Cloning

Now that genetically modified and copied mammals are a reality, biomedical researchers are starting to develop imaginative ways to use this technology

JOHN CHADWICK PHOTOGRAPHIC AND PPL THERAPEUTICS

RODDY FIELD *Roslin Institute*

In the summer of 1995 the birth of two lambs at my institution, the Roslin Institute near Edinburgh in Midlothian, Scotland, heralded what many scientists believe will be a period of revolutionary opportunities in biology and medicine. Megan and Morag, both carried to term by a surrogate mother, were not produced from the union of a sperm and an egg. Rather their genetic material came from cultured cells originally derived from a nine-day-old embryo. That made Megan and Morag genetic copies, or clones, of the embryo.

Before the arrival of the lambs, researchers had already learned how to produce sheep, cattle and other animals by genetically copying cells painstakingly isolated from early-stage embryos. Our work promised to make cloning vastly more practical, because cultured cells are relatively easy to work with. Megan and Morag proved that even though such cells are partially specialized, or differentiated, they can be genetically reprogrammed to function like those in an early embryo. Most biologists had believed that this would be impossible.

We went on to clone animals from cultured cells taken from a 26-day-old fetus and from a mature ewe. The ewe's cells gave rise to Dolly, the first mammal to be cloned from an adult. Our announcement of Dolly's birth in February 1997 attracted enormous press interest, perhaps because Dolly drew attention to the theoretical possibility of cloning humans. This is an outcome I hope never comes to pass. But the ability to make clones from cultured cells derived from easily obtained tissue should bring numerous practical benefits in animal husbandry and medical science, as well as answer critical biological questions.

How to Clone

Cloning is based on nuclear transfer, the same technique scientists have used for some years to copy animals from embryonic cells. Nuclear transfer involves the use of two cells. The recipient cell is normally an unfertilized egg taken from an animal soon after ovulation. Such eggs are poised to begin developing once they are appropriately stimulated. The donor cell is the one to be copied. A researcher working under a high-power microscope holds the recipient egg cell by suction on the end of a fine pipette and uses an extremely fine micropipette to suck out the chromosomes, sausage-shaped bodies that incorporate the cell's DNA. (At this stage, chromosomes are not enclosed in a distinct nucleus.) Then, typically, the donor cell, complete with its nucleus, is fused with the recipient egg. Some fused cells start to develop like a normal embryo and produce offspring if implanted into the uterus of a surrogate mother.

In our experiments with cultured cells, we took special measures to make the donor and recipient cells compatible. In particular, we tried to coordinate the cycles of duplication of DNA and those of the production of messenger RNA, a molecule that is copied from DNA and guides the manufacture of proteins. We chose to use donor cells whose DNA was not being duplicated at the time of the transfer [*see box on page 60*]. To arrange this, we worked with cells that we forced to become quiescent by reducing the concentration of nutrients in their culture medium. In addition, we delivered pulses of electric current to the egg after the transfer, to encourage the cells to fuse and to mimic the stimulation normally provided by a sperm.

After the birth of Megan and Morag demonstrated that we could produce viable offspring from embryo-derived cultures, we filed for patents and started experiments to see whether offspring could be produced from more completely differentiated cultured cells. Working in collaboration with

for Medicine

by Ian Wilmut

MEGAN AND MORAG (*above*) were the first mammals cloned from cultured cells. That basic technique has allowed the creation of cloned sheep carrying human genes. Such animals produce milk that can be collected and processed (*left*) to yield therapeutic human proteins.

PPL Therapeutics, also near Edinburgh, we tested fetal fibroblasts (common cells found in connective tissue) and cells taken from the udder of a ewe that was three and a half months pregnant. We selected a pregnant adult because mammary cells grow vigorously at this stage of pregnancy, indicating that they might do well in culture. Moreover, they have stable chromosomes, suggesting that they retain all their genetic information. The successful cloning of Dolly from the mammary-derived culture and of other lambs from the cultured fibroblasts showed that the Roslin protocol was robust and repeatable.

All the cloned offspring in our experiments looked, as expected, like the breed of sheep that donated the originating nucleus, rather than like their surrogate mothers or the egg donors. Genetic tests prove beyond doubt that Dolly is indeed a clone of an adult. It is most likely that she was derived from a fully differentiated mammary cell, although it is impossible to be certain because the culture also contained some less differentiated cells found in small numbers in the mammary gland. Other laboratories have since used an essentially similar technique to create healthy clones of cattle and mice from cultured cells, including ones from nonpregnant animals.

Although cloning by nuclear transfer is repeatable, it has

Is Quiescence the Key to Cloning?

All the cells that we used as donors for our nuclear-transfer experiments were quiescent—that is, they were not making messenger RNA. Most cells spend much of their life cycle copying DNA sequences into messenger RNA, which guides the production of proteins. We chose to experiment with quiescent cells because they are easy to maintain for days in a uniform state. But Keith H. S. Campbell of our team recognized that they might be particularly suitable for cloning.

He conjectured that for a nuclear transfer to be successful, the natural production of RNA in the donor nucleus must first be inhibited. The reason is that cells in a very early stage embryo are controlled by proteins and RNA made in the precursor of the parent egg cell. Only about three days after fertilization does the embryo start making its own RNA. Because an egg cell's own chromosomes would normally not be making RNA, nuclei from quiescent cells may have a better chance of developing after transfer.

A related possibility is that the chromosomes in quiescent nuclei may be in an especially favorable physical state. We think regulatory molecules in the recipient egg act on the transferred nucleus to reprogram it. Although we do not know what these molecules are, the chromosomes of a quiescent cell may be more accessible to them. *—I.W.*

limitations. Some cloned cattle and sheep are unusually large, but this effect has also been seen when embryos are simply cultured before gestation. Perhaps more important, nuclear transfer is not yet efficient. John B. Gurdon, now at the University of Cambridge, found in nuclear-transfer experiments with frogs almost 30 years ago that the number of embryos surviving to become tadpoles was smaller when donor cells were taken from animals at a more advanced developmental stage. Our first results with mammals showed a similar pattern. All the cloning studies described so far show a consistent pattern of deaths during embryonic and fetal development, with laboratories reporting only 1 to 2 percent of embryos surviving to become live offspring. Sadly, even some clones that survive through birth die shortly afterward.

Clones with a Difference

The cause of these losses remains unknown, but it may reflect the complexity of the genetic reprogramming needed if a healthy offspring is to be born. If even one gene inappropriately expresses or fails to express a crucial protein at a sensitive point, the result might be fatal. Yet reprogramming might involve regulating thousands of genes in a process that could involve some randomness. Technical improvements, such as the use of different donor cells, might reduce the toll.

The ability to produce offspring from cultured cells opens up relatively easy ways to make genetically modified, or transgenic, animals. Such animals are important for research and can produce medically valuable human proteins.

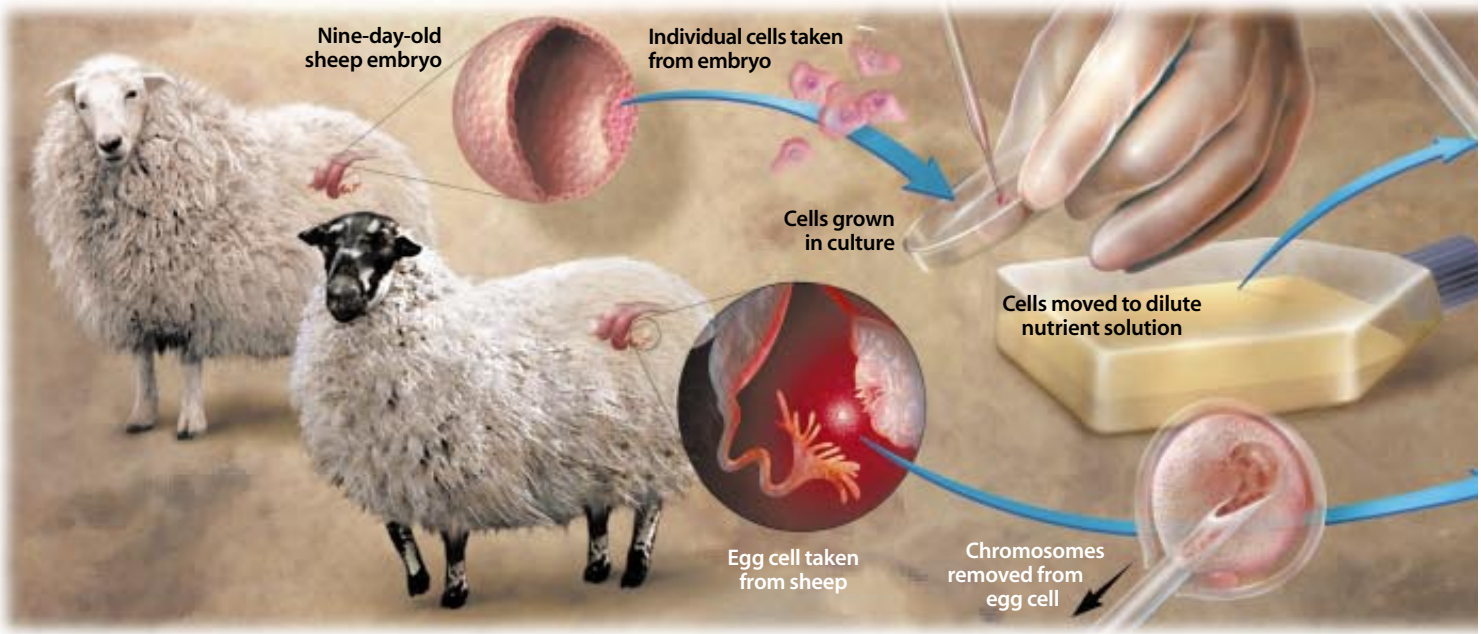
The standard technique for making transgenic animals is painfully slow and inefficient. It entails microinjecting a genetic construct—a DNA sequence incorporating a desired gene—into a large number of fertilized eggs. A few of them take up the introduced DNA so that the resulting offspring express it. These animals are then bred to pass on the construct [see "Transgenic Livestock as Drug Factories," by William H. Velander, Henryk Lubon and William N. Drohan; Scientific American, January 1997].

In contrast, a simple chemical treatment can persuade cultured cells to take up a DNA construct. If these cells are then used as donors for nuclear transfer, the resulting cloned offspring will all carry the construct. The Roslin Institute and PPL Therapeutics have already used this approach to produce transgenic animals more efficiently than is possible with microinjection.

We have incorporated into sheep the gene for human fac-

How Megan and Morag Were Made

Cultured cells were combined with egg cells to yield embryos that developed into cloned offspring.



KEITH KASNOT

tor IX, a blood-clotting protein used to treat hemophilia B. In this experiment we transferred an antibiotic-resistance gene to the donor cells along with the factor IX gene, so that by adding a toxic dose of the antibiotic neomycin to the culture, we could kill cells that had failed to take up the added DNA. Yet despite this genetic disruption, the proportion of embryos that developed to term after nuclear transfer was in line with our previous results.

The first transgenic sheep produced this way, Polly, was born in the summer of 1997. Polly and other transgenic clones secrete the human protein in their milk. These observations suggest that once techniques for the retrieval of egg cells in different species have been perfected, cloning will make it possible to introduce precise genetic changes into any mammal and to create multiple individuals bearing the alteration.

RODDY FIELD *Roslin Institute*

DOLLY (*right*) shot to worldwide fame in 1997 as the first mammal cloned from an adult's cells. Now mature, Dolly has given birth to a healthy lamb, Bonnie (*left*), the product of a normal mating and gestation.

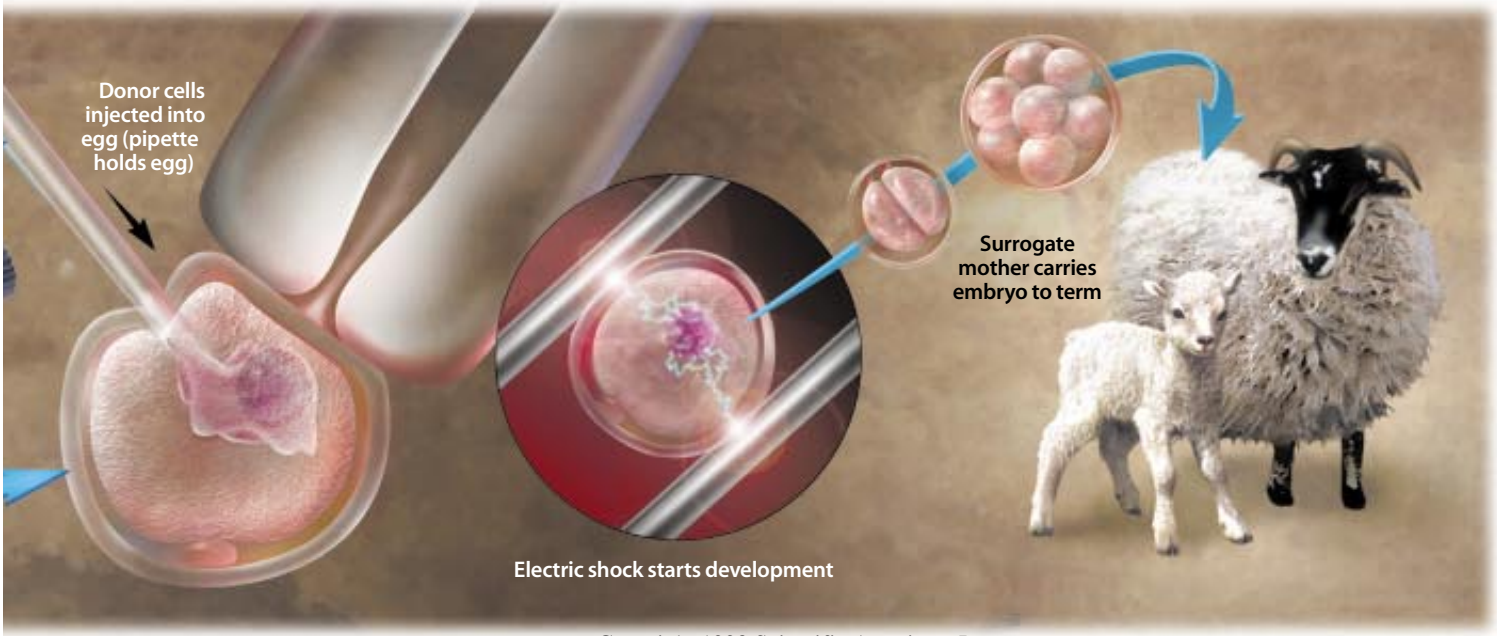
Cultures of mammary gland cells might have a particular advantage as donor material. Until recently, the only practical way to assess whether a DNA construct would cause a protein to be secreted in milk was to transfer it into female mice, then test their milk. It should be possible, however, to test mammary cells in culture directly. That will speed up the process of finding good constructs and cells that have incorporated them so as to give efficient secretion of the protein.

Cloning offers many other possibilities. One is the generation of genetically modified animal organs that are suitable for transplantation into humans. At present, thousands of patients die every year before a replacement heart, liver or kidney becomes available. A normal pig organ would be rapidly destroyed by a "hyperacute" immune reaction if transplanted into a human. This reaction is triggered by proteins on the pig cells that have been modified by an enzyme called alpha-galactosyl transferase. It stands to reason, then, that an organ from a pig that has been genetically altered so that it lacks this enzyme might be well tolerated if doctors gave the recipient drugs to suppress other, less extreme immune reactions.

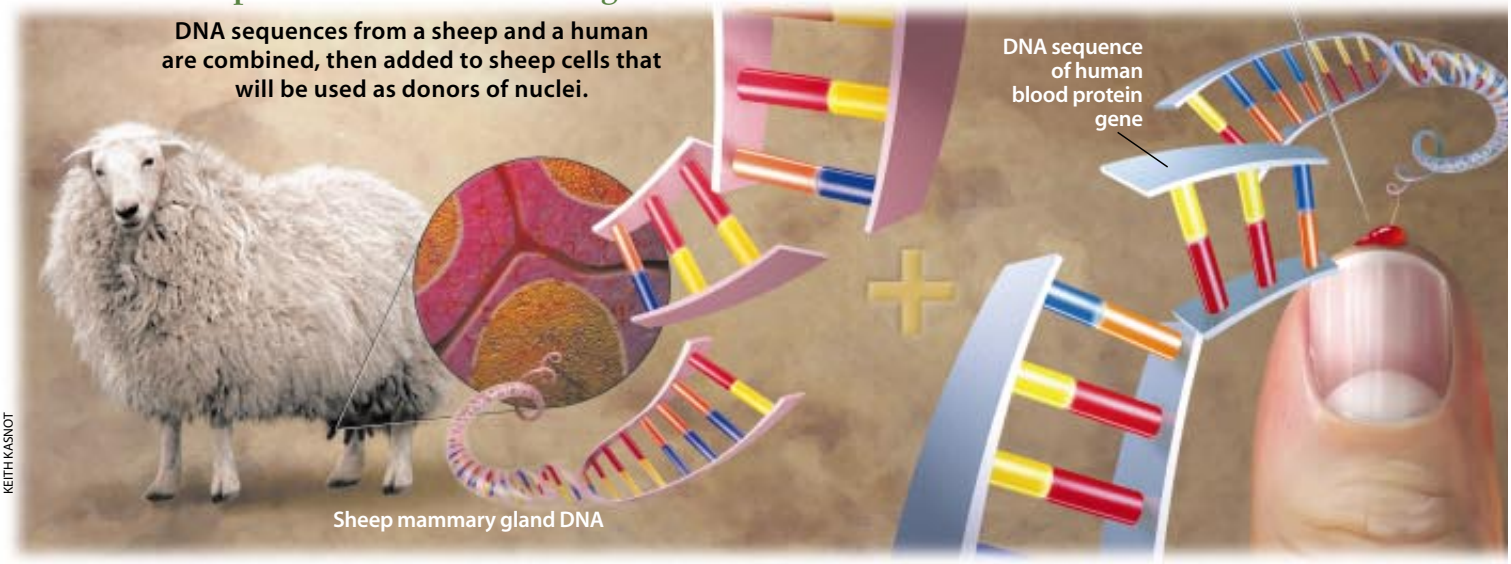
Another promising area is the rapid production of large animals carrying genetic defects that mimic human illnesses, such as cystic fibrosis. Although mice have provided some information, mice and humans have very different genes for cystic fibrosis. Sheep are expected to be more valuable for research into this condition, because their lungs resemble those of humans. Moreover, because sheep live for years, scientists can evaluate their long-term responses to treatments.

Creating animals with genetic defects raises challenging ethical questions. But it seems clear that society does in the main support research on animals, provided that the illnesses being studied are serious ones and that efforts are made to avoid unnecessary suffering.

The power to make animals with a precisely engineered genetic constitution could also be employed more directly in cell-based therapies for important illnesses, including Parkinson's disease, diabetes and muscular dystrophy. None of these conditions currently has any fully effective treatment. In each, some pathological process damages specific cell populations, which are unable to repair or replace themselves. Several novel approaches are now being explored that would provide



How to Prepare Cells to Make Transgenic Clones



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new cells—ones taken from the patient and cultured, donated by other humans or taken from animals.

To be useful, transferred cells must be incapable of transmitting new disease and must match the patient's physiological need closely. Any immune response they produce must be manageable. Cloned animals with precise genetic modifications that minimize the human immune response might constitute a plentiful supply of suitable cells. Animals might even produce cells with special properties, although any modifications would risk a stronger immune reaction.

Cloning could also be a way to produce herds of cattle that lack the prion protein gene. This gene makes cattle susceptible to infection with prions, agents that cause bovine spongiform encephalitis (BSE), or mad cow disease. Because many medicines contain gelatin or other products derived from cattle, health officials are concerned that prions from infected animals could infect patients. Cloning could create herds that, lacking the prion protein gene, would be a source of ingredients for certifiable prion-free medicines.

The technique might in addition curtail the transmission of genetic disease. Many scientists are now working on therapies that would supplement or replace defective genes in cells, but even successfully treated patients will still pass on defective genes to their offspring. If a couple was willing to produce an embryo that could be treated by advanced forms of gene therapy, nuclei from modified embryonic cells could be transferred to eggs to create children who would be entirely free of a given disease.

Some of the most ambitious medical projects now being considered envision the production of universal human donor cells. Scientists know how to isolate from very early mouse embryos undifferentiated stem cells, which can contribute to all the different tissues of the adult. Equivalent cells can be obtained for some other species, and humans are probably no exception. Scientists are learning how to differentiate stem cells in culture, so it may be possible to manufacture cells to repair or replace tissue damaged by illness.

Making Human Stem Cells

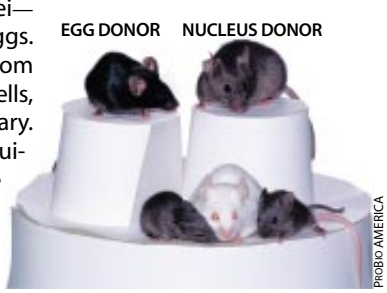
Stem cells matched to an individual patient could be made by creating an embryo by nuclear transfer just for that purpose, using one of the patient's cells as the donor and a human egg as the recipient. The embryo would be allowed to develop only to the stage needed to separate and culture stem cells from it. At that point, an embryo has only a few hundred cells, and they have not started to differentiate. In particular, the nervous system has not begun to develop, so the embryo has no means of feeling pain or sensing the environment. Embryo-derived cells might be used to treat a variety of serious diseases caused by damage to cells, perhaps including AIDS as well as Parkinson's, muscular dystrophy and diabetes.

Scenarios that involve growing human embryos for their cells are deeply disturbing to some people, because embryos have the potential to become people. The views of those who consider life sacred from conception should be respected, but I suggest a contrasting view. The embryo is a cluster of cells that does not become a sentient being until much later in development, so it is not yet a person. In the U.K., the Human Genetics Advisory Commission has initiated a major public consultation to assess attitudes toward this use of cloning.

Creating an embryo to treat a specific patient is likely to be an expensive proposition, so it might be more practical to establish permanent, stable human embryonic stem-cell lines from cloned embryos. Cells could then be differentiated as needed. Implanted cells derived this way would not be genetically perfect matches, but the immune reaction would prob-

Now, Cloned Mice

Recently Ryuzo Yanagimachi of the University of Hawaii at Honolulu and his colleagues successfully cloned mice by transferring donor nuclei—not whole cells—into eggs. The group took nuclei from cells called cumulus cells, which surround the ovary. These cells are naturally quiescent. So far we believe that no one has shown that offspring can be produced from differentiated cells that are not quiescent. —*I.W.*



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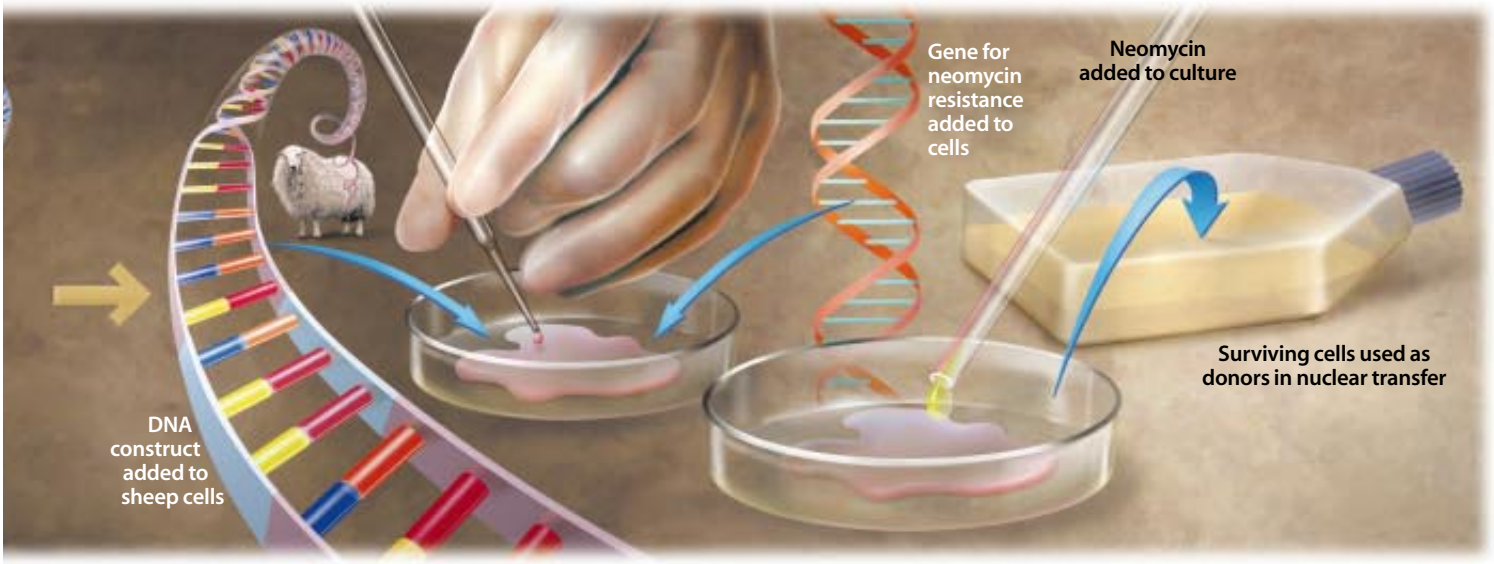
Surrogate mother (*center*) is flanked by cloned offspring of nucleus donor.



ably be controllable. In the longer term, scientists might be able to develop methods for manufacturing genetically matched stem cells for a patient by "dedifferentiating" them directly, without having to utilize an embryo to do it.

Several commentators and scientists have suggested that it might in some cases be ethically acceptable to clone existing people. One scenario envisages generating a replacement for a dying relative. All such possibilities, however, raise the concern that the clone would be treated as less than a complete individual, because he or she would likely be subjected to limitations and expectations based on the family's knowledge of the genetic "twin." Those expectations might be false, because human personality is only partly determined by genes. The clone of an extrovert could have a quite different demeanor. Clones of athletes, movie stars, entrepreneurs or scientists might well choose different careers because of chance events in early life.

Some pontificators have also put forward the notion that couples in which one member is infertile might choose to make a copy of one or the other partner. But society ought to be concerned that a couple might not treat naturally a child who is a copy of just one of them. Because other methods are available for the treatment of all known types of infertility, conventional therapeutic avenues seem more appropriate. None of the suggested uses of cloning for making copies of existing people is ethically acceptable to my way of thinking, because they are not in the interests of the resulting child. It should go without saying that I strongly oppose allowing cloned human embryos to develop so that they can be tissue donors.

It nonetheless seems clear that cloning from cultured cells will offer important medical opportunities. Predictions about new technologies are often wrong: societal attitudes change; unexpected developments occur. Time will tell. But biomedical researchers probing the potential of cloning now have a full agenda. SA

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POLLY (*left*) is a transgenic clone of a poll Dorset sheep. A gene for a human protein, factor IX, was added to the cell that provided the lamb's genetic heritage, so Polly has the human gene. The ewe that carried Polly (*right*) is a Scottish blackface.

The Author

IAN WILMUT pursues research on the genetic engineering of livestock at the Roslin Institute near Edinburgh in Midlothian, Scotland. After obtaining a Ph.D. from the University of Cambridge for research on methods of freezing boar semen, he did postdoctoral work at Cambridge on techniques for freezing animal embryos. Later Wilmut identified developmental and physiological causes of prenatal death in sheep and pigs, before turning to studies of ways to improve economically important animals.

Further Reading

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