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TRANSGENIC ANIMALS

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Abstract

Transgenic animals are animals that have been genetically modified by insertion of foreign DNA into their genomes. This paper provides an overview of transgenic animal construction techniques, their various categories of utilization, and describes the impact of this new technology on society via ethical and legal issues. We conclude that this new technology should continue to be supported by medical research especially in those cases that provide a clear medical benefit, while minimizing any animal suffering.

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Executive Summary

Chapter 1

Transgenic animals are animals in which a foreign gene has been inserted. Such animals are used for producing new drugs, for studying disease progression, and possibly for organ transfers. Although their medical benefits are enormous, several ethical and legal issues surround their use. Thus the purpose of this IQP is to discuss what this new controversial technology is, and how it affects society. Because these animals are created by inserting foreign DNA into their genomes, we begin with a brief discussion of DNA.

Every living creature in the world uses DNA or deoxyribonucleic acid as their genetic material. Some viruses use RNA as genetic material, but they are not considered living. DNA strands contain the genetic information for an organism. DNA is divided into protein coding regions called genes. Genes contain the information needed to create a specific protein, and each protein in an organism has a specific role.

The most common technique for inserting foreign DNA into an animal is microinjection into the male pronucleus of a newly fertilized egg. However this technique allows the DNA to incorporate randomly into the animal's genome, which can have detrimental effects. A more sophisticated technique for making a transgenic animal involves targeting the transgene to specific insertion sites, using a technique called homologous recombination. The term homologous in this case means a gene in the animal's genome is replaced with a different gene for the same kind of function. The homologous recombination process is usually applied to embryonic stem cells taken from the blastocyst stage of the animal, and then cultured to the blastocyst stage and reimplanted.

Retroviral infection of embryos is one of the three general techniques used for construction of transgenic mice. The purpose of retroviral infection of embryonic stem cells is to study cell lineage and random insertional mutagenesis. Unlike microinjections, retroviral infection can be performed at various stages of embryonic development. These stages range from preimplantation to midgestation. Retroviruses integrate into the host genome without deletions or rearrangements of the chromosomal DNA, making them very useful in random insertional mutagenesis studies. Retroviruses that infect embryonic stem cells allow for use of promoter trap and gene trap experiments. In this case, the infection allows for pre-selection of integration sites that may be of interest.

Another technique for delivering DNA into embryonic cells to create a transgenic animal is by way of liposome-mediated transfection. In this case the type of embryonic cells for use of delivery is embryonic carcinoma (EC) cells. These cells are excellent for studying early mammalian development.

Chapter 2

In chapter-1 we went over what transgenic animals are, and how they are made. Following the advent of transgenic technology, over the years a large variety of transgenic animals have been made. Some of these animals have obvious medical benefits, and some do not. The purpose of chapter-2 is to describe and categorize some examples.

The first category of transgenic animal discussed in this paper is disease models. These animals have been engineered to mimic some aspect of human disease, for the purpose of aiding our understanding of the human disease process. Alzheimer's mouse was used as an example of

an animal with enormous medical benefits, with no observed animal suffering, and the authors of this paper believe such experiments should continue in the future.

To solve a pharmaceutical production problem, scientists have taken a hint from Mother Nature. Over the ages, the mammary glands of goats and cows have evolved to pack proteins into breast milk, so that mother animals can nourish and protect their young. By introducing human protein genes under the control of promoters that target the gene's product to the mammary glands, these natural protein factories, now known as "transpharmers", can produce a near limitless supply of these drugs in the milk of the animal, eliminating the need to sacrifice the animal to obtain the drug.

Before transgenic animals, and even before the knowledge that genes exist, animals were still a large part of human life. The biggest reason being for food. Animals have been bred throughout history and techniques in raising farm animals have been nearly perfected so the kinds of food people want and need are grown efficiently. For the purpose of making larger animals for human consumption, with leaner meat, some transgenic animals have been engineered to express extra growth factor. However, when this approach was applied to pigs, the animals developed serious medical consequences, and had to be killed. Thus the authors of this paper agree with the self-imposed moratorium on performing these kinds of experiments.

Another group of transgenic animals discussed in this paper are xenotransplanters that are engineered to produce organs compatible with human recipients. Pigs are usually chosen for such experiments due to their similar physiology to humans. They are engineered to no longer express specific sugars on the surface of their cells to avoid immuno-rejection in human patients. The authors of this paper feel this is a promising first step to alleviating the extreme shortage of organ donors, and could eventually save thousands of lives. However, great care should be

implemented to avoid viral transmission to humans, so such animals should be carefully screened for all known viruses.

Chapter 3

With the use of such technological advances in the studies of biology, one must also consider the ethics surrounding this specific field of animal manipulation. Many questions arise concerning animal rights. Should the natural order of life be manipulated? It is difficult to determine what is right and what is wrong when it comes to altering life itself. With the use of transgenic animals, the concern lies with the life of that animal. Animal rights activists argue that it is cruel to manipulate the life of an animal, sacrificing animal life for the betterment of human life and others argue that it is crucial to the advancement of biological technologies. We authors feel that medical research on specific transgenic animals should proceed only in those cases where there are very strong clear medical benefits, with attention paid to minimizing any animal suffering.

Chapter 4

Ethical issues are not the only societal impact of this technology. Transgenic animals also have legal issues. The applicable regulatory structure depends more on the objective toward which transgenics are oriented, and less on the fact that transgenic technology is employed in that pursuit. This approach is a result of continuing adherence to the "Coordinated Framework for Regulation of Biotechnology," which was completed by the federal government in 1986. This document "...established the policy that a product of biotechnology should be regulated

according to its composition and intended use, rather than by the method used to produce it." (Vines, 2002). Biotech regulations consider product, not process.

In the United States, the Food and Drug Administration is mostly in charge of safety and labeling issues, while the Environmental Protection Agency deals with ecosystem and biodiversity questions. Neither of these agencies would be well-equipped to handle intellectual property disputes, so this becomes a task for the lawyers and the courts. The authors of this paper conclude that the legal regulation of transgenic animals falls under multiple governmental jurisdictions, which creates the possibility of enormous confusion in the future.

Project Objective

The intention of this paper was to investigate and elucidate the nature and current state of transgenic animal technology, and to determine the impact of this new technology on society by investigating its ethical and legal implications. Although the medical advances transgenic animals can facilitate are potentially enormous, several ethical and legal issues surround their use, thus the purpose of this IQP was to discuss what this new controversial technology is, and how it affects society. The report is primarily concerned with defining transgenic animals, as well as giving an overview of the science involved in their creation. The effect this technology has on the rest of the world is also of vital importance, and we approach a description thereof from the perspective of the various purposes transgenic animals may be directed towards.

Chapter 1 – Introduction to Transgenic Animals What Are They, and How Are They Made

Shawn Purcell & Mimy Truong

INTRODUCTION

Transgenic animals are animals in which a foreign gene has been inserted. Such animals are used for producing new drugs, for studying disease progression, and possibly for organ transfers. Although their medical benefits are enormous, several ethical and legal issues surround their use. Thus the purpose of this IQP is to discuss what this new controversial technology is, and how it affects society. Because these animals are created by inserting foreign DNA into their genomes, we begin with a brief discussion of DNA.

Every living creature in the world uses DNA or deoxyribonucleic acid as their genetic material. Some viruses use RNA as genetic material, but they are not considered living. DNA strands contain the genetic information for an organism. DNA is divided into protein coding regions called genes. Genes contain the information needed to create a specific protein, and each protein in an organism has a specific role. Though we now have the technology to interchange the genes of two or more different organisms, genetic engineering is not merely a simple cut and paste job.

The first step in transferring DNA is to create a transgene composed of the gene desired for transfer plus some extra vehicle DNA that allows the new gene to be grown in large quantities for purification, and to express correctly and at the right time in the new animal. The vehicle DNA is broken up into two parts, the promoter sequence, and the poly A sequence. Many genes are only expressed in certain parts of an animal or in particular tissue, the promoter sequence is what ensures correct gene function, and expression in the correct cells. The poly A

or termination sequence, is added to a gene to signal that the end of the gene sequence has been reached. Once the transgene has been completed it can be inserted in a variety of ways. ("Making a Genetically Modified Animal", 2003)

Figure-1. Diagram of a typical gene construct for inserting into the genome of an animal. The green region represents the promoter for specifying the beginning point for expressing the transgene. The pink portion represents the transgene to be expressed in the animal. The blue portion represents the termination sequence for the transgene. ("Making a Genetically Modified Animal", 2003)

MICROINJECTION INTO OOCYTES

Five main groups of scientists helped simultaneously develop the technique of inserting foreign DNA via microinjection into egg cells to create transgenic animals: Gordon, Brinster, Costantini and Lacy, Harbers, and Wagner. The first group to actually finish their creation was that of Jon Gordon and his colleagues (Gordon *et al.* 1980). Although the most scientifically important of the first animals came from T.E. Wagner's group who bread a mouse with the (β)-globin gene of a rabbit. The mouse produced rabbit proteins as they would be made in a rabbit. This experiment suggested that the function and regulation of proteins could be studied in different species. One year later, one specific trangenic mouse created by Richard Palmiter, Ralph Brinster, and colleagues sparked world wide interest in the field of transgenic animals. Their mouse, which expressed the rat growth hormone gene produced mice twice the size of their un-transgenic cousins (Palmiter *et al.* 1982) (Paigen 2003).

The technique used by these early pioneers of transgenic animals is known as microinjection. The process begins when two mother animals are prepared. One, the donor mother is given hormone treatments to make her superovulate, i.e. to produce many eggs at one

time. At the same time the recipient mother's oestrus cycle is synchronized with the donor mother's. The recipient's uterus swells as it is ready to receive a fertilized egg. Eggs are harvested from the donor mother by the use of a long needle inserted in the animals side trough her abdominal cavity. The surgeon is able to monitor their egg retrieval using a laparascope (a long tube with a light source and camera) inserted in the abdominal wall. The retrieved eggs are then fertilized by a male of the same color as the donor mother, but different than the recipient mother. This is done in case the baby is the same color as the recipient mother, in which case an accidental pregnancy has most likely occurred. The egg is then grown until it has two pronuclei, as seen in the drawing below.

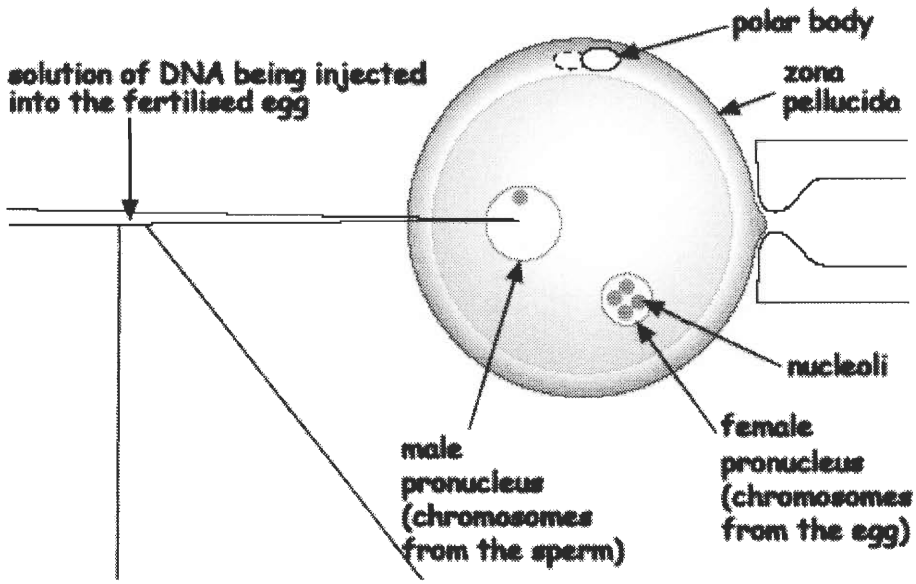

Figure-2. Diagram of microinjection into the male pronucleus. This is one of the most common techniques for creating a transgenic animal. (Green and Carlisle, 2003)

A solution containing the desired transgene is injected into the male pronucleus of the fertilized egg. The male pronucleus is chosen because its larger size allows for easier injection.

The egg is held in place with a suction pipet on the zona pellucida (the jelly-like coat of the egg), and the injection is made with a fine glass needle (Green and Carlisle, 2003).

In some cases, the egg is cultured to the blastocyst stage containing several hundred cells. The embryo is then transferred to the waiting recipient mother where it can implant in the uterus. The transgenic fetus will then grow and develop just as the normal offspring would, but this animal will have the transgene present in every cell in its body.

Although this process sounds like a straightforward way to produce transgenic animals it is actually quite inefficient. Of all mice born through the process of microinjection, only about 5% turn out to be transgenic. In farm animals the percentage is even worse, with less than 1% of animals born actually expressing the transgene. One other very important drawback to the microinjection process is that the insertion site in the genome is random, the result of which can mean little to no transgene expression, or unexpected tissue expression that occasionally causes unwanted abnormalities. A final reason why microinjection is hard in large mammals as opposed to mice is that their embryos are opaque, making locating the pronuclei difficult. (Transgenic Animals 2000)

HOMOLOGOUS RECOMBINATION

A more sophisticated technique for making a transgenic animal involves targeting the transgene to specific insertion sites, using a technique called homologous recombination. The term homologous in this case means a gene is replaced with a different gene for the same kind of function, located in the same place on the animal's genome. The most important part to this whole process is having transgenes with correct promoters that have correctly integrated with ES cells (Zinnen, 1997).

When a researcher adds a donor gene to a population of cells, there are at least three combinational possibilities that could occur within each cell. First, the donor gene could fail to insert, and the transgene would not be delivered into the cell. Second, the donor gene could also still randomly insert anywhere in the genome, just as in the microinjection technique. Third, when lucky, through homologous recombination, the donor transgene will insert at the site of its homologous gene and displace it.

Now a scientist has a formidable task, and that is to separate the cells that have exchanged genes from those that have not, and also to separate out the cell in which random insertions have occurred. Unfortunately, the donor gene is not easy to find when it inserts. Some genes have traits that make them easy to visibly see, these genes are called scoreable markers. Examples of scoreable markers are genes that create enzymes that make color, or light, or even fluorescent proteins. If we attach a scoreable marker to our gene of interest, it is then assumed that wherever we see one of the markers, that the important gene is there as well. (Zinnen, 1997)

A more precise way of separating cells that have undergone homologous recombination involves the use genes known as selectable markers. Selectable markers are inserted into the donor gene, and are special because they are resistant to certain types of antibiotics. A common selectable gene for resistance is called neo. Neo has a resistance to the G418 antibiotic. Recall that in our cell population many cells would not combine in any way with our transgene. When put in a G418 antibiotic solution all those uncombined cells would not survive. Those cells remaining would be our desired specific site insertion cells, and those in which the gene combined randomly. (Zinnen, 1997)

In order to separate the cells in which random gene insertion took place from those that underwent homologous recombination, we add a third gene to our donor transgene. The purpose of this gene is almost opposite of the selectable marker from above, this gene makes any gene it touches vulnerable to other chemicals or antibiotics. Often, the thymidine kinase (TK) gene is used. This TK gene's weakness is the antibiotic Gancyclovir. This gene is added next to but not within the region of homology. This means that if our three genes randomly insert themselves that this third gene will stay attached to our donor gene, and if treated with Gancyclovir would all be destroyed. But if homologous recombination occurs, this third gene, since it is not within the region of homology, is not inserted into the cell and therefore is not suceptible to the Gancyclovir, and that recombined cell lives. The point of this whole process is that starting with a population of cells growing in cell culture, one can generate, select for and obtain cells that have, through homologous recombination, exchanged a donor gene for an existing gene. (Zinnen, 1997)

Once the donor cells have been collected, they can be injected into a semipregnat recipient mother. Although the first offspring will be a Chimeric mouse, one in which not every cell expresses the transgene. It will have mixed offspring, some with no transgenic traits but some that will completely express the gene and start a new line of transgenic organisms.

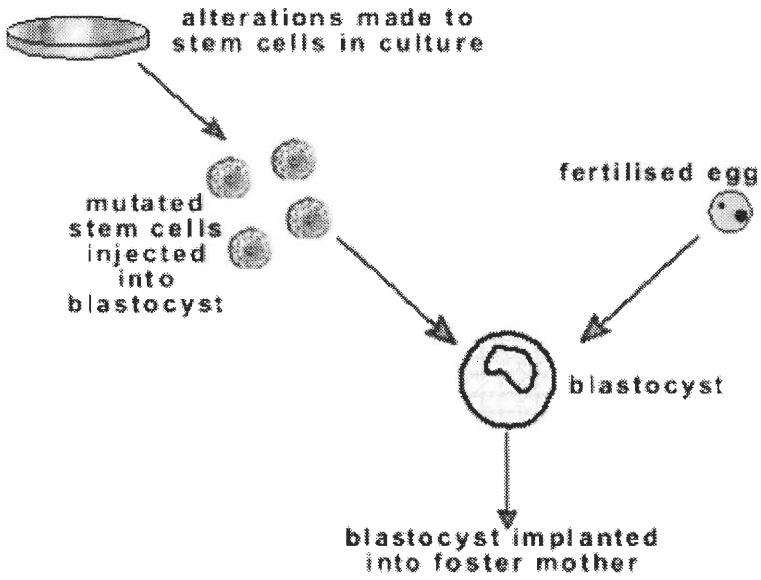

Figure-3. A diagram of homologous recombination for inserting a transgene into an animal's genome. ("Making a Genetically Modified Animal", 2003)

The true benefit of these genes with targeted expression is that by replacing a gene with another gene, we are actually removing or "knocking out" a gene from an animal's genome. Many times, by removing a gene, we are actually able to see what purpose a particular gene serves in this animal. Initial knock out experiments actually surprised most scientists in that the knock outs had little to no effect on animal. In an early example Wientraub and colleagues attempted to remove the gene that they thought caused muscle differentiation. Once removed the mouse created showed no signs of reduced muscle mass. It wasn't until later experiments that it was realized the gene in question was redundant to a second gene, and only when both genes were knocked out did it affect muscle differentiation. Though many times not what we expect, gene knock outs have been critical in answering many questions about the role of specific genes in the biology of other organisms, leading to a much better understanding of ourselves. (Paigen, 2003)

VIRAL DELIVERY

Retroviral infection of embryos is one of the three general techniques used for construction of transgenic mice. The purpose of retroviral infection of embryonic stem cells is to study cell lineage and random insertional mutagenesis. Unlike microinjections, retroviral infection can be performed at various stages of embryonic development. These stages range from preimplantation to midgestation. Retroviruses integrate into the host genome without deletions or rearrangements of the chromosomal DNA, making them very useful in random insertional mutagenesis studies. Retroviruses that infect embryonic stem cells allow for use of promoter trap and gene trap experiments. In this case, the infection allows for pre-selection of integration sites that may be of interest (Houdebine).

The following is a brief description of the technique for generating transgenic mice using retroviral infection of embryos: The DNA vector used for retroviral infection of embryos are recombinant or wild-type retroviruses. Introduction of DNA is done by infection after the removal of the cellular layer surrounding the mature unfertilized oocyte known as the zona pellucida. Embryo transfers for retroviral infection occurs in the uterus. The genotype of the founder mice is mosaic. Southern blots or PCR is used for screening of newborns. There is one copy of integrated DNA. The percentage of potential founders that are transgenic in retroviral infection of embryos ranges from 5 to 40% and there is poor expression of the new DNA. Integration is apparently random using retroviral long terminal repeats or LTRs. There is usually germ line transmission by founders in retroviral infection of embryos. The advantage of using the retroviral infection technique for constructing transgenic mice is that it results in single-copy integration using retroviral LTRs. The disadvantages concerning the technique are that there is

low-level expression and high titers can be difficult to achieve (Pinkert).

Transferring genes via viruses use receptor-mediated pathways as way of entry of foreign genetic elements into the target cells, or embryonic stem cells. Viral vectors are, for the most part, constructed with deletions in the virus that make replication a non-issue without the presence of a helper virus or wild-type virus (Joyner). There have been many different types of viral systems that have been used for construction of transgenic animals. These viral vectors include: adeno-associated virus, SV40, adenovirus, herpes virus, bovine papillomavirus, retrovirus, and vaccinia. However, most studies up to date have relied on retroviral systems as a means for viral delivery into mammalian cells. Some advantages in using the method of retroviral vectors include the following: broad host range, high efficiency transduction in cultured cells, stable integration, and helper virus-free. There are also disadvantages that include the following: random site of integration, inadequate control of expression, produced by mammalian cells, size limitation of approximately eight kilo bases for insert DNA, and integration requires target cell replication (Joyner).

In order to better understand the method of retroviral transfection, a protocol including materials and methods will illustrate exactly how this procedure is carried out. The protocol is entitled: GENE TRANSFER IN AVIAN EMBRYOS USING REPLICATION COMPETENT RETROVIRUSES. This procedure can be found in Molecular Embryology: methods and protocols edited by Paul T. Sharpe and Ivor Mason. The protocol for retroviral delivery into ES cells requires construction of the retroviral vectors, preparation of primary cultures of chick embryo fibroblasts (CEF), transfection of proviral constructs, collection of concentration of viral stocks, preparation of infected cells for grafting, and 3C2 immunostaining of virally infected CEF or QT6 cells. For the first part of the experiment, constructing the retroviral vectors, the

fragments that are to be subcloned can be done by either PCR or restriction digests. If using PCR to make the insert fragment cloning can be done by adding restriction sites to the short-chain polymers of interest. To let the fragment be directly cloned into the NcoI site of the adapter polylinker, NcoI site is introduced at the initiator ATG site. Next cutting of the PCR product is to be done and the product should be inspected to make sure there are no mutations. Next, partial or complete restriction enzyme digestion is done on the ClaI to ClaI fragment from the adaptor plasmid. It is then subcloned into the right RCASBP/RCANBP vectors by standard cloning procedures. Another example of retroviral-mediated gene transfer can be found in Transgenic Animal Technology: A Laboratory Handbook by Pinkert.

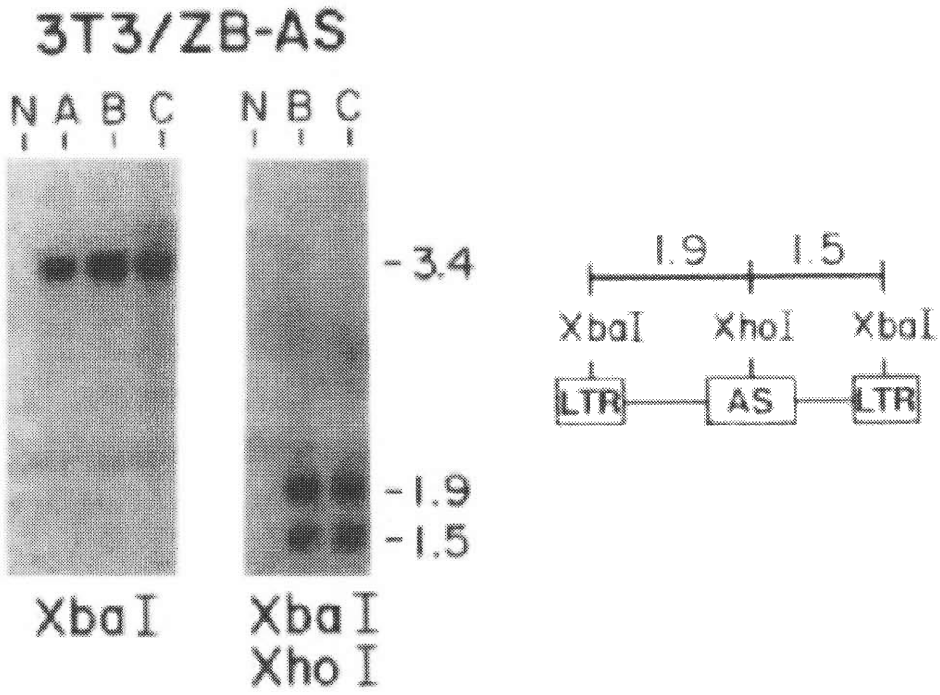

Figure-4. The above figure A is an example of what a blot would look like that shows whether or not the transfection was successful. The figure comes from Transgenic Animal Technology: A Laboratory Handbook by Pinkert. AS cDNA was used as the probe for this blot where (N) refers to noninfected, and (A, B, C) refer to infected 3T3 clones.

CHEMICAL DELIVERY

Another technique for delivering DNA into embryonic cells to create a transgenic animal is by way of chemical such as liposome-mediated transfection. In this case the type of embryonic cells for use of delivery is embryonal carcinoma (EC) cells. These cells are excellent in studying early mammalian development. Nowling et al (2002) performed a comparison of various ways of transfecting foreign DNA into EC cells. Their data is shown in Table 1 below.

TABLE 1. Transfection of F9 EC Cells Using Different Methods

Transfection method	Final cell number ($\times 10^6$)	Transfection efficiency (%)
Mock	2.67 ± .06	.16 ± .02
DOTAP	2.57	3.4
$CaPO_4$	.59 ± .16	3.01 ± 2.39
$CaPO_4$ wash	1.37 ± .60	4.19 ± 3.11
LipofectAMINE	2.51 ± .01	.99 ± .02
LipofectAMINE + PLUS	1.93 ± .21	20.55 ± 1.06
FuGENE 6	3.05 ± .78	19.4 ± 1.27

F9 EC cells were grown in 60-mm culture dishes at a starting cell density of 80,000 cells per dish in 3-ml DMEM + 10% FBS. Cells were harvested 48 hr after transfection. Total DNA used was 1.5 μg per dish. Amounts of transfection reagents used were: DOTAP, 30 μl; $CaPO_4$, 30 μl $CaCl_2$; LipofectAMINE, 12 μl; LipofectAMINE PLUS (PLUS), 8 μl; FuGENE 6, 5 μl. This experiment was repeated three times and similar results were obtained.

Table-1. The results of transfection efficiency using level of transfection obtained with calcium phosphate as a reference (Nowling et al, 2002).

This table shows that FuGENE 6 and LipofectAMINE-PLUS provided higher transfection efficiency than the other techniques tested, and provides an excellent example of the use of new chemical reagents for more efficiently inserting foreign DNA into cells. The data is shown in a different format in Figure-5 in which the two efficient treatments discussed above provided the

greatest expression of a fluorescent marker (x-axis).

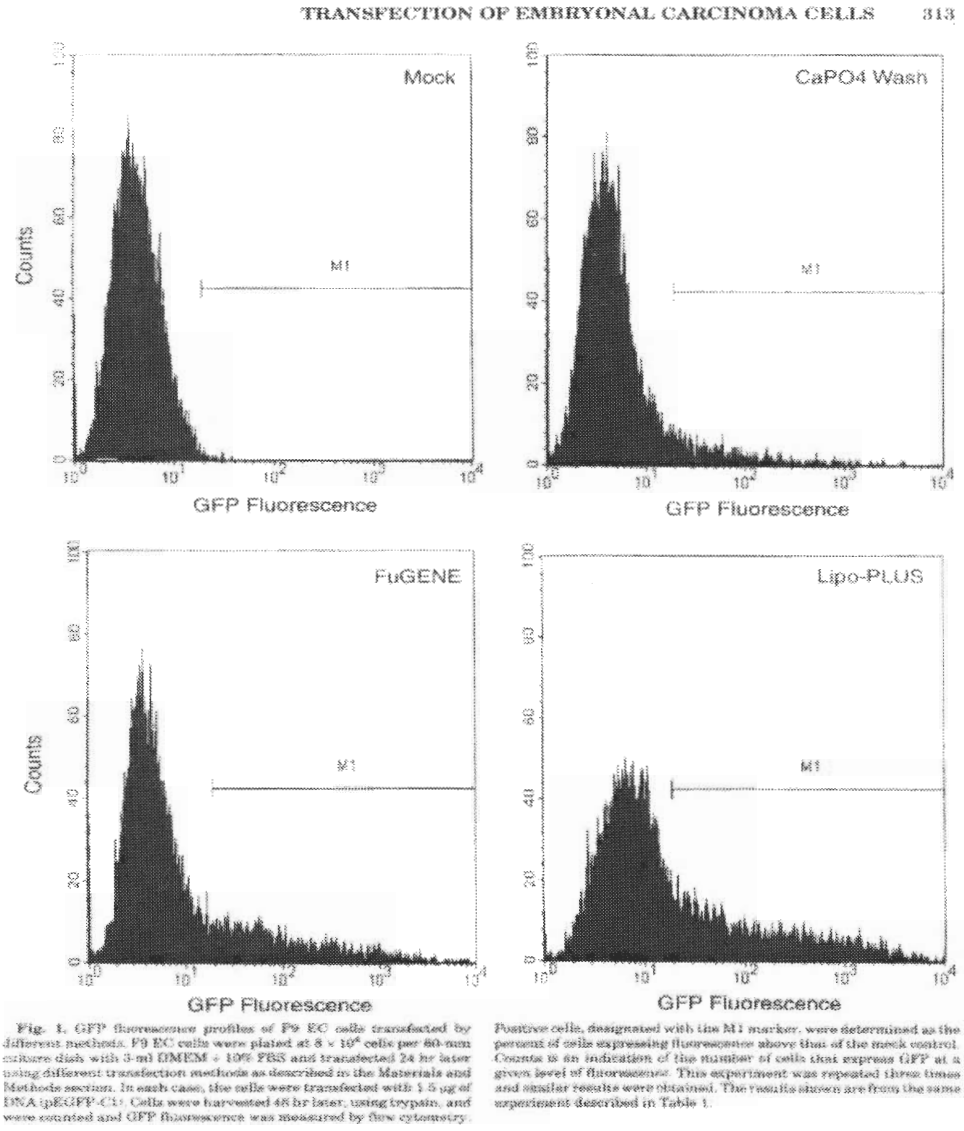

Fig. 1. GFP fluorescence profiles of F9 EC cells transfected by different methods. F9 EC cells were plated at 8×10^4 cells per 60-mm culture dish with 3-ml DMEM + 10% FBS and transfected 24 hr later using different transfection methods as described in the Materials and Methods section. In each case, the cells were transfected with 1.5 μg of DNA (pEGFP-C1). Cells were harvested 48 hr later, using trypsin, and were counted and GFP fluorescence was measured by flow cytometry. Positive cells, designated with the M1 marker, were determined as the percent of cells expressing fluorescence above that of the mock control. Counts is an indication of the number of cells that express GFP at a given level of fluorescence. This experiment was repeated three times and similar results were obtained. The results shown are from the same experiment described in Table 1.

Figure-5. GFP Fluorescence cell count (Nowling et al, 2002)

Lipofection as a chemical delivery method for transfection to create transgenic animals can be

used to transform cells of animals, yeast, plants, and bacteria. During the process of lipofection, the cells are treated to remove their cell walls, except in the case of animal cells, for they do not have cell walls. Special kinds of lipids that form tiny hollow bubbles are added to a solution. Into that solution the DNA of interest is combined. The cells to be transformed are then added to the solution containing the liposomes. These liposomes become part of the cell membrane of the cells and the liposome contents enters the cells (Pinkert).

Chapter 2 – Transgenic Examples

Shawn Purcell

In chapter-1 we went over what transgenic animals are, and how they are made. Following the advent of transgenic technology, over the years a large variety of transgenic animals have been made. Some of these animals have obvious medical benefits, and some do not. The purpose of this chapter is to describe and categorize some examples.

DISEASE MODELS

One heavy burden on the shoulders of science is that people expect to live healthy lives. Many ailments can be cured with modern medicines, and we have even gone so far as to create vaccines able to nearly extinguish some diseases, which used to run rampant around the world. For instance, MMR vaccinations prevent almost all new cases of the childhood diseases measles, mumps, and rhubella. Still some diseases have no cures and continue to spread all over the world.

Oncomouse

Often, prevention is the best cure. Because of this many animals are used to test every consumer product on the market today. As an example, products are tested to see if they produce cancer in human beings. Before transgenic animals, testing for these carcinogens would take at least 200 mice and take up to 2 years. There is a reason that hundreds of mice were needed to perform these test reliably, and that is that most mice are resistant to cancer formation (NIEHS Press Release, 2001) (NTP Factsheets, 2002). These animals are now susceptible to a type of

cancer which allows us to study the biology of tumor formation, and it allows fewer animals to be used to screen for potential carcinogens.

Alzheimer's Mouse

As described above, many diseases have non-transgenic models that can be used to test for disease treatments, however most human diseases do not. As an example, before the advent of transgenic animals, scientists relied on crude experiments to test new Alzheimer's drugs ("New Troopers..", 1995). A disease of the mind, Alzheimer's affects more than 4 million Americans. The disease, which generally affects the elderly, can also begin in hereditary cases as early as age 40 ("WPI Professor's..", 1995). Alzheimer's reduces mental ability, producing confusion, memory loss and impaired judgment, eventually leading to death. One of the primary causes of neurodegeneration is the formation of senile plaques, which are composed of amyloid protein. The first successful mouse model for Alzheimer's disease was created by Prof. Adams in WPI's BB department, in collaboration with the former Transgenic Sciences, Inc (Worcester, MA) (Games et al, 1995). The transgene used to create Alzheimer's symptoms in mice encodes human amyloid with a mutation to mimic an Indiana family with early onset disease. In mice, the human amyloid caused amyloid plaques to develop in the brain as they aged. This plaque damaged nerves and synapses in the rodents' brains, just as in human cases, proving that amyloid formation is a key cause of the disease. Prof Adams, when he introduced these mice to the scientific community, stated quite simply the magnitude of this mouse's importance when he said: "Researchers will now have mice that can be used to study Alzheimer's disease and test new treatments. Such experiments were formerly impossible without an inexpensive animal

model for the disease" (WPI Professor's...1995). The New York Times hailed the discovery as a "landmark in Alzheimer's research" (Kolata, 1995).

The creation of this disease model not only allowed the testing of a large variety of drugs on senile plaque formation, but also was a required first step in the development of a vaccine to clear the plaques from the brain (Schenk et al, 1999). Elan Pharmaceuticals used this mouse line to develop a vaccine which is currently in human clinical trials.

HIV Rodent

Many times when creating transgenic animals, the correct species can make all the difference. HIV (Human Immunodeficiency Virus) has been one of the most publicized viruses in recent decades. HIV is a virus only seen in humans since 1959, but since that time it has come to the forefront of disease research. In 2001 the first HIV-rat model was created (Reid et al. 2001). HIV attacks the cells of our immune system, which leads to the onset of AIDS. The virus mutates rapidly, a trait that has made it especially difficult for researchers to find effective treatments, or a vaccine. Of the several small-animal HIV models that have been created with mice, the mice have displayed skin lesions, hyperplasia and lymphoma, and some immunodeficiency due to loss of T cells. Unfortunately these are only some of the complex pathologies linked with HIV in humans, and the mice only display particularly high levels of HIV expression in the skin.

Reid's transgenic rats however, at only five to nine months of age develop AIDS characteristics, including cataracts, weight loss, skeletal muscle atrophy or "wasting", neurological abnormalities and respiratory difficulty. Transgene expression also occurs in lymph nodes, spleen, thymus, and blood. Because of these physical similarities to AIDS and the

immuno irregularities characteristic of HIV infection in humans, the rat will offer significant advantages as a small-animal model. The rat is also beneficial for blood and tissue samples, as mice provide only 2 to 3 millimeters of blood, rats 30 to 40 milliliters. Examining a rat's larger organs can also only benefit researchers in their quest to understand HIV. (Reid et al, 2001) (Kohn, 2001)

ANDi the Transgenic Monkey

As useful as mice and rats are as human stand-ins, sometimes the differences between rodents and humans are too great to make them useful tools for researching human disease. One example is that only primates have a monthly menstrual cycle, impacting the effects of breast and ovarian cancer. Rodents also lack a macula, a part of the eye lost in macular degeneration. An even bigger difference in mice and men is that rodent brains have not been shown to be complex enough to study the subtle effects of neurological diseases such as schizophrenia and Alzheimer's (Hayden, 2001). Thus, primates are now being sought as transgenic disease models.

After cloning a monkey in 2000 Anthony Chan and Gerald Schatten, along with their colleagues at the Oregon Regional Primate Research Center, set out to create the first transgenic primate. The transgene of choice was the green florescent protein gene from jellyfish. This well-studied gene actually does glow green under blue light. Using the retroviral technique, 224 rhesus-monkey eggs where given the transgene. Once fertilized with microinjection of sperm, 126 eggs grew to viable embryos. From this group Chan implanted the 40 best embryos in 20 surrogate monkey-mothers. Of the twenty, five pregnancies resulted. In the end three monkeys were born, and only one, born on October 2, exhibited signs of the green gene. This one special monkey called ANDi, which stands for "inserted DNA" backwards, is in the history books as

the first transgenic monkey. While ANDi does not glow as his transgene suggests, scientists have discovered traces of the transgene in his muscle, hair, cheek and blood cells. Whether or not ANDi can produce transgenic offspring we cannot not know right now. It will be four years before ANDi reaches sexual maturity at which point we will see if the transgene has made it into ANDi's reproductive cells. Even with the success of ANDi, transgenic monkeys are still a long way from becoming common research tools. The lack of efficient creation techniques, along with the expense and the ethical questions, will limit the use of transgenic monkeys as medical models. Drawbacks aside, the ability to study primates will enhance our knowledge of aging, neurodegenerative diseases, immunology and behavior. Monkeys will also be a great resource to developmental biologists, as they are suitable to fit in human diagnostic machines such as magnetic resonance imaging (MRI) machines. ANDi, and the monkeys to follow his footsteps will help fill in gaps in what we can learn from transgenic mouse models. (Begley, 2001) (Lemonick et al, 2001) (Vogel, 2001)

TRANSPHARMERS

Many of the hot new drugs available to consumers today are genetically engineered proteins that many people's bodies do not produce enough of on their own. These new drugs are usually created in big factories filled with bioreactors of gene-altered bacteria or culture dishes of animal cells (Ezzel,1991) Most of these drugs are amazing cures for many diseases that can benefit so many people, but cost and availability severely limit the number of people who can take advantage of such treatments. The reason companies are simply not building more bioreactor factories is that the cost to create these factories is upward of $300 Million, and can take 3 to 5 years to construct.

To solve this problem scientists have taken a hint from mother nature. Over the ages, the mammary glands of goats and cows have evolved to pack proteins into breast milk, so that mother animals can nourish and protect their young. By introducing human protein genes, these natural protein factories, now known as "transpharmers", can produce a near limitless supply of these drugs. Once a transpharming animal has been created, simply breeding more animals can increase output of the proteins. ("Brave New Worlds", 2002)

Now numerous human proteins are being produced in transgenic animals. The exact number is not known, says Paul Doering professor at University of Florida's college of Pharmacy "it is difficult to say how many tests of medicines from transgenic animals are under way, because many companies keep early trials under wraps for competitive reasons." (Clark, 1999)

A variety of animals are used to create the even wider variety of protein based drugs. And each animal has its own distinct advantages. Genzyme Transgenics Corporation in Framingham, Massachusetts, has special goats that have been engineered to produce human antithrombin III. This protein is used to control blood clotting. The drug will be useful to help patients undergoing coronary bypass surgery who are particularly vulnerable to excessive clotting and the danger of thrombosis ("Building to Order", 1997). The goat is a desirable transpharmer because it has a short gestation time of only 5 months. The lactating doe produces 4 liters of milk a day, 300 days a year. Goats are easy to manage having good temperament. Goats are also inexpensive to feed and maintain, as they are much smaller than cows. Goats also have a favorable disease history, without diseases like scrapie in sheep. (Ely, 1998)

Not that sheep are a bad source for human proteins either, Alan Colman of Pharmaceutical Proteins Ltd. has created sheep expressing human alpha-1-antitrypsin or AAT

(Ezzel, 1991). AAT is a protein produced in the liver that protect the lungs for a common enzyme which normally fights bacteria and cleans up dead lung tissue. A person with AAT deficiency does not create enough protein, and permanent irreversible lung damage is the result. The protein refined from these sheep could make affordable treatments available for the estimated 100,000 patients with an AAT deficiency, at the estimated annual cost of $60,000 to $80,000. The current source of AAT comes from blood plasma donations. (Clark, 1999)

The animals with the most promise as transpharmers are cows. Cows can produce thousands of liters of milk per year, meaning lots and lots of end product drugs. But the expense of collecting eggs from live cows, and then implanting them in such large animals initially slowed the transgenic cow process (Ezzel, 1991). Herman de Boer at Gene Pharming Europe B.V. in Leiden, now Pharming Holding NV, overcame these difficulties. Boer collected his eggs from slaughterhouses, and implanted 103 embryos with the gene to produce lactoferrin, into recipient cow mothers. In the end only 2 cows were born, and only one bull named Herman who displayed the transgene, became the first transgenic cow. Born in 1990, Herman, being a bull, could not actually produce the protein. Boer had to wait 4 years before Herman's first female offspring was milked, to finally prove his experiments as a success. (Spanjer, 1993)

Another first animal of its kind having transpharming success is Genie, the worlds first transgenic pig. Born in 1990, and created by William Velander at Virginia Polytechnic Institute. It was one year after her birth that scientists realized Genie could produce human protein C in her milk, an anticoagulant to treat blood clots. Other pigs created by Velander and William Drohan are milked for other profitable blood products such as Factor VII and fibrinogen which help our blood clot and is used to treat hemophilia. (Rosenfeld, 1998) ("Building to Order", 1997)

XENOTRANSPLANTATION

Transplantation is now widely viewed as the preferred treatment for end-stage organ failure. The survival rate for kidneys and hearts that have been transplanted for at least one year is 90%. Rejection rates are less than 30%. There are approximately 2,500 heart transplants and 12,500 kidney transplants performed every year in the US. The only thing currently preventing more transplants is a lack of donor organs. There are more than double those numbers of people on organ waiting lists than could ever receive one, and 10 people die per day waiting for a transplant.

The use of animal organs, xenotransplantation, has been considered for quite some time as a solution to the organ shortage, and three animal to human transplants have been attempted, all failing miserably. A young girl, Baby Fae, died 20 days after Californian surgeons gave her a baboon heart in 1984. Later two patients given baboon livers in 1992-93 both died within 10 weeks of transplant (Begely, 1993). Because of those failures, all future xenotransplantations have stopped, and much research is yet to be done before any other research involving humans is allowed. In fact, the baboon is not even the animal best suited for donor organs. This decision is based on concerns of compatibility in size, immunology, and pathology, which must all be taken into consideration. Primates are more closely immunologically related to humans, therefore, rejection could be more easily controlled with current drugs used in human to human transplants. Unfortunately, all apes of appropriate size, generally chimpanzee or larger, are on the endangered species list, and using them is not acceptable, even if possible to breed large numbers of them in captivity. The most abundant non-human primate is the baboon, but a baboon's heart is not big enough to support a full grown human. The kidneys and liver of a baboon could be

considered however. Unfortunately the baboon contains a number of potentially pathogenic organisms that would be very problematic if transmitted to humans. The risk is so great in fact, that the FDA has issued guidelines stating the use of non-human primates as donors poses unacceptable risks. (Logan and Sharma, 1999)

Therefore a seemingly unlikely candidate, the pig, has been selected as the best choice as a donor. The pig's organs are large enough to support human life. It was also thought that pigs could easily be raised in sterile captivity to be pathogen free, making a clear choice for scientists. The only hurdle left then to cross in making pig organ donors is the immunology and rejection concerns. Luckily there is one distinct advantage that the pig offers as a donor, and that is our ability to genetically modify the pig. (Logan,1999)

The problem with just putting a pig's heart in a human body is the process of hyperacute rejection, or HAR. HAR results in the complete loss of a graft and destruction of the donor organ within a few minutes to hours of transplantation. The process begins when antibodies in the recipient bind to the graft, destroying the foreign body as it would any invading bacteria. The antibodies begin a process called complement where the outer cells of the organ fill with plasma and cause destructive hemorrhaging. The human antibodies are attracted to the organ by the carbohydrate structure Galα-3Gal. Many mammals synthesize this substance, but humans and other primates do not. Galα-3Gal serves as an identifier, comparable to blood sugars in a humans signifying A, B, or O blood type. Once the Galα-3Gal is detected, only three proteins including decay accelerating factor (DAF), membrane cofactor protein (MCP) and CD59 can save the organ. Known as the complement regulatory proteins (CRP), these proteins can stop HAR from occurring. It is apparent that a pigs equivalents to CRP are non functional against human antibodies. By developing transgenic pigs which limited their Gal carbohydrate

expression, or that produced a significant amount of complement regulatory proteins, the antibodies causing HAR would either not be attracted to the graft or could be turned off upon detection of a foreign blood sugar. (Logan and Sharma,1999)

As early as 1993 scientists led by John Logan, at Duke and DNX of Princeton NJ, created the first pigs which expressed any of the human complement proteins. As predicted, the organs from these pig were able to mount a defense against HAR and organs survived longer in baboon recipients than control, non altered organs. (Begley,-1993) Insertion of different CRP into pigs continued throughout the 90's. In 1999 R. H. Chen and colleagues at Brigham & Women's Hospital created a line of transgenic pigs with the CD59 and DAF human complement proteins. While the heart of a normal control pig would last 20-80 minutes in immunosuppressed baboons, the transgenic hearts functioned for 85-130 hours. (Henderson, 1999) Later experiments by Chen, Adams, Kadner and Farivar, created a pig expressing the human membrane cofactor protein (MCP) and transplants to baboons occurred in 2001. Hearts were removed from their baboon recipients at 5 and 46 hours post-transplantation, and both hearts were functioning at time of removal. It turns out that hearts with the MCP can last for 16 days before rejection. (Adams et al, 2001) These two models are more proof that CRP's are vital to decreasing the effects of HAR of cardiac xenographs in baboons and potentially human recipients.

In 1997 Robin Weiss and colleagues at the Institute of Cancer Research in London actually caused the progress of xenotransplantation to fall back a step on its way towards clinical human trials. Weiss and team discovered a pig retrovirus that can infect human cells in vitro. A retrovirus is especially dangerous since they infect cells by integrating their genes into a host genome (Travis, 1997). The virus now known as the porcine endogenous retrovirus (PERV), although it can be transmitted to human cell cultures, has not been detected in any at risk

individuals. The test subjects include patients treated with pig tissue, and butchers, two groups most likely to come in contact with pig blood and PERV. Using highly sensitive screening methods no evidence was found of PERV-infection in humans potentially at risk. (Tacke et al, 2001)

Three big steps have been taken in the past year by one company in particular to advance the field of xenotransplantation. Immerge BioTheraputic's scientists Robert Hawley and Kenth Gustafsson, along with Liangxue Lai and colleagues, created the first miniature swine with the two genes called GGTA1 knocked out. Born on November 18, 2002 this pig, potentially will not posses the carbohydrate Galα-3Gal. As described above, this sugar is what human antibodies attach to allowing them to kill the donor organ. Since both copies of the gene are eliminated, the antibodies cannot attach, and hopefully will avoid the early rejection process ("Data presented", 2003). Another plus for this breed of swine is that the pigs of its herd possess a nontransmitting phenotype for PERV. Scientists have not been able to infect human cell cultures with the virus from this breed, so using this line of pig minimizes the theoretical risk of PERV infection in humans. ("Company Identifies Miniature Swine", 2002) Immereg also announced it has reversed insulin dependency in diabetic non-human primates through transplantation of porcine islet cells. The Cynomolgus monkeys were implanted with islets purified from the pancreas of adult pigs and miniature swine. The diabetes reversal lasted upwards of 70 days. Lastly Immerge scientists with Robin Weiss and Daniel Salomon also identified the receptors used by PERV to enter and infect a cell. This development could lead to ways to prevent PERV replication in human cells. ("Advances in Xenotransplantation Reported", 2003)

FOOD SOURCES

Before transgenic animals, before the knowledge that genes even existed, animals where still a large part of human life. The biggest reason being for food. Animals have been bred throughout history and techniques in raising farm animals have been nearly perfected so the kinds of food people want and need are grown efficiently. But there are disadvantages that come with the mass production of animals. One such problem is animal waste. Over 90,000 tons of phosphorus is released into the environment from animal waste each year. This phosphorus is washed into lakes and streams, causing algae blooms that deplete a lake's oxygen, killing many fish. The problems occur because pigs lack the enzyme phytase in their saliva, which is used to digest phosphorus in pig feed. (Gewolb, 2001) However pigs created by Cecil Fosberg as the University of Guelph, Ontario, enhanced with a mouse gene, excrete 75% less Phosphorus than standard control pigs. (Enviropigs,2001)

Land animals are not the only ones getting enhanced by genetics. One of the most successful transgenic food sources was discovered by accident around 20 years ago. When Choy Hew accidentally froze a tank full of a specific type of flounder, he had no idea that they would survive their de-icing. It turns out that the flounder have a gene, which in cold water turns on an anti-freeze like protein, keeping the fish alive. Hew and colleagues isolated that "turn-on" gene and attached it to the growth hormone in chinook salmon. As salmon live in cold water, the trans-salmon grew at nearly twice the rate of the common chinook salmon. While the transgenic salmon don't appear to grow any larger than the control salmon, they do reach a mature size in 18 months, while the control salmon take 24 to 30.

DEVELOPMENTAL MODELS

The final large group of transgenic animals is composed of animals designed to help us better understand human devotement. By carefully studying and evaluating these animals scientists have a clearer understanding of how we as humans develop. The genes in these animals could be "knocked out" to determine if certain deficiencies lead to developmental problems. Animals in this group could also have added genes to create "super" or enhanced animals, with above normal intelligence or physical strength, sometimes creating the most controversy as people fear the science fictional view of genetically enhancing humans. The pure scientific merit of such transgenic models is perfectly clear in the mouse affectionately called by its creators "Doogie". Doggie's creators, Joe Tsien and colleagues, named their mouse after the hit show Doogie Howzer, M.D., as their mouse, just like the young Mr. Howzer are both geniuses in their respective races. (Tsien, 2000)

Memory and learning are important processes, which require deep and in-depth understanding. What we learn and what we remember determine largely who we are. Our memories define us as an individual, and while ones physical appearance can be altered to be a near perfect copy of another individual, our memories are personal and can not be simply transferred from one person to another. The fundamental knowledge of learning began in 1949, when Canadian psychologist Donald Hebb proposed that a memory is produced when two nerve cells are connected creating a synapse. The chemicals between the cells, called neurotransmitters, flow from one cell to the next. It has been shown that these connections are controlled by NMDA receptors. The first successful transgenic mice with NMDA changes were produced by Tsien, with the help of Mattew Wilson, Patricio Huerta, Thomas McHugh and Kenneth Blum of MIT. They found that with the NMDA receptors knocked out from a section

of the brain's hippocampus, the mice show decreased memory function, as they could not remember their way around a water maze. (Tsien, 2000)

Not satisfied by the tests, Tsien, with help from Guosong Liu, and Min Zhuo, created mice with enhanced NMDA receptors of children to replace the receptors of adults. They found that synapse connections could stay open nearly twice as long as normal adult mice. The results are echoed by the well know fact that children seem to learn better than adults. The new mice could remember objects 4 to 5 times longer than control mice; they also learned incorrect paths in shock experiments more than twice as fast. The results of Tsien's experiments prove that NMDA is a key piece in the course of human development and learning. (Tsien, 2000)

Chapter 3 – Transgenic Ethics

Mimy Truong

With the use of such technological advances in the studies of biology, one must also consider the ethics surrounding this specific field of animal manipulation. Many questions arise concerning animal rights. Should the natural order of life be manipulated? It is difficult to determine what is right and what is wrong when it comes to altering life itself. With the use of transgenic animals, the concern lies with the life of that animal. Animal rights activists argue that it is cruel to manipulate the life of an animal, while others argue that it is crucial to the advancement of mankind, and to find cures for human diseases, sacrificing animal life for the betterment of human life.

Animal rights activists take a strong stand and can be very convincing. One philosopher, Dave Kopel, makes an interesting comparison (Kopel, 1998). Take for example an extremely retarded child, this child still has rights yet remains incapable of higher level thought; on the other hand consider a chimp who's very capable and surpasses the extremely retarded child in intellect. What sense is it that the child has rights but the chimp's life is in the hands of scientist to manipulate? A more classic example, and perhaps more convincing would be to consider the question "Can it suffer?" first noted by 19th century philosopher Jeremy Bentham. If one were to witness a cattle prod being used on a human being would one consider his rights to be violated? Take that same instance and put a farm animal in the human's place, is there no pain then? Of course not. The animal feels pain as well. But because they do not communicate with words as do human beings do, is it our place to disregard animals' safety? No. That would be considered cruel (Kopel, 1998). The late C.S. Lewis observed, "Man's power over nature is really the power of some men over others with nature as their instrument."

Genetic engineering has sparked an ongoing controversy among both the non- science and science community. Take a step back into time and consider the words spoken by our 26th President of the United States of America, Theodore Roosevelt:

> Some day we will realize that the prime duty, the inescapable duty of the good citizens of the right type is to leave his or her blood behind him in the world; and that we have no business to permit the perpetuation of citizens of the wrong type. The great problem of civilization is to secure a relative increase of the valuable as compared with the less valuable or noxious elements in the population ... The problem cannot be met unless we give full consideration to the immense influence of heredity ... I wish very much that the wrong people could be prevented entirely from breeding; and when the evil nature of these people is sufficiently flagrant, this should be done. Criminals should be sterilized and feebleminded persons forbidden to leave offspring behind them... The emphasis should be laid on getting desirable people to breed.
>
> (Howard and Rifkin, 1977, pp 47)

This form of thinking called eugenics was a movement that spanned thirty years and is now lost within the history books. Reading the statement above, one may be thinking that it was a radical idea and should be left in the past. However, the above statement contributes in the argument that perhaps by allowing the use of transgenic animals in genetic engineering, will make way for future use in human beings and hence making the statement a reality. Eugenics, the thought of breeding perfect humans (Howard and Rifkin, 1977). Who decides what genes are better than others?

Now consider the other side of the argument for the use of transgenic animals in the advancement of genetic engineering. Look at transgenics as another weapon against the never-ending war against disease. Genetic engineering is a tool to be used for the greater good. Like any weapon, if fallen into the wrong hands can mean disaster. For this reason strict laws and regulations have and are going to help police the technology. Among the general population, little is understood about the technology itself and what its goals are and how important it is to continue the research, which means use of animals. Many scientists consider species as a

population or reproductive community, and altering some genes in that animal for research purposes does not interfere with the integrity of that species; they exist in nature as reproductive communities not as individual creatures (Macer, 1990). The use of animals is extremely vital in research of biological interests. Without it, the advancement and knowledge of biotechnology would be extremely slow moving or even non-existent.

So how do we combine the positive medical benefits to society that transgenic animals bring with sensitivity to animal rights? The authors of this paper support animal rights, but believe that in those specific transgenic cases where there are very clear medical benefits, and little or no animal suffering, such experiments should proceed.

Chapter 4 – Transgenic Legal Issues

Daniel Schmidt

INTRODUCTION

Although ethical concerns surrounding transgenic animals are of high importance, the concrete and stringent nature of legal issues lends them an immediate relevance. Whereas ethics is a discussion of what one *ought* to do, laws dictate what one *must* do. Therefore in this chapter our focus is with the various requirements and restrictions governments place upon the creation and utilization of transgenic animals. In practice, governmental dictums come in the form of laws, which indicate required conduct, and guidelines, whose recommendations are only suggestions. However, the onus created by a guideline, while not mandatory, still differs from the ethical *ought* because it remains of legal importance.

> "Guidelines are suggestions or recommendations of acceptable practice. There are no legal penalties for not following guidelines (Marois et al, 1991). There may be institutional penalties for not following guidelines, such as loss of funding. If guidelines are not followed and damage results, there will be liability for damages. Guidelines tend to be more easily changed than standards, as more information becomes available." (Stallman, 1992)

One of the reasons for the varying formats of legal statements regarding transgenic animals is the rapid development of the technology. Legislative machinery cannot keep pace with scientific advances in terms of maintaining a comprehensive tailor-made legal framework. The result is that transgenic issues are approached from the direction of extant laws and jurisdictions, and responsibility ends up somewhat diffused among multiple agencies. The following chart, while not specific to transgenic animals, illustrates the capacity for biotechnology regulation confusion.

Examples of Agency Involvement in Agricultural Biotechnology Regulation		
Trait	**Agency Involved in Regulation**	**Agency ensures the product is:**
Insecticide producing food crop	USDA	Safe to grow
	EPA	Safe for the environment
	FDA	Safe to eat
Herbicide resistance in food crop (i.e. Roundup Ready® soybean)	USDA	Safe to grow
	EPA	Accompanied by safe use of companion herbicide
	FDA	Safe to eat
Viral resistance in food crop	USDA	Safe to grow
	EPA	Safe for the environment
	FDA	Safe to eat
Modified oil content in food crop	USDA	Safe to grow
	FDA	Safe to eat

Source: APHIS-USDA

Chart source: (Vines, 2002)

In the end, the applicable regulatory structure depends more on the objective toward which transgenics are oriented, and less on the fact that transgenic technology is employed in that pursuit. This approach is a result of continuing adherence to the "Coordinated Framework for Regulation of Biotechnology," which was completed by the federal government in 1986. This document "...established the policy that a product of biotechnology should be regulated according to its composition and intended use, rather than by the method used to produce it." (Vines, 2002). Biotech regulations consider product, not process.

With these divisions in mind, we have organized our examination of transgenic laws and guidelines into three areas based largely on the role transgenic technology takes within that area. These regulatory categories are:

1) Safety and testing
2) Intellectual property protection

3) Implications regarding ecosystems, biodiversity, and sustainable development

Under *Safety and testing* fall all regulations that deal with the fitness of transgenic animals for food or drug purposes, as well as any inherent risks to humans they may pose sans consumption. The *Intellectual property* section regards the patenting and protection of animals as inventions that belong to their creator. Finally, the *Implications regarding ecosystems* segment is focused on the rules that control the interactions that transgenic animals may have with the environment at large.

Among these categories it is fairly easy to see how regulatory responsibility can be dispersed to different agencies and government sectors. In the United States, the Food and Drug Administration is mostly in charge of safety and labeling issues, while the Environmental Protection Agency deals with ecosystem and biodiversity questions. Neither of these agencies would be well-equipped to handle intellectual property disputes, so this becomes a task for the lawyers and the courts. The presence of transgenic animals at the core of all these issues link them together, but the distinct modes of regulation and liability between domains aptly differentiate them.

SAFETY AND TESTING

Tests are performed on transgenic animals and products thereof to determine whether they are safe for consumption or use by humans. The regulation of this testing is determined largely by the type of use the transgenic product is being put to. Since the two major categories of usage are as food and drugs, the USDA and FDA share jurisdiction over this area.

A significant portion of the promise of transgenic animals is that they can be made to grow faster and more efficiently than natural animals, thus resulting in greater food production

and less waste. Both human and animal feed would become more affordable, but decreased cost is no benefit if the resulting product is more dangerous than the existing alternative. To avoid this eventuality, governmental approval must be obtained before any field testing, large scale cultivation, or consumption of transgenic animals may occur. The FDA controls "safety and labeling of drugs and the nation's food and feed supply, excluding meat and poultry." (USDA, 2003). Since pursuit of transgenic animals as food occurs largely within the realms of meat and poultry, the FDA cedes significant authority here to the USDA.

Specifically, the USDA regulates vaccines, research, production, and movement. Even without any consideration of a transgenic animal as food, the USDA is concerned with its possible impact on other areas of agriculture and food production, so they are responsible for monitoring creation and field testing of transgenic animals. Likewise any transportation thereof must be USDA approved, especially if it is across state lines. (US-FDA, 1999) Responsibility for regulating these areas falls to the Animal and Plant Health Inspection Service (APHIS), a subdivision of the USDA. The primary mission of APHIS is to protect US agriculture from pests and diseases, and this gives it the authority to regulate vaccines, making it most relevant to transgenic technology. As mentioned before, it is the product, not the process, that is regulated, and in the case of a transgenic animal which has been engineered to be immune to a certain disease, the immunity is the product. The transgenesis creating the immunity is viewed as a type of vaccination, and therefore falls under USDA-APHIS jurisdiction.

The other USDA agency which regulates transgenic animals is the Food Safety and Inspection Service (FSIS). "Under the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act, FSIS inspects all meat, poultry, and egg products sold in interstate commerce and reinspects imported products, to ensure that they meet

U.S. food safety standards." (USDA-FSIS, 2001). Clearly this gives the FSIS authority to inspect transgenic meat and poultry sold as food, even though no specific directive is stated regarding the biotechnological aspects thereof. However, the FSIS does go beyond their strictly defined parameters and performs research and analysis to enhance the safety of biotechnological as well as traditional food animals. For instance, it produces publications such as "Guidance on Risk Reduction During Animal Production" (USDA-FSIS, 2002), and "Points to consider in the food safety evaluation of transgenic animals from transgenic animal research" (USDA-FSIS, 1994), which discuss transgenic technologies as directly relevant to their overall mission.

As mentioned above, the FDA is the other US agency responsible for ensuring the safety of the food supply, having jurisdiction over all products other than meat, poultry and eggs. Where this is most evident is in the regulation of transgenic fish and shellfish, since those more simplistic organisms are "most amenable to [the] current level of technology." (US-FDA, 1999) Of course, as yet no transgenic animals have yet been approved as human food, not even aquatic organisms. For this reason the FDA's most evident regulatory impact is demonstrated in their control over drugs resulting from transgenesis. Both animal and human drugs are regulated by the FDA, but since it is "product, not process," some ambiguity exists. When it comes to transgenic animals, any modification of the animal that has a therapeutic objective is deemed to be an animal drug. This does not include animal vaccines, which, as discussed, are regulated by the USDA-APHIS. An example of a transgenic animal which would fall under the FDA's animal drug authority would be a fish which was engineered to produce a greater quantity of growth hormone. Even though the modification is inherent, it is still considered a drug. Another example might be transgenically benign bacteria which are used to colonize the digestive tracts

of farms animals so as to competitively exclude pathogenic bacteria. "When these products carry disease prevention claims, they are animal drugs." (US-FDA, 1999)

Another area of FDA concern is in the production of human drugs via transgenic animals. This process is sometimes known as Animal Pharming, in reference to the animal producing "a certain pharmaceutical protein in its milk, urine, blood, sperm, or eggs, or [growing] rejection-resistant organs for transplant." (Rittner and Cummings, 1999). These proteins are usually complex enough that they must be produced by higher organisms such as sheep or cows rather than bacteria. The economic and humanitarian potential for this technology clearly seems to be very great, but at the same time so does the potential for disaster. Towards avoiding such, in 1994 the FDA published "a report titled 'Points to consider in the manufacture and testing of therapeutic products for human use derived from transgenic animals.' This report set guidelines for the development, maintenance and disposal of transgenic animals." (Rittner and Cummings, 1999). In fact, beyond simple guidelines, the FDA has strict requirements for this development, maintenance and disposal, which it has the authority to enforce. Only last February (2003), the FDA began an investigation into the release of transgenic pig offspring to a livestock dealer. Both the FDA and USDA agree that the incident posed no threat to public health, but the violation remains serious enough to warrant investigation and possible action. (US-FDA, 2003a)

The FDA's guidelines seem to be fostering the development of transgenic animal pharmaceuticals; the table below from 1999 indicates some of the drugs that were already in development just 5 years later. However, as with transgenic food, no animal pharmed products have yet been approved for sale on the open market. (BBC, 2003)

Animal	Drug/protein	Use
sheep	alpha1 anti trypsin	deficiency leads to emphysema
sheep	CFTR	treatment of cystic fibrosis
sheep	tissue plasminogen activator	treatment of thrombosis
sheep	factor VIII, IX	treatment of hemophilia
sheep	fibrinogen	treatment of wound healing
pig,	tissue plasminogen activator	treatment of thrombosis
pig	factor VIII, IX	treatment of hemophilia
goat	human protein C	treatment of thrombosis
goat	antithrombin 3	treatment of thrombosis
goat	glutamic acid decarboxylase	treatment of type 1 diabetes
goat	Pro542	treatment of HIV
cow	alpha-lactalbumin	anti-infection
cow	factor VIII	treatment of hemophilia
cow	fibrinogen	wound healing
cow	collagen I, collagen II	tissue repair, treatment of rheumatoid arthritis
cow	lactoferrin	treatment of GI tract infection, treatment of infectious arthritis
cow	human serum albumin	maintains blood volume
chicken, cow, goat	monoclonal antibodies	other vaccine production

(Rittner and Cummings, 1999)

INTELLECTUAL PROPERTY

The issue of transgenic animals as intellectual property is on par with food and drug safety in terms of importance, and is a far more contentious area of discussion. The creation of transgenic animals is of course a complex and highly technological procedure which requires not only extremely skilled employees but also extensive infrastructure. In addition the process is fairly wasteful, with the target genes being expressed in a number of healthy adult animals representing only a small fraction of the quantity of cells originally manipulated. This percentage is increasing with continued research and development, but those also require significant expenditures, and with no directly profitable results. The costs of producing transgenic animals require investment, and assurance that investors will receive appropriate

returns. This cannot be done without intellectual property protection of advances and discoveries made by transgenic animal researchers.

However, the fact that the end product of transgenic technology is a living organism introduces complications that do not appear when discussing the protection of knowledge regarding a mechanical device or a chemical compound. The issue is also different from that of owning a living creature, such as a farm animal. In the latter case the animal is a concrete object, and all rights of disposal thereof can be said to reside with its owner. For a transgenic animal, some of those rights exist with the owner of the animal, and others belong to the owner of knowledge of how to create the animal.

Ex Parte Allen, 1987

One of the first controversial legal decisions on animal patents was made on April 3, 1987, when "the Board of Appeals in *Ex parte Allen* refused to grant a patent on a process to make more edible oysters by putting them under pressure." (Woessner, 1999) The reason cited was that the introduction of a different environment for the oysters did not mitigate the fact that they remained a creation of nature, not of man. This decision however did not in any way disallow other attempts to patent multicellular animals, as long as they would not exist without human intervention. In fact, only days later, on April 21, 1987, the Patent and Trademark Office "announced that it would accept applications for 'nonnaturally occurring nonhuman multicellular living organisms, including animals.' The PTO stated that, to be patentable, the animals must be 'given a new form, quality, properties or combination not present in the original article existing in nature in accordance with existing law.'" (Woessner, 1999) And applications did come in.

Oncomouse, 1988

Less than a year later, on April 12, 1988, a patent was issued to Philip Leder and Timothy A. Stewart of Harvard University for the 'oncomouse' (Leder and Stewart, 1988). This was a transgenic mouse engineered to be especially susceptible to cancer, and produced for sale to cancer researchers.

The door for the Harvard oncomouse was opened in 1980 by the US Supreme Court case of Diamond v. Chakrabarty. This case focused on a bacteria which while not transgenic, had been genetically engineered to break down crude oil. An application for a patent on the bacteria itself was rejected by the patent office "on the ground that living things are not patentable subject matter under [Title 35 U.S.C.] 101. The Court of Customs and Patent Appeals reversed, concluding that the fact that micro-organisms are alive is without legal significance for purposes of the patent law." (Diamond v Chakrabarty, 1980). The Supreme Court agreed. Since the bacteria was non-naturally occurring, it was acceptable to deem it a work of human ingenuity worthy of protection. This position of considering vitality as irrelevant to patents had in fact been presaged by such previous legislation as the "1930 Plant Patent Act, which afforded patent protection to certain asexually reproduced plants, and the 1970 Plant Variety Protection Act" (Diamond v Chakrabarty, 1980).

However, the word of the US Supreme Court is only final within the United States, and other countries can have varying views of the issues. For instance, on December 5, 2002 the Supreme Court of Canada handed down the decision that Harvard's oncomouse is not itself patentable. This is not a total denial of any intellectual property rights to modifying life though. "The court did not rule out Harvard's claims on the process used to make the oncomouse...and the university says it will continue to pursue that claim." (Check, 2002).

Other Patented Animals

Other patents for transgenic animals have been granted, including "(a) a mouse that develops an enlarged prostate gland (U.S. Patent No. 5,175,383), (b) transgenic mice depleted in mature T-cells (U.S. Patent No. 5,175,384)...(c) a virus-resistant mouse that produces *beta*-interferon (U.S. Patent No. 5,175,385)...[and] (U.S. Patent No. 5,183,949)...'a rabbit infected with HIV-1 virus, said rabbit produced by the infection of human T-cells infected *in vitro* with HIV-1' [not technically transgenic]" (Woessner, 1999) Despite these and other patents, afforded protection by the Supreme Court's decision, the patenting of life forms continues to court controversy. "The issue is more recently illustrated by the case now before the Canadian courts over the patentability of higher life forms (a transgenic mouse for research), and by the 1998 European Union *Directive on the Legal Protection of Biotechnological Inventions.* The latter precludes, as contrary to public order or morality, patents for transgenic animals when their creation is likely to cause animal suffering without substantial medical benefit." (Industry Canada, 2000)

ECOSYSTEMS, BIODIVERSITY, AND SUSTAINABLE DEVELOPMENT

The final major area of regulatory concern regards the implications transgenic animals have for their ecosystems. Since transgenic technology is so new, and the possibilities for modification of organisms appear virtually limitless, the effects that transgenic animals could have on the environment are cause for serious concern. The alterations humans have been able to make to the Earth by manipulation of only naturally occurring plants, animals, and materials remain potentially devastating. The variability that changing life itself adds to the system could vastly increase the damage. Some of this the responsibility for ameliorating these risks is held

by the USDA, as discussed above. APHIS is charged with regulating field testing of transgenic animals to maintain control over their release into the environment at large. This is also part of the reason the FDA was so concerned with the release of the aforementioned bioengineered pigs to the livestock dealer. No one knows for certain the interactions a particular transgenesis will have with all or any environments, and we have learned much from our ecological mistakes of the past.

Beyond agricultural concerns, the EPA is charged with protecting the environment as a whole, and protecting it from transgenic animals is clearly a part of that. In the past the EPA's concern with genetically modified organisms has been primarily confined to crop plants such as corn. Just this year (2003), "the Environmental Protection Agency (EPA) fined biotech company Pioneer $72,000 for failing to immediately report crops that tested positive for genetic material from last year's controversial misplanting of experimental corn." (Center for Science in the Public Interest, 2003) However the EPA is also responsible for "regulating pesticidal substances produced through biotechnology, and also pesticide resistant plants. It works with APHIS to oversee the large scale testing of both, and with FDA on the safety to humans of the plants or their products. EPA must assess the risks to human safety and the fate of the substance in the environment, including effects on 'non-target' species." (Wellcome Trust, 2002) In keeping with the "product not process" dictum, a transgenic bacteria that infects agricultural pests would be considered a pesticidal substance, and fall under EPA jurisdiction. The intended effect of the bacteria is not even actually required to be a pesticide. In fact, Vines (2002) explicitly states that the "EPA oversees testing of GM microorganisms that could impact the environment upon release."

Although the EPA casts a wider regulatory eye than the USDA or FDA, this enhanced jurisdiction can lead to some murkiness and ambiguity over exactly what they should be responsible for directly, and what they should work with other departments to police. So far the concerned agencies have cooperated well, and the vagueness in liability can actually result in double-checked regulation of situations with particularly hazardous potential. On the other hand, the capacity for holes in the net certainly exists, and were the agencies to have a less cordial working relationship in the future, transgenic animals could cause serious damage with no one accountable for keeping control. There is an ongoing battle between lobbying groups pushing for more or less regulation of transgenics, but what both sides have in common is a demand for well-defined legal parameters for propitious development and utilization of transgenic animals.

Chapter 5 – Conclusions

Daniel Schmidt

In this paper we have sought to convey an overview of the technology of transgenic animals, both the science of their construction and the issues surrounding their place in the world. Our discussion has focused on four major areas, corresponding to each of the previous chapters.

1) Design and construction
2) Classification and examples
3) Ethics
4) Legalities

Based upon the authors' analyses of each of these areas, our overall conclusion is that transgenic animal technology has vast potential for favorable consequences, but if misused, the potential for harm as well. Transgenic science provides exciting new tools for deciphering and manipulating the biology of virtually all organisms, and the benefits such ability could provide are endlessly diverse. Beyond the readily apparent applications to diagnosis and treatment of human diseases, transgenic animals can be used to improve agriculture, economies, the environment, and simply the state of human knowledge.

However, these advantages do need to be weighed against the both the possible and the inevitable drawbacks of transgenic research. The fact remains that development of the science requires animal suffering and sacrifice; these must be justifiable by benefits to humans. From our investigations, we the authors have concluded that the ethics and legalities surrounding transgenic experimentation are complex enough that instances should be considered on a case-

by-case basis. In each case, the benefits to humans (or the environment, or other animals), should clearly outweigh any pain and suffering the experiments require.

In the case of transgenic animal pharming, the production of pharmaceuticals in milk provides very significant human advantage, while not having little adverse effect upon the animal producing them. Transpharming experiments should continue, most especially where the drugs concerned are difficult to synthesize via chemical or naturally organic means.

In the case of disease models, we feel such experiments should continue only when all possible efforts are made to limit platform animal suffering with painkillers and/or euthanasia. Similar limitations must be placed on the pursuit of transgenic xenotransplantation, although in these cases the benefit to humans can be much more concrete and immediate than disease models, justifying a corresponding relaxation in sensitivity to animal rights. Strictures of a different nature must be observed though, to avoid the possibility of transmitting diseases through organ transplantation that were previously witnessed only in the donor species.

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