Published in final edited form as:
Nature. 2009 May 28; 459(7246): 515–516. doi:10.1038/459515a.

DEVELOPMENTAL BIOLOGY:

Transgenic primate offspring

Gerald Schatten and **Shoukhrat Mitalipov**
Gerald Schatten is in the Departments of Obstetrics, Gynecology and Reproductive Sciences, and of Cell Biology, Physiology and Bioengineering, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213, USA. Shoukhrat Mitalipov is in the Division of Reproductive Sciences, Oregon National Primate Research Center, Oregon Health and Sciences University, Beaverton, Oregon 97006, USA

Abstract

Genetically engineered monkeys carrying a foreign gene that is passed on to their offspring provide a potentially valuable bridge between mouse models of disease and treatment for human disorders.

The development of transgenic mice — in which foreign DNA is inserted into the mouse genome — meant that the functions of human genes could be studied rigorously in living animals rather than in cell culture. Developments in transgene technology, complemented by advances in reproductive cloning, have followed in other mammals, including rats, rabbits, pigs, cows, and even cats and dogs. Sasaki *et al.*[1] (page 523 of this issue) now report a breakthrough in primate research — the generation of transgenic monkeys that pass the foreign gene on to their offspring and so could be used to establish specialized primate colonies for the study of human disease.

Mouse models of disease have been used in research into disorders ranging from anaemia and asthma to autism and schizophrenia. But not every human disease can be modelled faithfully in rodents. Mice engineered to express the cystic fibrosis gene, for example, do not develop the lung problems that typify this disorder (a pig model of cystic fibrosis[2] proved more useful). Disorders of higher brain function, such as Alzheimer's disease, are especially challenging to reproduce in rodents, and here, as with many other diseases, it is our closest animal relatives — the non-human primates — that offer potentially invaluable biological models.

In the United States, research with non-human primates can be conducted with stringent local and federal oversight. Consequently, this decade has seen the generation of a transgenic rhesus monkey[3] and the first primate model of a human disorder, Huntington's disease, also in rhesus monkeys[4]. In another study, the transfer of a transgenic rhesus embryo generated a transgene-expressing placenta[5]. But no study has shown transmission of foreign DNA to gametes — the sperm and egg — which is essential for the generation of transgenic offspring. These offspring could then be bred to create transgenic-primate strains.

Sasaki *et al.*[1] build on this work, and introduce several innovations. Instead of rhesus monkeys, the authors studied the common marmoset (*Callithrix jacchus*) — small creatures that reach sexual maturity in just over a year, and that often bear twins after a relatively short gestation period. They found that naturally produced embryos, flushed from the reproductive tracts of mated females, were better transgene carriers than embryos generated by *in vitro* fertilization

gschatten@pdc.magee.edu; mitalipo@ohsu.edu.

(IVF). After injection of the transgene, which encoded a green fluorescent protein (GFP) as a reporter of gene expression, nearly 100% of the natural embryos expressed the gene compared with about 70% of the IVF embryos — four of the five transgenic marmosets developed from these natural embryos.

To improve the efficiency of transgene delivery, the authors shrank the egg within its outer coating by placing it in a sugar solution, freeing up space for the injection of more transgene-containing particles. After transferring 80 embryos to 50 surrogate females, seven pregnancies were established, resulting in five offspring. The GFP transgene was incorporated into several sites in the offspring's genome and was expressed in various tissues, as confirmed by the green glow of the fluorescent protein. Furthermore, Sasaki *et al*. followed these animals until sexual maturity and found that the transgene was in their gametes, affording the tantalizing prospect of obtaining transgenic offspring through germline transmission. This hope came to fruition when the first infant conceived by the GFP-transgenic founder member also expressed GFP in its skin.

The birth of this transgenic marmoset baby is undoubtedly a milestone. The cumber-some and often frustrating process of making a transgenic animal from scratch need now only occur with founder animals. Subsequent generations can be produced by natural propagation, with the eventual establishment of transgene-specific monkey colonies — a potentially invaluable resource for studying incurable human disorders, and one that may also contribute to preserving endangered primate species. The study of transgenic primates may also help to answer fundamental questions about stem-cell biology. Primate stem cells have recently been generated from adult cells by nuclear cloning[6], and a comparison of these cells with patient-specific induced pluripotent stem cells — also derived from adult cells — will be enlightening.

Box 1 | Considerations before establishing colonies of primate disease models

- Optimize the initial protocol for disease modelling.
- Direct research primarily at incurable diseases for which there are potential treatments in the pipeline for preclinical testing.
- Ensure the disease under study cannot be modelled in transgenic mice or other non-primates.
- Attempt to develop transgenic animals with features that allow rapid and informative research[13,14], for example the use of:
  - Transgenes with inducible promoters, meaning that genes can be switched on or off.
  - Reporter transgenes that are sensitive to particular metabolic states.
  - Gene-trap sites in the target genome, similar to the *Rosa26* locus in mice, that allow efficient integration and strong expression of inserted sequences.
  - Cre-lox technology, which can be used to excise the transgene from the target genome.
  - Gene targeting by homologous recombination for the creation of animals in which specific genes are knocked out.
  - Transgene reporters suitable for non-invasive imaging by magnetic resonance imaging, positron emission tomography, luminescence and other whole-body approaches.

- Isolate primate colonies to prevent contamination with other research colonies.
- Clarify CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and other regulatory practices to enable sharing of molecular and cellular research resources while still protecting endangered species.
- Foster public debate about the strengths and limits of these technologies[11,12].

Transgenic marmosets are potentially useful models for research into infectious diseases, immunology and neurological disorders, for example. Marmosets engineered to express single-gene defects, such as the mutated gene that causes muscular dystrophy, might accelerate the translation of discoveries from mouse research[7] to patients who have few treatment options. However, marmosets do have limitations as research models. They are New World primates, and are less closely related to humans than are Old World primates such as rhesus macaques and baboons. Because of biological differences, diseases such as HIV/AIDS, macular degeneration and tuberculosis can be studied only in Old World primates.

Also, despite the commendable success rates achieved by Sasaki *et al.*[1], their results pale in comparison with those achieved with mice. As in other primate studies, the authors use a virus vector to carry the transgene into the genome of the embryo after injection. Consequently, the transgene inserts into random sites in the target DNA. This is much less satisfactory than in transgenic mice, which are now routinely generated using embryonic stem cells. Here, the transgene is directly targeted to integrate into, or mutate, a specific site in the embryonic stem-cell genome by exploiting a natural genome repair process known as homologous recombination[8]. Random transgene integration probably resulted in some of the marmoset miscarriages; and as it could theoretically activate silent cancer-causing genes, or endogenous viral sequences that are part of the host genome, monitoring transgene inheritance in subsequent generations will be necessary.

As with all animal experimentation, genetic modification in primates raises concerns about animal welfare. We suggest that various considerations should be taken into account before colonies of primate disease models are established (Box 1). There are also bioethical concerns, which raise themselves anew with this work. Foremost among them is the prospect of unwarranted and unwise application of transgenic technologies to human gametes and embryos for reproductive purposes. Transgenic technologies are still primitive and inefficient, with unknown risks for animals, let alone people. Hence the very real need for the existing guidelines framed by professional societies and regulatory authorities (for example, those issued by the UK Human Fertilisation and Embryology Authority[9]), which prevent germline genetic modifications in humans. Perhaps even the use of techniques for generating embryonic stem cells from human embryos[10] that have been genetically altered to prevent implantation, and that are therefore devoid of reproductive potential, needs to be weighed carefully so as to avoid going down any kind of slippery slope involving human transgenesis.

With recent breakthroughs in stem-cell research and these latest advances in primate developmental biology, increased attention will naturally be focused on the practices of human assisted reproductive technologies — hence the need to consider calls[11,12] to establish realistic policies for governing work with human embryos. Although the future for using transgenic primates for medical and translational research looks bright, scientists need to engage with the public in informed bioethical debate about genetic modification and innovation in reproductive biology.

References

1. Sasaki E, et al. Nature 2009;459:523–527. [PubMed: 19478777]

2. Rogers CS, et al. Science 2008;321:1837–1844. [PubMed: 18818360]
3. Chan AWS, Chong KY, Martinovich C, Simerly C, Schatten G. Science 2001;291:309–312. [PubMed: 11209082]
4. Yang S-H, et al. Nature 2008;453:921–924. [PubMed: 18488016]
5. Wolfgang MJ, et al. Proc. Natl Acad. Sci. USA 2001;98:10728–10732. [PubMed: 11553810]
6. Byrne JA, et al. Nature 2007;450:497–502. [PubMed: 18004281]
7. Lim LE, Rando TA. Nature Clin. Pract. Neurol 2008;3:149–158. [PubMed: 18268530]
8. Nagy, A.; Gertsenstein, M.; Vintersten, K.; Behringer, R. Manipulating the Mouse Embryo: A Laboratory Manual. Vol. 3rd edn. Cold Spring Harbor Lab. Press; 2003.
9. www.hfea.gov.uk/docs/SCAAC_Genetic_ModificationJan09.pdf.pdf
10. Hurlbut WB. Stem Cell Rev 2005;1:293–300. [PubMed: 17142870]
11. Schatten G. Nature Cell Biol 2002;4:s19–s22. [PubMed: 12479610]
12. Berg P. Nature 2008;455:290–291. [PubMed: 18800118]
13. Raymond CS, Soriano P. Dev. Dyn 2006;235:2424–2436. [PubMed: 16724325]
14. Rochefort NL, Jia H, Konnerth A. Trends Mol. Med 2008;14:389–399. [PubMed: 18701348]