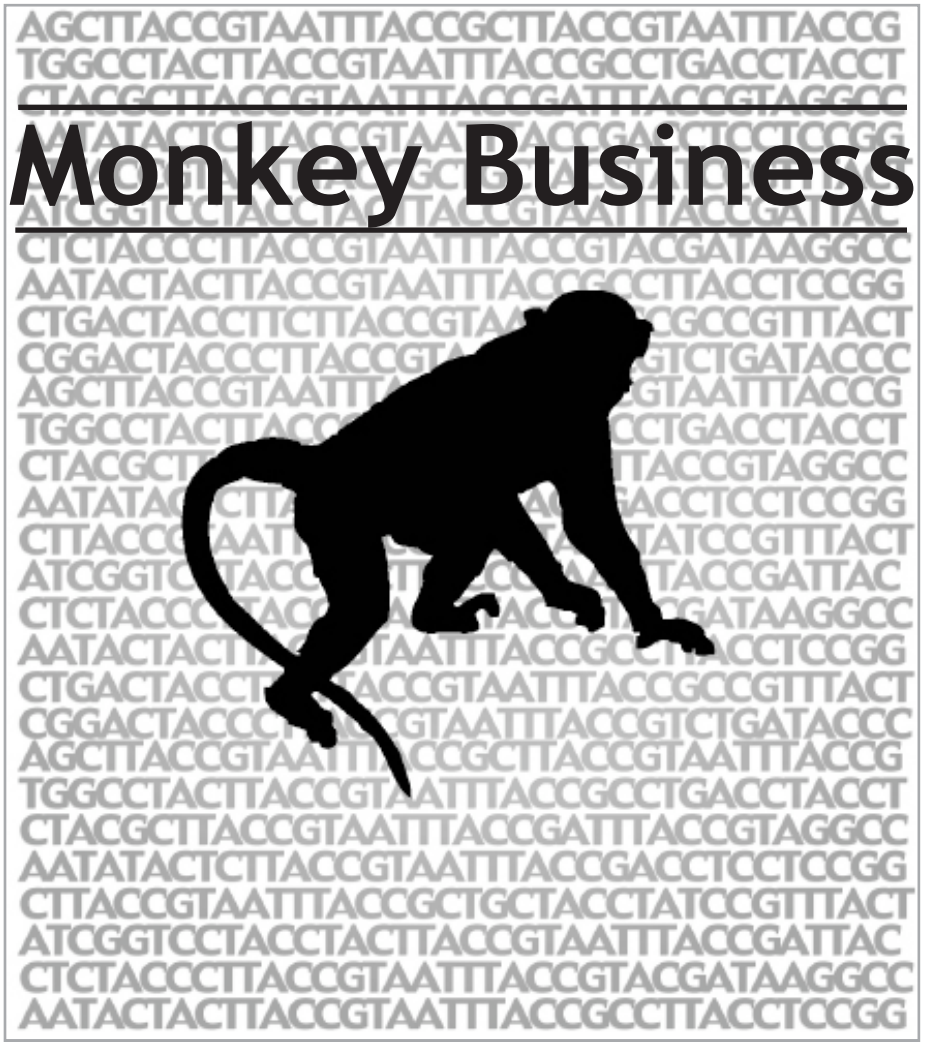


credit: Jane Ades, NHGRI

Monkey Business

Transgenic primates and approaching ethical concerns

By Matthew Coates

Picture a hospital nursery. The faces of fifteen new lives peak through their swaddling blankets. Now, picture the babies fluorescing bright green in the soft blue morning light of the room. Such an odd image of alien-like babies belongs in science-fiction movies, right? Believe it or not, glowing babies, though an absurd use of transgenics, are well within the realm of possibility. In fact, green rhesus monkey babies have been a reality for just over ten years. In 2000, scientists at Oregon Health Sciences University were able to modify the DNA of several rhesus monkey eggs to cause the expression of green fluorescent protein (GFP), normally found in jellyfish, to create the first transgenic primate (1). Now that the first few generations of transgenic primates have had time to mature and reproduce, the field of transgenics has begun to show promise in some of our close relatives. In the last half of the decade, scientists have engineered marmosets that pass their modified genes to their offspring and macaques that have a condition analogous to Huntington's disease (2,3). The fortieth anniversary of the first human production of recombinant DNA molecules is fast approaching. As we move into the next decade of genetic research, we bring with us expectations of a better understanding of human disease, but also the ethical concerns that genetic manipulation provokes.

Beginnings and Methods

In the early 1970s, several researchers, including future Nobel Prize winner Paul Berg, were first able to use restriction enzymes to cut strands of DNA and have them recombine into plasmid vectors (4). That is, they used pieces of DNA called plasmids, typically found in bacterial cells, as a way to incorporate and then replicate specific foreign DNA. To start this process, proteins called restriction enzymes are used to cut the sequence of DNA to be inserted, and a space in the plasmid is similarly cut to allow for the new sequence to be entered into the plasmid. Then, bacteria with the recombinant plasmid can be grown (Figure 1), and the DNA from the plasmid can be purified for introduction into a target organism.

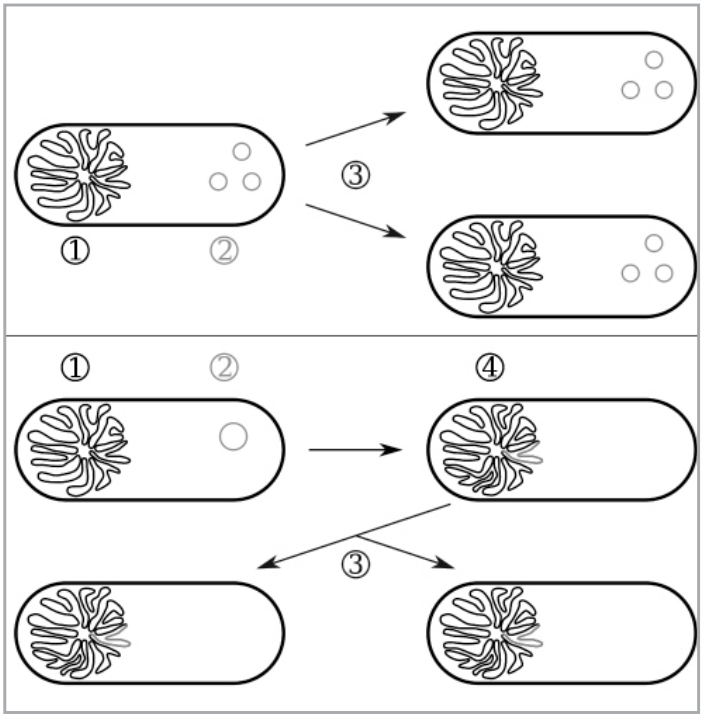
There are several methods for incorporating foreign DNA into new organisms, though a select group of these are particularly important in creating transgenic animals. Researchers can inject vector DNA into a fertilized egg using a virus that incorporates the DNA into the new cell, or they can introduce DNA to embryonic stem cells in an organism that will eventually produce offspring with the altered genome. In particular, the use of retroviruses, which implant their genetic codes into host cells to make the host produce more of the virus, has proven instrumental in creating higher-level transgenic animals and shows potential in gene therapy treatments (5).

Growing Applications

The accomplishments of the 1970s and early 1980s, from incorporating animal genes into bacteria to creating transgenic mice and flies, paved the way for the developments that have led transgenics to where it is today (4). In between, however, transgenic organ-

isms have been created for a myriad of purposes, including commercial use. The agriculture industry has harnessed the power of transgenics to create and farm insect-resistant and herbicide-resistant crops. In fact, over eighty-five percent of the corn, soybeans, and cotton grown in the United States are genetically engineered (6).

Despite the large amount of attention that genetically modified foods garner in the public, often from the organic food movement, plants are not the only successfully engineered farm products. Companies have been seeking FDA approval for modified fast-growing salmon for several years, but concerns about consumer safety and environmental impacts have blocked approval (7,8). Similar efforts to create pigs with low amounts of fat and high growth rates succeeded but resulted in organ enlargement and health problems for the animals, indicating that successful gene regulation is an additional barrier to genetically modified animals becoming approved food sources (9).



▲ **Figure 1.** Growing the recombinant plasmid. The top image shows bacterial replication without the incorporation of the plasmid, while the bottom image shows incorporation, then replication. (1) indicates bacterial DNA. (2) indicates the plasmid. (3) indicates cell replication.

Several animals, however, have been used to create products for medicine instead of food. In 2009, the FDA approved an anti-clotting agent produced in transgenic goat milk, the first drug in the United States to be produced in a transgenic animal (10). While animal products have proven helpful in this way, researchers are attempting to find ways by which part of the animal itself, namely organs, can be used in medicine. Animal organ transplant into humans (xenotransplantation) has been largely a dream, partly because of the immune responses that humans have to foreign bodies. However, genetic engineering could produce animals without the markers that are present in cells of non-humans (11). The medical field will no doubt benefit from research like this, but much of the progress in animal transgenics over the past decade has come from the improvement of animals as model organisms for disease, genetics, and neurobiology; it is in these areas that groundbreaking discoveries using transgenic primates are possible.

Manipulating Monkeys

Science has come a long way in understanding evolution and genetics due to model animals like *Mus musculus* (the mouse) and *Drosophila melanogaster* (the fruit fly). In fact, transgenic mice have even been engineered with Alzheimer's disease for neurological studies (12). But at the end of the day, there is a limit to the information about humans that we can obtain from simply sequencing and understanding genes of these animals, as only certain genes and functions are evolutionarily conserved in humans (13). Dissimilarities in genetics, physical structure, and the mechanisms of behavior and disease present inherent limits on the ability of scientists to apply discoveries in model organisms to the problems that humans face. Developments in the last decade, however, have shown that the next step may involve much closer relatives: non-human primates.

The results of the first successful transgenic primate experiment were published in 2001, after the birth of a transgenic rhesus macaque aptly named ANDi. The name comes from "inserted DNA" in reverse order, alluding to reverse transcription, a process in which viruses create a strand of DNA from their RNA. This is the technique retroviruses use to transplant their genetic code into hosts, the process of which ANDi's creators took advantage. Researchers injected the retroviral vector into eggs, which were then fertilized and implanted into females. Several pregnancies failed or had complications, leaving ANDi as the only macaque baby that carried the new transgene, the green fluorescent protein from a jellyfish, in all of his tested tissues. The scientists were then forced to wait several years until ANDi reached maturity to see if the transgene could be passed along through ANDi's germ cells to his sperm and subsequently his offspring (1). This did not occur.

While some progress in creating transgenic primates occurred in the years shortly following ANDi's birth, scientists were unable to create transgenic primates that could pass their altered genetic make-up to their young until 2009. This is when researchers in Japan first showed transmission of a transgene to a transgenic primate's offspring. Instead of macaques, the scientists used marmosets, which are good model organisms for several reasons.

First, their rate of reproduction is much faster than that of macaques and larger primates. Second, they give birth to between four and eight times more offspring than their larger relatives. And last, marmosets are smaller, which makes them easier to use in a laboratory setting. However, marmosets differ in these ways, in part, because they are more distant relatives of humans, unfortunately limiting the parallel traits and diseases that can be studied. The germline transmission that this study shows is certainly a step towards transgenic primate colonies that could be used to study disease. However, several challenges remain. The retrovirus used for this study could only carry a relatively small amount of genetic material. 8.5 kilobases of DNA is enough to carry the GFP gene given to the marmosets, but it may not be enough to confer certain human genetic disorders to the primate models (2).

Figure 2. The skulls of several primates. From left to right: macaque, orangutan, chimpanzee, human. Macaques are useful model organisms because they are primates, but they are more distant relatives of humans than chimpanzees or orangutans.

One genetic disorder that has been successfully transferred to monkeys is Huntington's disease. Previous models of Huntington's disease in mice have been promising, but inherent differences between mice and humans have caused dissimilarities in the expression of the disease in mice, limiting their practicality as models. In 2008, the successful incorporation of a repeated area of genetic material that causes Huntington's disease in rhesus macaques enabled the use of a better model organism. Not only did the macaques all express both the GFP and Huntington's repeat that was given to them, but they also showed the characteristic behaviors of Huntington's disease in humans. Although the macaques have not yet had the opportunity to show that they can confer their transgenes to offspring, the researchers at Emory University remain optimistic that they can breed a group of transgenic monkeys with which to further study Huntington's disease (3).

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The ability to study human disease in non-human primate models should enable a better understanding and treatment of conditions that have previously been incurable or uncontrollable. Each of the developments in transgenic primates of the past decade has been a step toward this ultimate goal of understanding human disease. But what cost are we paying to expand our knowledge?

Ethical Questions

Making monkeys glow is one thing, but giving them a deadly neurological disease is another. The ability to manipulate genomes has provoked ethical debate since its fledgling days as a practice. It makes sense that as the genetic engineering gets more complex and approaches human subjects, so too do the stakes for ethical responsibility grow higher.

In the 1970s, the focus was on the containment of genetically altered organisms to the laboratory. The risks of releasing genetically altered organisms, especially pathogens, into the environment were relatively unknown. As a better understanding of the process developed, alongside several years of safe research, regulations were relaxed (14). But as the engineered organisms became animals instead of bacteria, moral, ethical, and religious concerns were magnified.

Several basic objections to genetically modified (GM) organisms exist. First, by engineering new life, some say that we are diminishing the intrinsic quality of life and nature that already exists. In other words, does our meddling tarnish the sanctity of nature? Second,

▲ **Figure 3.** Macaque taken from a laboratory by the Animal Liberation Front, a group against treating animals as property (16)

Credit: http://commons.wikimedia.org/wiki/File:Britches-after-treatment-by-the-ALF.jpg

the risk of accidentally creating organisms that are dangerous to humans and destructive to the environment is a constant danger. We are creating organisms that have never coexisted with those found in the natural world. How can we know the extent of their future interactions outside of the lab? Transgenic organisms, like certain invasive species, could cause extinction or harm to fragile ecosystems. Third, the effects that GM crops have on economies and consumers are not equitably distributed. Most countries with well established transgenic crop strains are already economically prosperous. The added advantage of hardy crops in wealthy countries threatens the success of already hindered developing nations in becoming economically stable. Last, there is no transparency in decision-making that allows for educated public choices regarding the approval of new transgenic projects. The information asymmetry between genetic engineering corporations, research organizations, and the general public disallows effective democratic regulation of genetic engineering and research (14). While some of these ethical objections to transgenics can be mitigated by cautious experimentation, transparent decision-making, and legal regulation, some opposition based on the sanctity of nature and life remains.

Because of the rapid progress and tangible benefits that genetic engineering has seen, altogether repealing the practice on ethical grounds is almost certainly not part of our near future. But concerns about suffering and life can still be mitigated. Organizations have been established to evaluate the ethics of studying deadly diseases in non-human primates. In 2006, a panel organized by Britain's Royal Society, Medical Research Council, Wellcome Trust, and Academy of Medical Sciences found that the promise of treating millions of humans with potentially controllable diseases outweighs the suffering that primate model organisms face. This tough decision aside, limiting the organisms to monkeys instead of great apes, minimizing the number of animals used, and reducing the suffering of the animals are all requirements that are meant to reduce ethical concerns (15). Research agencies around the world continuously face similar ethical judgments. These decisions will only grow more complex as transgenics penetrates the realm of possibility in humans. Certainly, new gene therapy techniques may be beneficial for treating human disease, but testing genetic treatments on human subjects can be risky or unsafe. In addition to evaluating issues of safety, ethicists must draw a distinct line between treating disease with techniques like gene therapy and genetically "improving" humans in a sort of eugenics movement. The power of genetic manipulation makes it clear that, as our capabilities expand to the bizarre, like creating glowing babies, and the life-saving, like implementing treatments for genetic disorders, we will require a dynamic field of ethics to continue accompanying scientific innovation on the genetic frontier. H

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References

1. Chan, A.W.S., et al. (2001) Transgenic monkeys produced by retroviral gene transfer into mature oocytes. Science 291(5502): 309-312.
2. Sasaki, E., et al. (2009) Generation of transgenic non-human primates with germline transmission. Nature 459: 523-528.
3. Yang, S., et al. (2008) Towards a transgenic model of Huntington's disease in a non-human primate. Nature 453: 921-924.
4. National Human Genome Research Institute. Understanding the Human Genome Project. URL: http://www.genome.gov/25019879
5. Hartl, D.L. and Jones, E.W. (2009) Genetics: Analysis of Genes and Genomes. Sudbury, MA: Jones and Bartlett Publishers.
6. USDA Economic Research Service. "Data Sets: Adoption of Genetically Engineered Crops in the U.S." URL: http://www.ers.usda.gov/Data/BiotechCrops/
7. Chern, W.S., et al. (2002) Consumer acceptance and willingness to pay for genetically modified vegetable oil and salmon: A multiple-country assessment. AgBioForum. 5(3): 105-112.
8. Pollack, A. Genetically altered salmon move closer to your table. New York Times. 26 June 2010: A1.
9. Pursel, V.G., et al. (1997) Transfer of Ovine Metallothionein-Ovine Growth Hormone Fusion Gene into Swine. Journal of Animal Science 75: 2208-2214.
10. Kling, J. (2009) First US approval for a transgenic animal drug." Nature Biotechnology 27: 302-304.
11. Lai, L., et al. (2002) Production of α-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science. 295: 1089-1092.
12. Crowther, R.A. (1995) Steps towards a mouse model of Alzheimer's disease. Bioessays 17(7): 593-595.
13. Miklos, G.L.G. and Rubin G.M. (1996) The role of the genome project in determining gene function: Insights from model organisms. Cell. 86: 521-529.
14. Peterson, M.J. (2010) Case study: Asilomar conference on laboratory precautions when conducting recombinant DNA research. International Dimensions of Ethics Education Case Study Series, 2010. URL: http://www.umass.edu/sts/ethics/asilomar.html
15. Experts defend experiments on monkeys. The Guardian. 12 December 2006. URL: http://www.guardian.co.uk/uk/2006/dec/12/animalwelfare.research
16. Mission Statement. Animal Liberation Front. URL: http://www.animalliberationfront.com/ALFront/mission_statement.htm