A Fantastical Experiment

The science behind the controversial cloning of Dolly

By JOHN TRAVIS

In 1938, just a few years before his death, the famous German embryologist Hans Spemann pondered a long-debated idea called nuclear equivalency. Spemann and his contemporaries knew that all animal cells contain a nucleus, the membrane-bound structure that houses a cell's genes.

During the earliest stages of life, when an embryo consists of fewer than a dozen or so cells, the genes inside every nucleus have their fullest potential. Each embryonic cell is, in the jargon of biologists, totipotent: It has the ability to give rise to cells that make up the eyes, the liver, the brain, or any other part of an adult animal.

Yet as an embryo develops, cells lose this ability. By ordering genes to turn on or off, cells begin to specialize. One group of embryonic cells may commit itself to constructing the nervous system, while another acts to create muscles.

This gradual specialization of cells, also known as differentiation, poses a provocative question. Does a nucleus from a differentiated cell retain the know-how to construct an entire organism? Scientists have generally dismissed this idea of nuclear equivalency, suggesting that as cells specialize, they irreversibly alter their DNA or even discard whole genes.

Spemann wasn't so sure. In a 1938 article, he suggested an experiment that might resolve the debate: Remove the nucleus from an unfertilized egg and replace it with one from a differentiated cell.

This "fantastical" experiment, as Spemann called it, lay beyond the technological reach of his time. Yet today, almost 6 decades after Spemann proposed the strategy of nuclear transplantation, Scottish scientists have startled the world by using that process to create a lamb named Dolly (SN: 3/1/97, p. 132).

With this first cloning of a mammal from an adult nucleus, the fantastical has become reality, and biologists are struggling to explain Dolly and to discover whether the method used to make her can be put to practical use.

One of the first issues raised by Dolly's birth is the troublesome question of what is meant by "cloning."

"Cloning is not really a scientific word, and unfortunately it has many, many meanings," notes W. Richard Dukelow of Michigan State University in East Lansing, who conducts in vitro fertilization research in nonhuman primates.

In its most common use, cloning means creating an organism genetically identical to another organism. Some scientists use the word to include a procedure more accurately called embryo splitting (SN: 2/5/94, p. 92). In this process, which has been available to animal breeders for decades, scientists take a young embryo, still composed of totipotent cells, and divide it in half or thirds or quarters. Each of these portions can give rise to a normal animal, so the procedure creates twins, triplets, or quadruplets.

The experiment Spemann envisioned—replacing the nucleus of an unfertilized egg with the nucleus from another cell—is what most scientists refer to as cloning. Nuclear transplantation is nothing new, notes Robert M. Moor of Babraham Institute in Cambridge, England, who 2 years ago reviewed the field in CURRENT TOPICS IN DEVELOPMENTAL BIOLOGY.

In 1952, scientists working with frogs transferred nuclei into unfertilized eggs whose own nuclei had been removed. Those first experiments used donor nuclei from early frog embryos, whose cells were thought to be totipotent. The resulting frogs made it only to the larval stage before dying.

Since then, other scientists have perfected the approach in a variety of species—frogs, mice, sheep, cows, and most recently monkeys (SN: 3/8/97, p. 142). In each case, when a nucleus from a very early embryo replaces an unfertilized egg's nucleus, the offspring generated grows into a fertile adult.

The feat is typically performed by removing the egg's nucleus with a fine, hollow needle. A donor cell is fused to the egg by pulses of electricity, which break down the donor cell's outer membrane and allow the egg to envelope its new nucleus.

While transplantation of early embryonic nuclei proved successful, researchers almost always failed with transfers of older nuclei—those from the cells of a late-stage fetus or an adult. Such results seemed to confirm that the nucleus undergoes irreversible changes during development.

A few experiments provided hints to the contrary, however. Notably, in the late 1950s and early 1960s, John B. Gurdon of the University of Oxford in England showed that nuclear transplants from tadpole cells could produce fertile adult frogs. He also established that transplants of nuclei from adult frogs generated tadpoles, though the animals died before fully maturing.

Gurdon interpreted his results as proof that nuclei of differentiated cells can regain their totipotency.

In light of this rich history of the nuclear transplantation field, the cloning of Dolly from an adult cell should come as only a "mild surprise," argues Matthew P. Scott, president-elect of the Society for Developmental Biology.

Still, how did the Scottish scientists manage to create Dolly, when attempts to clone mice from adult cells have invariably failed and even Gurdon's frog experiments were not completely successful?

The key to nuclear transplantation with adult cells seems to be a phenomenon that scientists call either nuclear reprogramming or chromatin remodeling. "Chromatin is really just a word for the mixture of proteins and DNA and small molecules that constitutes the substance of chromosomes," explains Scott.

While it seems that most nuclei do not suffer irreversible changes during differentiation, researchers have found that chromatin inside the nucleus of an adult cell differs considerably from that inside an egg cell or an early embryo. As cells differentiate, clusters of atoms, such as methyl groups, latch onto DNA; some of these clusters deactivate specific genes on chromosomes. In addition, some of the proteins forming the core of chromatin appear to change over time.

Consequently, for an adult nucleus to behave like a totipotent one requires the genetic equivalent of the Fountain of Youth. Such a rejuvenation formula seems to exist inside the egg cell. Proteins and other molecules floating within the egg can interact with a transplanted nucleus to restore its vitality, says Alan P. Wolffe of the National Institute of Child Health and Human Development in Bethesda, Md.

The specific molecular mechanisms by which an egg exerts this influence on

a nucleus are just beginning to be revealed. In the Nov. 1 EMBO JOURNAL, for example, Wolffe and a colleague describe how one egg protein induces the nucleus of an adult frog cell to shed certain chromatin proteins and incorporate others more suited to an egg nucleus. This exchange of proteins turns on several genes that had been shut down formerly.

To clone an animal from an adult cell, scientists had to find ways of helping the egg with its nuclear reprogramming. That's exactly what Ian Wilmut and his colleagues at the Roslin Institute in Edinburgh, the team that created Dolly, believe they've found.

Their trick is to starve the adult cells. The strategy, developed by Wilmut's colleague Keith H.S. Campbell, involves depriving the intended donor cells, mammary cells in the case of Dolly, of almost all nutrients for 5 days.

This diet induces the cells to abandon their normal cycle of growth and division and enter a quiescent stage. Such cells appear to have few, if any, genes activated, and Wilmut suggests that the starvation prompts some initial chromatin remodeling that makes it easier for the egg cell to finish the job.

Nonetheless, Wilmut and other researchers warn that Dolly could still turn out to be a fluke. After all, the Roslin group fused 277 donor cells to eggs and produced just one viable animal.

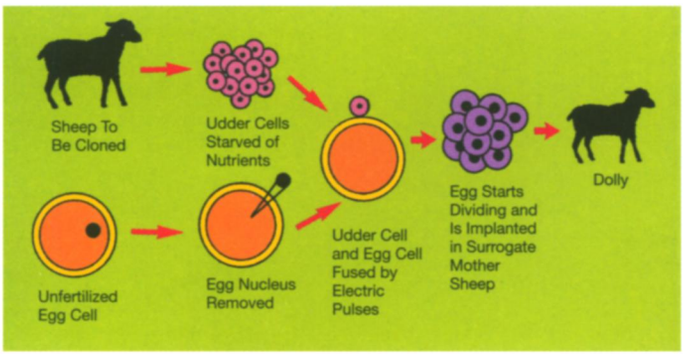
Nutrient deprivation "is the only aspect of the whole study that is novel to Wilmut's group. It might be the key, but when you have only one young out of hundreds of tries, it's difficult to know," says Moor.

Why have Wilmut and his colleagues persisted for years in attempts to create cloned sheep from embryonic, fetal, and now adult cells? To a large extent, commercial interests motivated the effort. The cloning research was funded by PPL Therapeutics, a Scottish biotech firm interested in genetically altering female animals to secrete valuable drugs into their milk. By adding human genes to sheep, for example, the company has already created animals that provide large, easily recoverable quantities of a protein that holds promise in the treatment of cystic fibrosis.

This strategy has been around for years, but companies such as PPL have faced a major hurdle in creating drug-producing animals. The traditional method is to inject multiple copies of a human gene into a newly fertilized egg—an approach that resembles playing roulette, however. The injected genes rarely integrate properly into the egg's genome, and few drug-producing offspring result.

Wilmut and his coworkers hope to have laid the foundation for a more efficient approach to genetically altering animals. In their cloning method, there is a short period during which researchers can grow donor cells in the laboratory before transplanting the nuclei into eggs. During that time, Wilmut's group plans to manipulate the cells, either adding or deleting genes. The scientists will then transplant only the nuclei of cells that have been effectively altered, thereby dramatically increasing their odds of creating a commercially useful animal. They might then use that animal to create a herd, cloning it over and over again.

In addition to drug-producing livestock, new animal models of human diseases may result from the development of this cloning technology. Wilmut told a Senate hearing last month that he is particularly interested in adding to large animals the mutant human gene that causes cystic fibrosis. "Sheep will provide an outstanding model for this disease," he testified.



The strategy that made Dolly.

Another much-discussed use of cloning is to tailor animals, most likely pigs, so that their organs can be transplanted into people. Controversial for many reasons, this technique depends upon finding ways to make animal organs appear less foreign to the human immune system (SN: 11/4/95, p. 298). With an effective cloning technique, researchers might delete from pigs the genes whose proteins tell the human immune system to reject the foreign organs.

On the most speculative front, some scientists suggest that the recent cloning work may provide medically useful information about reprogramming the genes in cells. Such knowledge might enable them to produce whole organs from undifferentiated cells or to trick cancer cells into assuming a less harmful form.

Wilmut has repeatedly stressed that cloning technology is still in its infancy and that many questions remain unanswered.

In one sense, Dolly isn't even a true clone—she does not share all of her genes with her donor. As many scientists have pointed out, a few dozen genes reside in the energy-producing organelles called mitochondria. Since only the nucleus of an adult ewe cell was transferred to the egg, Dolly's mitochondrial DNA comes from the recipient egg.

Wilmut and his colleagues do not know whether this mixing of genes is important. Nor do they know whether Dolly will be fertile or have a normal life span.

Regarding the latter concern, the nucleus that created Dolly was 6 years old. It's unclear whether the chromatin remodeling of the transplanted nucleus reset its age. If it didn't, Dolly's life might be historic but brief.

Moreover, Dolly was the only survivor of 277 cloning attempts with adult sheep cells. One explanation for the group's low success rate may be the electric pulses that trick the egg into developing, says Moor. These pulses don't always trigger the same signals inside an egg that a sperm's arrival does, he and other researchers have found.

The most obvious unsettled issue surrounding Dolly is whether she is a precursor to human cloning. From the very beginning, Wilmut has stressed that there is no reasonable justification for cloning humans. Moreover, given the current lack of knowledge about how cloning works, it would be too dangerous to try, he says. "In the past, we have had lambs that were born that were quite deformed."

Fundamental scientific barriers could prevent human cloning via nuclear transplantation, Wilmut and other scientists suggest. One may be the differing speeds at which developmental events occur among species. A developing embryo doesn't immediately make use of its genes, for example. During the first few cell divisions, it depends upon proteins and genetic instructions the unfertilized egg had already prepared.

When an embryo switches on its genes varies among species, notes Richard M. Schultz of the University of Pennsylvania in Philadelphia. Mouse embryos activate their genes quickly, sheep wait much longer, and human embryos fall somewhere in between, he explains.

The sheep embryo's relatively late activation of its genome may have been crucial to the success of cloning Dolly because it allowed more time for the transplanted nucleus to be reprogrammed. Human embryos may not provide enough time for this chromatin remodeling, thus preventing the cloning of humans using adult cells, says Schultz.

This potential limitation may offer solace to people who do not want to address the ethical dilemmas of human cloning and may allay fears that this fantastical experiment will soon become routine throughout the animal kingdom. □