their release into the environment. Although there are guidelines on experimentation on normal animals to control their treatment and living conditions in the lab, these do not as of yet apply to transgenic animals.

The development of transgenic animals holds many hopes for the future, and this IQP team has concluded that the benefits of this technology outweigh public concerns so long as tight restrictions are placed on their production. Ethical review boards composed

of a variety of experts and laymen should be set up to establish how strong the medical benefits are likely to be for given experiments, as well as the likelihood of animal suffering, and the use of these animals in labs. Hopefully such a committee will be able to assess what will be affected by transgenics, and be able to qualm the fears of the public.

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