Cloning: Pathways to a Pluripotent Future

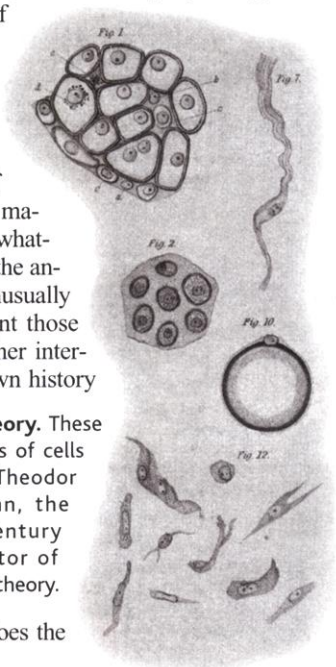
Anne McLaren

Over the years, clones and cloning have meant different things to different people. Gardeners have always known that their vegetatively propagated plants formed a clone, but this only hit the headlines when all the plants of a well-known raspberry clone, distributed all over the world, started dying off at the same time. There was a brief flurry of excitement in the 1970s when John Gurdon's spectacular photograph of 30 little albino frogs, cloned by nuclear transfer from an albino tadpole, reminded the newspapers of Aldous Huxley's *Brave New World*. The advent of recombinant DNA enabled molecular biologists to clone genes, but for most people, the word "clone" has had more to do with less expensive versions of IBM PCs than with anything biological. That wide usage of the term since the 1980s has helped change its meaning even in biological circles: "Clone" no longer signifies a group of identical members; it signifies a single member of such a group.

Frogs galore. This array of 30 cloned frogs spurred headlines in 1977.

In 1997, cloning topped the charts of scientific and social discourse. That's when the news broke that Dolly, the Scottish cloned sheep, had been born. In reality Dolly represented just one stage in a whole series of experiments carried out in different labs by different teams of scientists, and all duly published in the scientific literature. But for the general public, and indeed for many scientists whose attention was focused elsewhere, Dolly came like a bolt from the future. Because the nucleus that gave rise to Dolly came from an *adult* sheep (not even in frogs had an adult been cloned from adult cells), and because this feat of replication had been achieved in a *mammal*, the idea that *people* might also be cloned lost its air of fantasy. Making human clones became a real possibility.

There are two distinct scientific motivations that account for the creation of Dolly. The first is the fundamental desire to know whether the hereditary material in the nucleus of each cell remains intact throughout development, whatever the fate of the cell. The second relates in particular to farm animals: the ancient and ongoing desire to replicate those rare animals that possess an unusually favorable combination of genetic characteristics. The desire to augment those characteristics still further by genetic manipulation introduces still another interweaving strand—stem cell biology (see sidebar on p. 1778)—with its own history and its strong biomedical implications for the future.



Cell theory. These drawings of cells are by Theodor Schwann, the 19th century originator of the cell theory.

In this essay, I propose to consider Dolly not as a sheep but as a node, with scientific input streams flowing in, and scientific, social, and ethical consequences as outputs.

The Role of the Nucleus

One of the questions that has inspired the science leading to and emerging from Dolly is: Does the hereditary material in the nucleus remain intact as the embryo develops? In other words, what role does the nucleus play in development *(1)*?

BORDER ILLUSTRATIONS: MATT ZANG; CREDITS: (TOP TO BOTTOM) J. GURDON, *PROC. R. SOC. LONDON, SER. B.*; SCHWANN, SYDENHAM SOCIETY, 1847

A Cloning Timeline

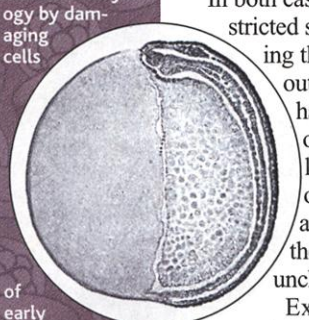
1839
Theodor Schwann lays the foundation of what becomes known as the cell theory of biology.

1855
Rudolf Virchow states that all cells arise from cells.

1865
Gregor Mendel first reports the results of his pea plant experiments, from which he discerned his fundamental laws of heredity.

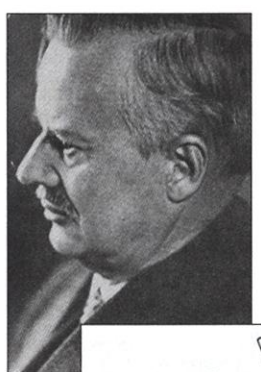
1885
August Weismann incorrectly proposes that the genetic information of cells diminishes as the cells differentiate during development.

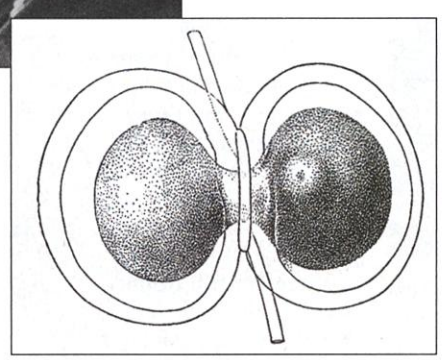
1888
Wilhelm Roux helps initiate the field of experimental embryology by damaging cells of early embryos and observing the developmental consequences. The half-embryos that result seem to confirm Weismann's ideas.



This part of the story really begins in 1839, when Theodor Schwann launched the cell theory, later to be encapsulated by Rudolf Virchow in his famous slogan *Omnis cellula e cellula* (All cells come from cells). As applied to development, the cell theory requires two antagonistic properties: *cell heredity* and *cell differentiation*. Did every cell division produce two identical daughter cells, or did they differ? After the first cleavage division, could each cell on its own produce a whole embryo, or would one produce a left and one a right half, or one a front and one a back?

In 1888, Wilhelm Roux attempted to answer this question by damaging one cell of a two-cell frog embryo with a hot needle. The cell stayed in place but did not develop further; its partner developed into a left or right half-embryo. Sadly, this pioneering effort gave a misleading result, and August Weismann (who was more of a philosopher than an empirical scientist) used it to support his long-held, erroneous belief that all development and cell differentiation depended on loss of hereditary material *(2)*. Weismann's theory was soon disproved by Hans Driesch, who in 1892 separated the two-cell and even the four-cell sea urchin embryo into separate cells: Each developed into a small but perfect larva *(3)*. Similar results were obtained a few years later by others. One of them was Hans Spemann, who in 1901 found that if the first two cells of amphibian embryos were separated, two normal tadpoles could develop. It seemed that Roux's result was an unfortunate artifact due to the inhibitory effect of the damaged cell. Some invertebrates including nematodes, however, showed *mosaic* rather than *regulative* development: When separated, the first two cells really did have different fates.





First steps. Using a hair to tie a fertilized frog egg into two halves, Hans Spemann (*top*) took early steps toward cloning.

The nucleus, containing the chromosomes, soon became recognized as the carrier of heredity. Was it also the engine of differentiation?

In order to explore nuclear, rather than cellular, potential, Spemann and Jacques Loeb carried out ingenious primitive nuclear transfer experiments in Amphibia and sea urchins, respectively. In both cases, the fertilized egg was constricted so as to give a portion containing the nucleus and a portion without. When the nucleated portion had cleaved to eight to 16 cells, one of these nuclei was allowed to reenter the portion of original uncleaved cytoplasm. Both portions were able to give rise to normal embryos, showing that the developmental potential of the nuclei remained unchanged at least to the 16-cell stage.

Experiments along these lines emboldened Spemann in 1938 to propose a "thought experiment," which, as he put it, "appears at first sight to be somewhat fantastical" *(4)*. He wondered what would happen if a nucleus from a differentiated cell, even an adult cell, were to be somehow introduced into an egg whose own nucleus had been removed. It was 14 years before his *gedanken* experiment could be carried out. For one, Spemann lacked the know-how and techniques to carry out such a traumatic series of manipulations. And within a year, World War II had begun. It wasn't until 1952 that the necessary nuclear transfer technology was devised for Amphibia *(5)*. It took another 23 years before an adult amphibian nucleus was successfully transferred. And not until 1996, 58 years after Spemann articulated his fantastical experiment, was Dolly born.

In 1952, Robert Briggs and Thomas King electrified the biological world by reporting successful nuclear transfer in the frog *Rana pipiens (6)*. The nuclei came initially from undifferentiated blastula cells; they were transferred to unfertilized eggs from which the nuclei had previously been removed. Once the eggs had been stimulated to develop, some produced normal tadpoles. Over the next few years, this group published a paper per year, each one detailing ever more ambitious experiments. When nuclei were taken from gastrula cells, the next developmental stage after blastulae, the proportion of normal tadpoles was much lower. From gut cells it was lower still, and nuclei from still later tail-bud stage embryos gave no normal development. By 1960, Briggs and King had concluded that differentiation was accompanied by progressive restriction of the capacities of nuclei to promote all the various types of differentiation required for normal development.

Meanwhile, across the Atlantic, the Swiss embryologist Michael Fischberg was working in Oxford with two younger colleagues, Thomas Elsdale and John Gurdon, on a different frog, *Xenopus laevis*. In many ways *Xenopus* was easier to work with than *Rana*. There was no need to remove the recipient egg nucleus, as it seldom took part in subsequent development. What's more, the researchers had a cell marker (the number of nucleoli) distinguishing donor and recipient strains, so there was never any doubt as to whether the nuclear transplantation had succeeded.

Already in their first paper, nuclei from early *Xenopus* blastulae were shown to support development not only to the tadpole stage, but through metamorphosis to give sexually mature adults *(7)*. Gurdon followed up this work: He found that nuclei from later stages could also support development to adults but less frequently—30% from blastulae, but only 6% from hatched tadpoles, and 3% from swimming tadpoles. Did these nuclear changes reflect what was going on in normal development, or were they the result of transplantation?

Experiments became more refined, and knowledge about what underlay the observed nuclear changes grew. Marie Di Berardino started looking at chromosomes. In 1967, she and King reported more than 1200 transfers using *Rana* nuclei taken from differentiated neural cells. Only four out of the whole set had normal chromosomes, and three of these showed developmental abnormalities. They concluded that all the abnormal development they were seeing was attributable to chromosome aberrations occurring soon after transplantation. To give the nuclei a long period of exposure to the new cytoplasmic environment before they were required to replicate, Di Berardino started transplanting them to oocytes rather than to mature eggs. Eventually in 1983 Di Berardino and Nancy Hoffner showed that adult *Rana* red blood cell nuclei transferred to oocytes could support development up

CREDITS: (TOP TO BOTTOM) NLM; NLM; SPEMANN (REF. 4); SPEMANN (REF. 4)

to the swimming tadpole stage. The same nuclei put into eggs got no further than the early gastrula.

In *Xenopus*, Gurdon greatly improved his success rate by doing serial nuclear transfers, rescuing normal nuclei from arrested embryos. He and his colleagues were able to produce fertile adult frogs using the nuclei of differentiated epithelial cells from the guts of feeding tadpoles. To prove that even nuclei from terminally differentiated cells had not lost their developmental potential, they showed that nuclei from specialized adult skin cells, identified with antikeratin antibody, could support development up to the swimming tadpole stage. That meant they must still retain the genetic information required for heart, muscle, brain, eyes, and all the rest *(8)*. These results were impressive, but still nobody had succeeded in making an adult amphibian by transplantation of an *adult* nucleus to an egg or oocyte.

Nuclear transfer experiments in mammals also had been going on. Live young were obtained from single blastomeres isolated at the two-cell stage in rats as early as 1942, and up to the eight-cell stage in rabbits in 1968. Following a few earlier attempts to induce development of enucleated mouse eggs by virus-induced fusion of somatic cells, Derek Bromhall obtained blastocysts by microsurgical introduction of early embryonic nuclei into enucleated rabbit eggs *(9)*. Yukio Tsunoda injected genetically marked nuclei into fertilized mouse eggs; development continued up to the blastocyst stage when nuclei were taken from morulae or from the blastocyst inner cell mass, but failed when nuclei from the already differentiated trophectoderm were used. Because the recipient eggs were not enucleated, the blastocysts were tetraploid and did not develop further.

The first claim to obtain live young after nuclear transfer in mammals was by Karl Illmensee and Peter Hoppe in the mouse using genetically marked inner cell mass nuclei microsurgically introduced into enucleated fertilized mouse eggs *(10)*. The results have never been successfully repeated, despite determined efforts by James McGrath and Davor Solter. These two researchers succeeded in obtaining young after transferring nuclei between unfertilized mouse eggs at the one-cell stage, using virus-induced fusion *(11)*, but nuclei taken later, even at the two-cell stage, were unable to support development.

Cloning for Replication

Embryo splitting in sea urchins, Amphibia, or mammals can give clones of two, four, or eight individuals. Neither Driesch nor Spemann carried out their experiments to increase numbers. Others did, however. From 1979 onward *(12)*, Steen Willadsen successfully reared to adulthood single cells from eight-cell sheep and cattle embryos, in full awareness of the economic benefits that could accrue from rapid multiplication of genetically superior breeds.

Nuclear transfer was not seen by Briggs and King as a means of replicating frogs. On the other hand, the first *Xenopus* nuclear transfer paper mentioned that a number of monozygotic frogs had been obtained from single donors, and in 1962 Gurdon published a picture of 20 cloned male frogs, of uniform color pattern. Two were small and sterile, the rest were of uniform size. By 1977, an albino *Xenopus* mutant became available and was used for the well-known picture of 30 small albino frogs *(8)*. They were made by transferring albino nuclei into the eggs of a dark female.

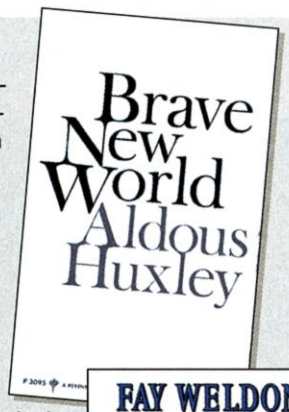
Nuclear transfer experiments proved more successful in cattle *(13)* than they had been in mice. From the first, these experiments were designed to multiply the numbers of valuable animals rather than to examine the role of the nucleus per se.

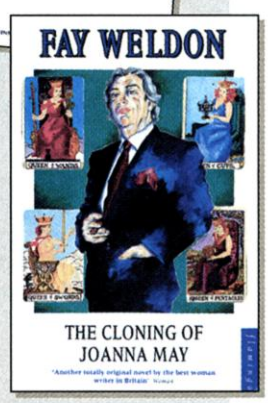
In 1991 Willadsen and colleagues reported 101 nuclear transfer calves, using nuclei derived from cattle morulae. Further cattle studies yielded clones of up to eight calves ("octuplets") generated from a single donor embryo, and successes were reported with nuclei taken from cultured blastocyst cells. Unfortunately, many of the calves developed abnormally and were pathologically overweight at birth, so the procedure has not yet proved economical for cattle breeding.

Megan and Morag. The first mammals cloned from differentiated cells.

Art Imitates Life

The picture on page 1775 instantly conjures up thoughts of the Hatchery in Aldous Huxley's *Brave New World,* in which fertilized eggs destined to develop into Gamma, Delta, and Epsilon humans were subjected to Bokanovsky's Process, each giving rise to up to 96 perfectly formed embryos, and each embryo developing into a fully grown adult.





Huxley's novel was published in 1932, 6 years before Hans Spemann's "fantastical experiment" and decades before John Gurdon's frogs. Where did Huxley's idea come from? No hint of cloning is seen in H. G. Wells's science fiction writings nor in Mary Shelley's *Frankenstein.* Huxley describes the Bokanovsky Process as a series of arrests to development, induced by x-rays, followed by cooling, and finally alcohol. At each arrest the egg buds, then the buds bud and bud again like a plant, producing up to eight embryos each time. So perhaps it was plants that fired his imagination.

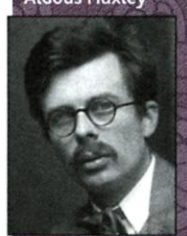
Other cloning novels appeared in the 1970s and 1980s. David Rorvik's *In His Image: The Cloning of a Man* (1978) purported to be a true story based on Derek Bromhall's nuclear transfer experiment in rabbits. (Bromhall sued Rorvik and the publishers.) Ira Levin's *The Boys From Brazil* (1976), about the cloning of Hitler, was made into a film. Fay Weldon's *The Cloning of Joanna May* (1989) is the best of these novels and illustrates how very unidentical human clones would probably be. Cloning literature is sure to expand in both the fiction and nonfiction genres. **–A.M.**

1892
Hans Driesch shows that each cell of a two-cell or four-cell sea urchin embryo can develop into separate, perfectly formed embryos, which goes against Weismann's theory. Roux's earlier results were likely due to damage from the hot needle.

1894
Jacques Loeb conducts early nuclear transfer experiment, in which the nucleus of one cell is transferred to a piece of egg cytoplasm containing no nucleus.

1914
Hans Spemann conducts nuclear transfer experiments with newts and later (1928) with salamanders.

1932
Aldous Huxley



publishes *Brave New World,* which describes a kind of human husbandry.

1938
In his book *Embryonic Development and Induction,* Spemann proposes a "fantastical" thought experiment: to introduce the nucleus from a differentiated cell into an egg whose own nucleus had been removed and then to see what would develop. The first of these experiments began 14 years later.

CREDITS: (BOTTOM, LEFT TO RIGHT) DESIGN & PRINT SERVICES/THE ROSLIN INSTITUTE; E. O. HOPP/CORBIS

1952
Robert Briggs and Thomas King electrify the biological world by reporting the development of normal *Rana pipiens* tadpoles by transferring nuclei from embryonic cells to eggs from which nuclei had been removed.

1953
James Watson and Francis Crick report the structure of DNA.

1958
Michael Fischberg, Thomas Elsdale, and John Gurdon perform nuclear transfer in South African frogs leading to sexually mature adults.

1962
Gurdon announces cloning frogs using the nuclei of fully differentiated adult intestinal cells.

1963
J. B. S. Haldane uses the term "clone" in a speech titled "Biological Possibilities for the Human Species of the Next Ten-Thousand Years."

1970s
Fictionalized accounts of cloning technology proliferate. Examples include David Rorvik's *In His Image: The Cloning of a Man* (1978), Ira Levin's *The Boys From Brazil* (1976), about the cloning of Hitler, and Fay Weldon's *The Cloning of Joanna May* (1989), which illustrates how very unidentical human clones probably would be.

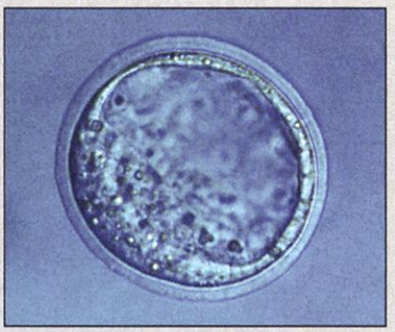
1977
Gurdon's image of 30 cloned frogs sparks public coverage that associates cloning research with *Brave New World*.

Stem Cells: Golden Opportunities With Ethical Baggage

If all cells come from cells, as Rudolf Virchow postulated in the 1850s, all but the most short-lived animals must harbor a reserve of cells to replace those that die or are damaged. This reserve consists of stem cells *(18)*. They are defined as those cells which can divide to produce a daughter like themselves (self-renewal) as well as a daughter that will give rise to specific differentiated cells. Stem cells in the body may be unipotent, like spermatogenic stem cells (which are responsible for the continuing production of spermatozoa), or they can be multipotent, like neural or hemopoietic stem cells, which give rise respectively to all the varied cell types in the nervous system or in the blood and immune system. Given the possibility of directed differentiation of stem cells, these multipotent somatic stem cell lines may prove to be of significant clinical value *(19)*.

Experimentally, it has also proved possible to create immortalized pluripotent stem cells. In 1981, pluripotent embryonic stem (ES) cell lines derived from mouse blastocysts were reported *(20)*. These will proliferate indefinitely in vitro as undifferentiated cells, but will also differentiate when the culture conditions are modified, and when introduced back into an embryo, they will successfully colonize every cell lineage including the germ line. However, pluripotent stem cells cannot on their own make an embryo, that is, they are not *totipotent*. Undifferentiated ES cell lines have been extensively used in mice for genetic manipulation, including the introduction of new genetic material as well as knocking out and replacing genes. Later, similar pluripotential stem cell lines were derived from mouse embryonic germ (EG) cells *(21)*. Despite energetic attempts, it proved extremely difficult to make ES or EG cell lines from any species other than the mouse.

That changed in 1998 when James Thomson and colleagues in Wisconsin reported that they had derived human pluripotential stem cell lines from surplus blastocysts donated by patients undergoing infertility treatment involving in vitro fertilization *(22)*. In the same year, John Gearhart and colleagues reported the derivation of human EG cell lines from aborted human fetal material *(23)*. All these lines are now owned by Geron Corp. of Menlo Park, California; some others have been made elsewhere and are being studied in Australia.



Cellular clay. Stem cells like this one can give rise to different cell types.

Intense activity is now being focused on both mouse and human pluripotential stem cells, in an attempt to induce directed differentiation to defined cell types in culture, for example, by exposing the cells to signaling molecules such as retinoic acid and cytokines, as well as by genetic manipulation *(24)*. The ultimate aim here is to supply transplant surgeons with a readily available supply of any tissue for the repair of damaged or diseased organs so that the need for organ donors would drop. Harold Varmus, until recently director of the National Institutes of Health (NIH), stated before Congress: "There is almost no realm of medicine that might not be touched by this innovation." Among the many medical possibilities are the use of cardiac muscle cells for heart problems, pancreatic islet cells for diabetes, liver cells for hepatitis, and neural cells for Parkinson's or Alzheimer's disease. In animal models, some successes have already been achieved: ES cell–derived cardiac muscle cells have been incorporated into damaged rat hearts, and neural cells introduced into the brain of a mouse model of multiple sclerosis have differentiated into appropriate cell types *(25)*.

In mice, EG cells introduced into embryos have led to some abnormalities, so they may be less suitable than ES cells for clinical use *(26)*. ES cells raise ethical problems, however, as they are derived from early human embryos. Some people believe that fertilized human eggs and early embryos are already persons. They will therefore object to their use for research, even for such ends as cell and tissue therapy to reduce human suffering and disease. Others argue that, because the donated blastocysts will never be transferred to a uterus, it is preferable for them to be used for a beneficent purpose than to merely be left to perish. NIH is now prepared to fund research on human pluripotential stem cells that have been derived according to certain guidelines, but they will not fund the derivation of such lines.

–A.M.

The Roslin Institute: Before and After Dolly

Ian Wilmut at the Roslin Institute in Scotland *(14)* was seeking a way to modify the genetic constitution of sheep and cattle more effectively than by the rather hit-or-miss method of injecting genes into the fertilized egg. Mouse embryonic stem (ES) cell lines made from the blastocyst inner cell mass were amenable to genetic modification, and nuclei from the inner cell mass of cattle had successfully been used to make nuclear transfer calves. Combining these two approaches offered a possible way forward. Keith Campbell, Wilmut's colleague, was impressed by the amphibian evidence that nuclei retained their full developmental potential even in differentiated cells. Also, he had worked on the cell cycle, and he was convinced that synchronization between donor and recipient cell cycles was the key to successful nuclear transfer.

The Roslin team first tried and failed to make immortalized and undifferentiated sheep ES cell lines. That frustration may have been an important factor in their subsequent successes. Unperturbed by the fact that the cells were differentiating, they continued to culture blastocyst inner cells. To optimize the chances of successful nuclear transfer, they put their cultured cells into a state of quiescence, which approximated the cell cycle stage of the recipient unfertilized sheep egg. Transfers were done, using electrical stimuli both to fuse the cultured cell with the enucleated egg and to kick-start embryonic development. From 244 nuclear transfers, 34 developed to a stage where they could be placed in the uteri of surrogate mothers. In the summer of 1995, five

CREDIT: I. WILMUT, ROSLIN INSTITUTE/*NATURE* **385**, 810 (1997)

lambs were born, of which two—Megan and Morag—survived to become healthy fertile adults *(15)*. Megan and Morag were the first mammals cloned from differentiated cells.

The next season, Wilmut and Campbell became more ambitious. They repeated the transfers of nuclei from embryonic cells. They included a group using nuclei taken from cultured fetal fibroblasts, which give chromosomally stable cell cultures. And they also used nuclei from cultured mammary gland cells taken originally from a 6-year-old ewe and stored, frozen, in liquid nitrogen. The first group produced four live lambs, the second two, and the third just one—Dolly *(16)*.

Dolly was the sole survivor from 277 transfers of adult nuclei. The procedure has now been extended to cattle, goats, and mice, but the success rate remains very low, seldom more than 3%. Of those that are placed in surrogate mothers, many die in utero. Others die at birth, often with abnormalities. The reason for this high mortality rate is not known. Perhaps the extensive reprogramming that the adult nucleus requires is incomplete.

By 1997 the project had moved on yet again. Polly was born, cloned from a fetal fibroblast into which had been inserted a gene for a valuable pharmaceutical protein, the human blood-clotting factor IX. Subsequent progress along these lines remains shrouded in commercial secrecy and the confused state of patent law.

Each month, Britannica.com enhances the Pathways of Discovery essay with links to relevant items within and without *Encyclopedia Britannica*'s vast stores of information. To access this month's Pathways essay and all previous ones, go to www.britannica.com and click on the "Science" channel.

Whither Cloning?

Just as the exploration of nuclear potential and the desire for replication have been two distinct strands in the evolution of cloning, so they remain distinguishable factors that will influence possible future lines of development, both in animals and in humans.

1. *Replication.* In farm animals, cloning by nuclear transfer could replicate large numbers of genetically elite individuals that have highly advantageous combinations of genes, brought together either by traditional breeding methods, by transgenic technology, or by in vitro gene targeting, cell selection, and nuclear transfer. Without cloning, these unique gene combinations would be dissipated by genetic recombination. In the plant world, this approach is routine.

Missy. This aging dog could become the first pet to be cloned.

CREDIT: THE MISSYPLICITY PROJECT

Cloning by nuclear transfer could be used for replicating household animals as well. Many people will likely request to clone their much-loved cats and dogs. A project to investigate nuclear transfer cloning in dogs, the "Missyplicity Project," has already been funded by a wealthy Californian seeking to clone his dog Missy. Pet owners may be disappointed, however. Genetic identity by no means ensures identity of personality or temperament.

When techniques of nuclear transfer cloning have been improved and extended, it might even be possible to recover species that have become extinct, provided some of their cells were preserved by freezing. This possibility provides a strong incentive to maintain tissue banks for endangered species.

Humans are animals too, so what works with other animals will probably work with humans. Reproductive cloning, where a human embryo derived by somatic cell nuclear transfer is placed in a woman's uterus to develop into a baby, is out of the question at present. The large numbers of deaths and abnormalities that accompany reproductive cloning in mice and farm animals make any extension to humans totally unacceptable, at the moment anyway. But if the procedure became safer and more efficient, and if it worked in humans (it has not yet worked in rabbits), and if enucleated oocytes and surrogate mothers were available, what would be the ethical and social implications?

Many people find cloning human beings entirely unacceptable ethically, but there are many different reasons why people might wish to clone themselves or others. Some reasons seem more ethically objectionable than others.

The genetic constitution of anyone cloned from an existing person could not be confidential. Also, for the cloned child, the weight of expectation to be like his or her progenitor could become intolerably great. Neither of these objections would apply in the case of a couple who had finally achieved a pregnancy only to find that the fetus was ectopic, or the baby dead through an accident at birth. Nuclear transfer from the fetus or baby might offer their best chance of a replacement pregnancy. But any attempt to clone a talented violin player or a famous sportsman could lead to problems. The child might disappointingly dislike music, or despise sport, and be made deeply unhappy.

For couples whose infertility is so extreme that one partner is entirely lacking in germ cells, somatic nuclear transfer from one or the other might be their only means of having their "own" child. As the child grew up and came to resemble its progenitor (the nucleus donor) at the time when the couple first met, however, their relationship might suffer. For lesbian couples, or single women who have failed to find a man whom they wish to father their children, nuclear transfer from one of their own somatic cells, perhaps using their own eggs and their own uterus, would offer an interesting alternative to donor insemination or adoption.

Then there are people who fear death or desire immortality. From the ethical point of view, this would mean treating children as a commodity, merely as a means to an end, rather than desiring them for their own sake. But even with conventional reproduction, people have always had children for all sorts of reasons, not always for the sake of the children.

From the philosophical point of view, none of the ethical objections seem conclusive. The strongest arguments against human reproductive cloning are perhaps the social ones. It runs counter to our present culture, it would wreak havoc with family law, and, at least in Europe, public consultations have produced an overwhelmingly negative response: People don't want it.

2. *The role of the nucleus.* We know almost nothing about how cloning by somatic cell nuclear transfer works. Increased understanding can come only from basic research using laboratory animals.

A differentiated nucleus has a set genetic program that has to be canceled and replaced by the genetic program of a fertilized egg at the very beginning of embryogenesis. How is this

1979
Steen Willadsen begins successfully rearing to adulthood single cells from eight-cell sheep and cattle embryos, in full awareness of the economic benefits that could accrue from rapid multiplication of genetically superior breeds.

1981
Karl Illmensee and Peter Hoppe claim to obtain live young via nuclear transfer using genetically marked inner cell mass nuclei microsurgically introduced into enucleated fertilized mouse eggs. But no one can replicate the experiments.

Several researchers report generating pluripotent embryonic stem cell lines from mouse blastocysts.

1983
James McGrath and Davor Solter succeed in obtaining young mice after transferring nuclei between unfertilized mouse eggs at the one-cell stage, using virus-induced fusion.

1984
McGrath and Solter fail to clone mice and claim that the cloning of mammals by simple nuclear transfer is impossible.

1986
Willadsen clones a sheep from embryo cells using nuclear transfer. This is the first such success to clearly stand the test of time.

1990
The Human Genome Project officially begins.

1995
Using differentiated cells of sheep embryos, Ian Wilmut and Keith Campbell of the Roslin Institute create Megan and Morag, the world's first sheep cloned from differentiated cells.

1997
Wilmut and colleagues report Dolly, the world's first creature to be cloned from adult cells.

President Clinton proposes a 5-year moratorium on both federally and privately funded human cloning research.

Polly is born at the Roslin Institute. She is cloned from a fetal fibroblast into which had been inserted a gene for a valuable pharmaceutical protein, the human blood-clotting factor IX.

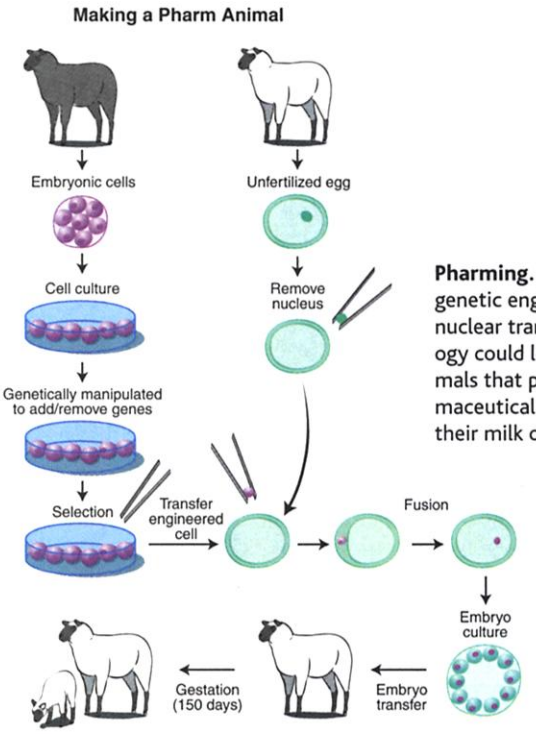
Richard Seed announces plans to clone a human being before regulatory laws could be enacted.

A wealthy Californian seeking to clone his dog Missy funds the "Missyplicity Project."

1998
James Thomson and colleagues report deriving human pluripotential stem cell lines from supernumerary blastocysts donated by patients undergoing infertility treatment involving in vitro fertilization.

John Gearhart and colleagues report the derivation of human embryonic germ cell lines from aborted human fetal material.

The following Web site offers another relevant timeline: library.thinkquest.org/24355/data/timelinenav.html



Pharming. Combining genetic engineering and nuclear transfer technology could lead to animals that produce pharmaceutical agents in their milk or urine.

genetic reprogramming achieved? What are the crucial factors in egg cytoplasm? If telomere length *(17)* is not restored by reprogramming, does it matter? Is the mismatch between nuclear and mitochondrial genes ever a problem? Do some somatic nuclei reprogram more readily than others? Is the prior quiescence of the donor nucleus important? Why are there so many deaths and abnormalities, and so few born alive?

These questions will occupy scientists for many decades. If the cytoplasmic factors in the egg cytoplasm that are responsible for reprogramming are identified, it might be possible to reproduce them in vitro. Perhaps reprogramming could then be brought about without requiring the participation of a mature egg.

As basic research progresses, so too will technology. The genotype of cultured cells can be altered by recombinant DNA technology, including gene targeting to remove or replace genes. The use of nuclear transfer from such cultures to make animals of the desired genotype opens up many new perspectives in animal breeding. Not much is known about the genetics of quantitative characters such as growth and fertility, except that they are complex and probably under the control of many genes. Disease resistance could be an early target, but the most immediate impact of nuclear transfer cloning that we are likely to see will be animals producing valuable pharmaceutical products in their milk or even urine ("pharming"), or producing milk lacking the proteins to which babies are allergic, or milk or meat with enhanced nutritional value (see diagram).

New medical treatments may be the most exciting single outcome of cloning by somatic cell nuclear transfer. If the biomedical uses of pluripotent human stem cells can be realized, cell and tissue therapy for many serious diseases will become available. But because the patients may still reject the transplanted cells, they will have to take immunosuppressive drugs, along with the associated risks of infection and cancer. Maybe the patients could be rendered tolerant, or maybe the cells could be genetically manipulated to make them nonantigenic.

Or maybe not. The alternative would be to use somatic cells from the patients themselves for nuclear transfer, so that the early embryo and any pluripotent stem cells derived from it would be genetically and antigenically identical to the patient, 100% compatible. No question of transplant rejection could then arise. This approach is certainly not on the immediate agenda and would require a fair amount of prior research, but it appears technically feasible and could greatly reduce suffering. Because no reproductive cloning is involved, the ethical objections outlined earlier would not apply.

There would of course still be people who believe that personhood is present from the very beginning of embryonic life, so that using an embryo for any purpose other than making a baby is tantamount to murder. The stroke victim, the multiple sclerosis patient, the person crippled with rheumatoid arthritis may, on the other hand, believe that they have every right to use what are effectively their own cells.

The 21st century will see many deep ethical conflicts, but it will also see unprecedented biomedical advances that will benefit all humankind.

References and Notes

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Anne McLaren, a Principal Research Associate at the Wellcome/CRC Institute in Cambridge, has worked for many years on the genetics, reproductive biology, and developmental biology of mice, first for the U.K. Agricultural and then for the Medical Research Council. She is a member of the European Group on Ethics that advises the European Commission on social and ethical implications of new technologies, and also a member of the Human Fertilisation and Embryology Authority, which regulates in vitro fertilization and human embryo research in the U.K.

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