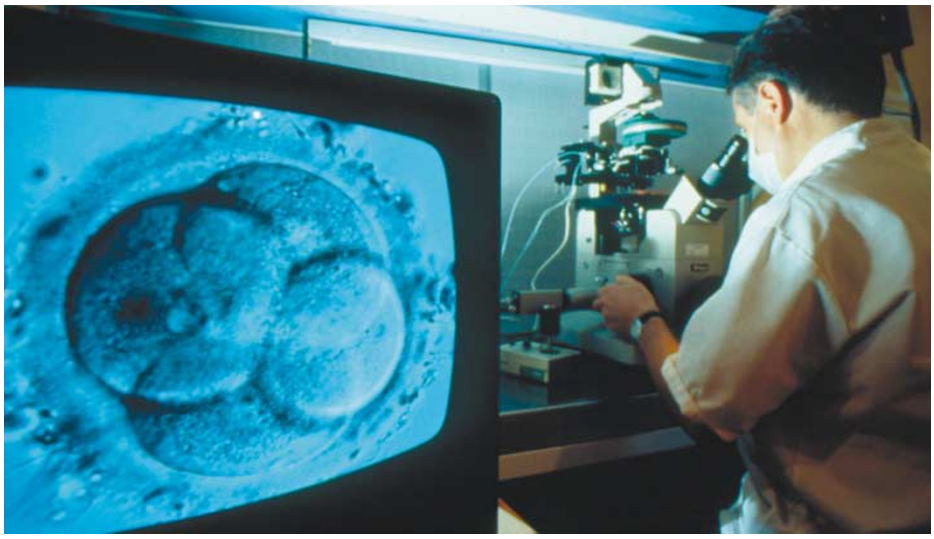
Biology's last taboo



P. PLAILLY/EURELIOS/SPL

Will gene therapy ever extend to inducing changes in humans that can be inherited down through generations? Maybe so, if the concerns over safety can be ironed out. Jonathan Knight considers the technical challenges and the ethical arguments.

For a few days in January, a young rhesus monkey called ANDi became one of the most famous animals on the planet, his endearing features plastered across countless newspapers. Engineered by a team at the Oregon Regional Primate Research Center near Portland so that each of his cells carries a gene for a glowing green protein, ANDi is the world's first transgenic primate[1]. His name derives from 'inserted DNA' spelt backwards.

Some reports suggested that ANDi is just one step removed from 'designer' human babies. But if anything, he shows that transgenic people are still far from a practical reality. ANDi gives out not even the faintest green glow — the introduced gene was active in two other fetuses that aborted spontaneously, but in ANDi it seems to lie silent. Nor do ANDi's creators hope to develop a method for use in people. Rather, they are interested in creating genetically modified monkeys for use in medical research. "We discourage any extrapolations to people," says Gerald Schatten, the team's leader, now at the University of Pittsburgh.

But when asked to look a decade or more into the future, many experts believe that 'germline' gene therapy, which aims to produce babies carrying altered genes that will be inherited down the generations, might become part of mainstream medicine. Given advances in basic research, they argue, the safety concerns that currently put germline gene therapy off-limits might be overcome.

Objections to human germline manipulation run deeper than the safety issues. For some people, tampering with our genetic inheritance in this way is fundamentally wrong. Others fear that what starts as an attempt to cure inherited diseases will soon mutate into a programme of genetic 'improvement'. But previous experience suggests that such concerns may not restrain those intent on pushing back the boundaries of reproductive and genetic medicine (see 'The unregulated frontier', overleaf). "Altering the genome is essentially the endpoint of the whole genomic revolution," claims Gregory Stock of the University of California, Los Angeles, an expert on the societal impact of emerging genetic technologies.

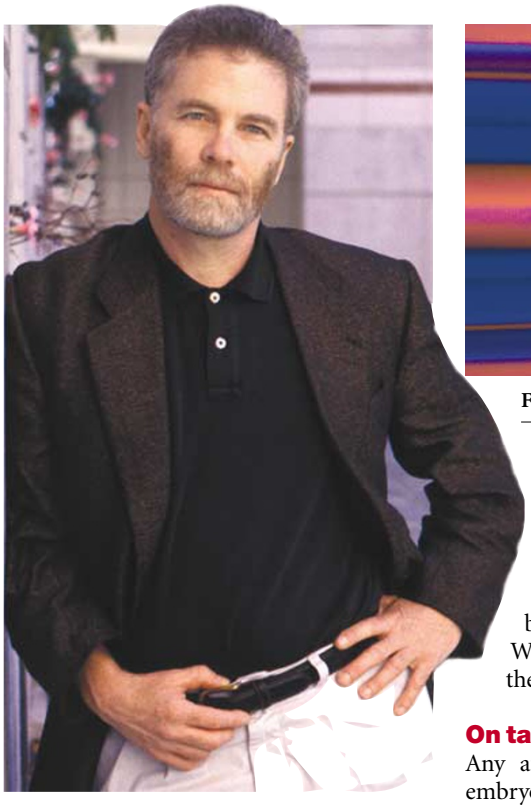
Tackling the taboo

In March 1998, Stock organized a conference in Los Angeles that threw a spotlight on human germline manipulation, then considered a taboo subject. At the meeting, leading scientists spoke in favour of the idea, arguing that excessive regulation could hamper research of medical value[2]. Last year, a report from the American Association for the Advancement of Science dampened the enthusiasm, calling for a moratorium on attempts to change a person's genes in ways that could affect future generations[3]. But it left the door open: with safe methods, proper supervision and adequate public discussion, the report concluded that germline gene therapy might one day be acceptable.

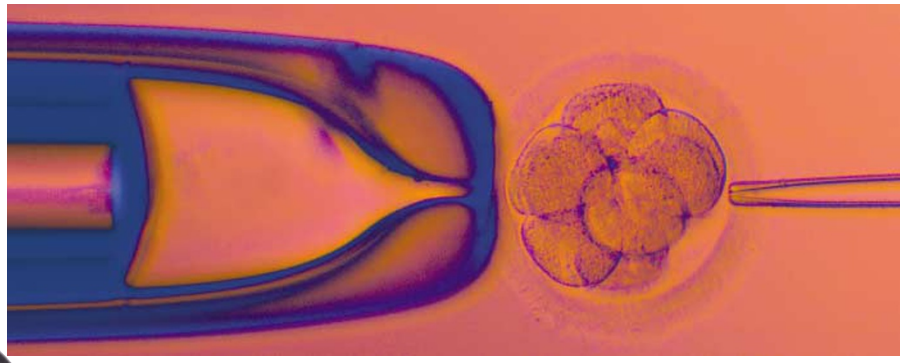
Any future attempt at human germline gene therapy will draw on experience with transgenic animals. The most basic procedure, injecting DNA fragments into one of the unfused nuclei in a newly fertilized egg, was pioneered in mice more than two decades ago[4]. The

NEWSMAKERS

Monkey business: ANDi, the first genetically modified primate, was born containing a jellyfish gene.



Gregory Stock sees altering the genome as the 'endpoint' of the genomic revolution.



P. GOETGHELUCK/SPL

Fine tuning: an eight-celled human embryo, created by IVF, has one cell removed for screening.

inserted DNA only occasionally works its way into a chromosome. Even when it does, it may fail to switch on — of the embryos that survive to be transferred to a surrogate mother, about one in a hundred incorporates a working copy of the gene. At present, there is no way to be sure of the outcome before transferring the embryo to the womb.

In 1998, Robert Bremel and his colleagues at the University of Wisconsin in Madison devised a way to boost the efficiency of trangenesis by using a retrovirus to deliver the genes[5]. Retroviruses insert their genetic material into their host's chromosomes, before hijacking its cellular machinery to make copies of themselves. By modifying a retrovirus so that it could still carry a transgene into the genome of unfertilized cows' eggs, but disabling its ability to replicate, the researchers found that all four embryos that developed to term carried a working copy of the transgene. This technique was used by the team that created ANDi. Researchers in Hawaii have since achieved similar efficiency in mice by injecting eggs with sperm mixed with the transgene[6].

But there is a bigger problem with techniques that rely on inserting genes into newly fertilized eggs: the consequences are not always easy to predict. When researchers at the US Department of Agriculture added a growth-hormone gene to pigs they got fast-growing livestock. But most of the animals suffered lethargy, arthritis and gastric ulcers — and the boars lost their sex drive[7].

Such problems are probably caused by the random insertion of the transgene into the genome. Multiple copies might be incorporated, and transgenes may also land next to regulatory sequences that suppress or enhance their normal activity or cause them to be expressed in the wrong tissues. Worse, random insertion can also lead to the disruption of other important genes.

On target

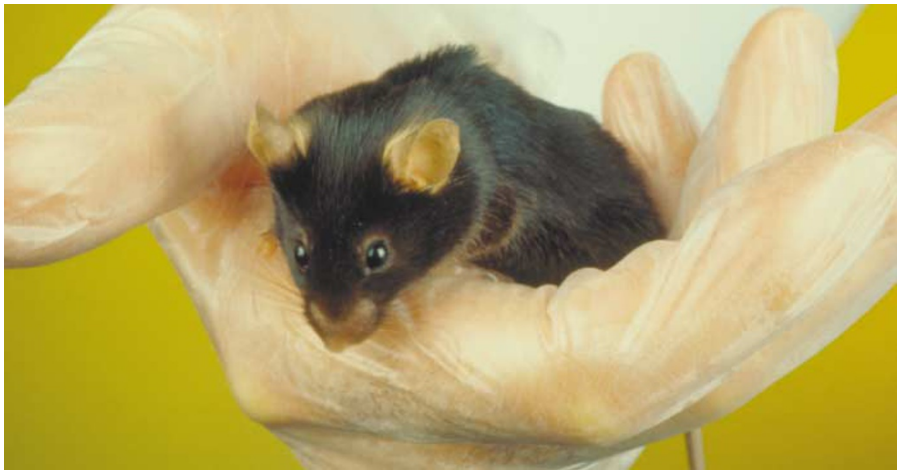
Any attempt to repair genes in human embryos would require a way to ensure that a single copy of the transgene is incorporated in exactly the right position, neatly displacing the target 'faulty' gene. In mice, such gene targeting is now standard practice. It relies on a phenomenon called homologous recombination, in which a gene flanked by sequences that match those bordering a target site in the genome is occasionally inserted in that position by the cell's DNA-repair enzymes, replacing the target gene.

Homologous recombination is a rare event. But in the mid-1980s, the pioneers of gene targeting hit on the idea of adding the DNA to cultured mouse embryonic stem (ES) cells, selecting those in which the gene had incorporated, and then injecting these cells into mouse embryos. Because ES cells can develop into any type of cell in the body, this results in mouse 'chimaeras' consisting of some cells derived from the original embryo and others from the ES cells. These mice can then be mated with one another to produce pure transgenic mice[8].

But producing chimaeras is hardly an acceptable scenario for human reproduction. So if society ever decides that tinkering with the human genome is desirable, we will need a new technology. What might it be?

One possibility is an artificial chromosome. Several researchers are manufacturing chromosomes that can function in mouse and human cells. These consist of three basic components: the centromere, the central region to which the microtubules that pull chromosomes apart during cell division attach; the telomeres, which cap chromosome ends; and the intervening DNA that carries genes. Placing transgenes in artificial chromosomes means there is virtually no limit to the size of the genes inserted. It also carries no risk of genetic disruption caused by random insertions into the genome.

The technology, still in its infancy, is being developed for transgenic animal production and gene therapy, rather than for human germline modification. Huntington Willard, director of the Research Institute of University Hospitals in Cleveland, Ohio, and his colleagues at Case Western Reserve University, also in Cleveland, have engineered an artificial human chromosome from



J. C. LEVY/SPL

Handy: a transgenic mouse with no dystrophine in its muscles, created for muscular dystrophy research.


PPL THERAPEUTICS

Taking a gambol: is the technique used to produce gene-targeted lambs at Roslin the way forward?

scratch, using only synthetic DNA. When they inject their artificial centromeres together with gene-bearing DNA fragments into human cells, the chromosomes assemble spontaneously and seem to behave like the real thing[9].

Meanwhile, the Canadian company Chromos in Burnaby, British Columbia, has built artificial chromosomes around portions of mouse centromeres. The chromosomes replicate normally in cells from several species, including cows and humans[10]. Transgenic mice can be created by simply injecting an artificial chromosome into a fertilized egg, and these mice can subsequently pass the chromosome to their offspring[11].

Dynamic duos

This hints at the possibility of applying the technology to human germline manipulation. But as Chromos only endows its mice with a single chromosome, rather than a pair, only half the offspring end up with a copy. To get artificial chromosomes to inherit normally would require bearers to carry two copies. During the chromosomal dance that takes place in meiosis — the cell divisions that give rise to eggs and sperm — the artificial chromosomes would have to recognize themselves as members of a pair, and segregate accordingly. How this process occurs in natural chromosomes is poorly understood, so there seems to be little chance of making it work in artificial versions any time soon. "We are a very long way from thinking about meiosis," says Willard.

In any case, therapies that rely on giving people extra chromosomes raise another difficult question. Would people engineered to have 24 pairs of chromosomes instead of the usual 23 be able to reproduce with any partner? Experience with Chromos's mice suggests that they might produce viable offspring. But unless they mated with another individual carrying two copies of the extra chromosome, their offspring would only inherit one copy. Their grandchildren, in turn, could not be sure of inheriting the chromosome at all.

Given these complications, some experts believe that the most likely route to human germline manipulation will involve the nuclear transfer technology used to create Dolly the cloned sheep. The basic idea is to take a cell, introduce a transgene by homologous recombination, and then transfer the resulting nucleus to an egg cell stripped of its own chromosomes. Last year, members of the Dolly team at PPL Therapeutics in Roslin, near Edinburgh, created transgenic sheep by this method[12].

For human germline gene therapy, the procedure would be slightly different. Clinicians would isolate ES cells from embryos made by *in vitro* fertilization (IVF) with the parents' eggs and sperm. Once the ES cells have multiplied in a dish to a population of several million, a technician would deliver a transgene to all the cells, and select the few in which it had integrated by homologous recombination. Up to this point, the procedure resembles that used to make gene-targeted mice. But rather than making chimaeras, the Dolly procedure would then be applied. The resulting embryo would be genetically identical to the original one created by IVF, with the exception of the modified genes.

> Does this amount to sacrificing one life in order to generate another? Or is it the same life all along?

The procedure raises an interesting ethical question. Does it amount to sacrificing one life in order to generate another? Or is it the same life all along? In a sense the original embryo was never destroyed, but recreated by cloning from its own cells.

This ethical conundrum aside, safety is the real barrier. The success rate of nuclear-transfer cloning in animals is still extremely low. Most embryos die before or soon after birth. Even if the miscarriages and infant mortality could be prevented, hidden problems may remain. In July, researchers led by Rudolf Jaenisch at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, reported that mice cloned from ES cells show unpredictably altered levels of gene expression[13]. Until this problem can be solved, experts agree, the procedure cannot be countenanced in humans.

Other biologists argue that there is little point in trying to solve these problems, given the existence of the simpler, safer technique of pre-implantation genetic diagnosis[14].

Inheritance taxed

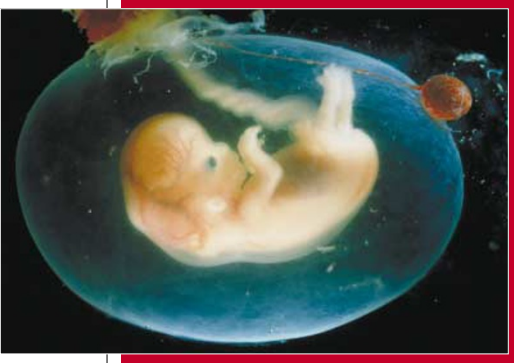
Although it might at first seem sensible to want to repair the single-gene defects that cause diseases such as cystic fibrosis, couples who both carry a mutation in the gene can instead choose IVF, and then have the embryos screened for the mutation. Only those with at least one normal copy are transferred to the mother's uterus.

In rare cases, pre-implantation genetic diagnosis offers no hope of preventing a heritable disorder. A few Huntington's disease patients, for example, carry two copies of the faulty gene[15]. A single copy of the Huntington's gene is enough to cause the fatal neurological disorder, the symptoms of which usually appear during middle age. People with two copies suffer only slightly worse symptoms, but all their children will get at least one copy of the gene.

Such cases are tragedies, but are far too rare to inspire the concerted effort required to develop techniques of germline gene therapy. Any impetus is more likely to come from the discovery of genes that heighten susceptibility to more common diseases. Mutations in the gene *BRCA-1*, for example, can increase the chance that a woman will get breast cancer in her lifetime to 80% (ref. 16).

As the list of known susceptibility genes grows, eliminating them through pre-implantation genetic diagnosis becomes impractical. If a couple carries four disease-susceptibility genes between them, finding an embryo free of all four means screening 16 embryos on average — requiring more eggs than can be taken from a woman in a typical IVF egg-retrieval procedure. On the other hand, the combination of gene targeting and nuclear transfer has the potential — if the problems with miscarriages and infant mor-

The unregulated frontier



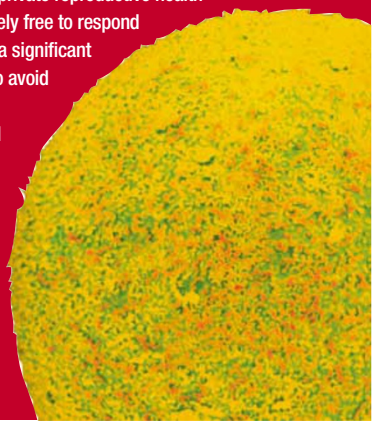
G. MOSCOSO/SPL

Human germline genetic engineering? Didn't I read somewhere that it had already been done? If that was your initial reaction to this feature, you are probably thinking of the fuss that in March greeted a paper[18] from researchers led by Jacques Cohen at the Institute for Reproductive Medicine and Science of Saint Barnabas in Livingston, New Jersey.

Cohen and his colleagues reported that they had helped infertile women to have children by 'rejuvenating' their eggs with a shot of cytoplasm from a younger woman's egg. Along with the cytoplasm came a handful of mitochondria, the components that generate energy for our cells and which contain a small number of their own genes. The resulting children each had mitochondria from their mother as well as from the donor, and the authors claimed this amounted to a germline genetic modification.

But this is not what most biologists think of when they use this term. Mixing mitochondria does not risk damaging important chromosomal genes, nor could it endow children with eugenic traits. To call it germline engineering, says Gregory Stock of the University of California, Los Angeles, who has written extensively on the topic, is "playing games with words". Nevertheless, the Cohen paper may provide a preview of how the first genetically modified human child will be born. In some countries, private reproductive health clinics are tightly regulated. But in the United States, they are largely free to respond to the demands of their patients — and surveys have shown that a significant minority of the public is open to the idea of engineering children to avoid disease and even to possess 'desirable' characteristics.

Cohen's work attracted further controversy when it emerged that his paper had failed to mention that two fetuses created by the technique had suffered from a rare chromosomal abnormality — one woman miscarried, the other chose an abortion. To some bioethicists, this underlines the need to regulate private clinics so that they cannot experiment freely with true germline procedures.



Y. NIKAS/SPL

Quick change: a human cell one day after fertilization, right, and a human fetus at seven to eight weeks, top.

tality can be solved — to repair or replace multiple genes in a single embryo.

But even with safe techniques for making such alterations, critics warn that the genome is far too complex to predict all of the consequences. Gene variants that contribute to one disease may protect against another — the classic example being the mutation in the gene for haemoglobin that keeps malaria at bay if its bearer carries one copy, but causes sickle-cell anaemia in people unlucky enough to inherit two. Whether a gene variant helps or hurts may depend on its genetic surroundings. In women who are protected from cancer by certain other genes, the risky *BRCA-1* gene might conceivably fend off some other disease.

UPPA

Mary Warnock: worried about effects on children.

The problem, say critics, is that we would not know until it was too late that a known disease-susceptibility gene has some beneficial effect. "You need to be careful before you start fiddling around with a delicate system that has evolved over millennia," says Patricia Baird, a geneticist at the University of British Columbia in Vancouver.

But current transgenic animal technology includes methods that would allow the damage to be undone, should problems arise. "It has to be reversible," says Mario Capecchi of the University of Utah in Salt Lake City, one of the pioneers of gene targeting in mice. Reversible transgenes have been used in mice for years, he points out.

To achieve this in mice, the transgene is placed between the target sequences for a site-specific recombinase, an enzyme that precisely clips out a particular segment of DNA and then reseals the chromosome[17]. In mouse experiments, the recombinase can be activated in specific situations such as the production of sperm cells, allowing the next generation to be bred free of the transgene. For use in human germline gene therapy, the recombinase gene could be included with the transgene and be linked to a regulatory sequence that would be activated if the patient took a specific drug.

Ethical issues

Although such systems might address the safety objections to germline manipulation, they leave other questions unanswered. Germline gene therapy to replace genes such as *BRCA-1* is seen by some ethicists as being just a short step from manipulations to imbue children with genes predisposing to high intelligence, tall stature or socially desirable behaviour.

Such children might feel like machines manufactured to specifications, says Mary Warnock, a moral philosopher who headed Britain's Committee of Enquiry into Human Fertilisation in the early 1980s — the deliberations of which fed into subsequent legislation on reproductive technology. "If you tinker with the genes of a child to make it turn out any way you want, then there is an intolerable burden on that child," she says.

Clearly, any attempt to manipulate the human germline would provoke a fierce controversy. Those scientists who believe that the technology will one day be perfected agree on one thing: it is far better to hold the necessary ethical debate now, at leisure, before the techniques to make it happen are a practical reality, than later, in haste, when they are entering the clinic. ■

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1. Chan, A. W. S., Chong, K. Y., Martinovich, C., Simerly, C. & Schatten, G. *Science* **291,** 309–312 (2001).
2. Wadman, M. *Nature* **392,** 317 (1998).
3. Frankel, M. S. & Chapman, A.R . *Human Inheritable Genetic Modifications: Assessing Scientific, Religious, and Policy Issues* (American Association for the Advancement of Science, Washington, 2000).
4. Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A. & Ruddle, F. H. *Proc. Natl Acad. Sci. USA* **77,** 7380–7384 (1980).
5. Chan, A. W. S., Homan, E. J., Ballou, L. U., Burns, J. C. & Bremel, R. D. *Proc. Natl Acad. Sci. USA* **95,** 14028–14033 (1998).
6. Perry, A. C. F. *et al. Science* **284,** 1180–1183 (1999).
7. Pursel, V. G. *et al. Science* **244,** 1281–1288 (1989).
8. Thompson, S., Clarke, A. R., Pow, A. M., Hooper, M. L. & Melton, D. W. *Cell* **56,** 313–321 (1989).
9. Harrington, J. J., Van Bokkelen, G., Mays, R. W., Gustashaw, K. & Willard, H. F. *Nature Genet.* **15,** 345–355 (1997).
10. Telenius, H. *et al. Chromosome Res.* **7,** 3–7 (1999).
11. Co, D. O. *et al. Chromosome Res.* **8,** 183–191 (2000).
12. McCreath, K. J. *et al. Nature* **405,** 1066–1069 (2000).
13. Humpherys, D. *et al. Science* **293,** 95–97 (2001).
14. McLaren, A. *Nature* **392,** 645 (1998).
15. Wexler, N. S. *et al. Nature* **326,** 194–197 (1987).
16. Ford, D. *et al. Lancet* **343,** 692–695 (1994).
17. Chambers, C. A. *Bioessays* **16,** 865–868 (1994).
18. Barritt, J. A., Brenner, C. A., Malter, H. E. & Cohen, J. *Hum. Reprod.* **16,** 513–516 (2001).