Mini-Review **MR-143E**

THE DOLLY PHENOMENON: "CLONING" IN MAMMALS

Thomas Curran
Science and Technology Division

3 April 1997
Revised 30 May 1997

Library of Parliament
Bibliothèque du Parlement

Parliamentary Research Branch

The Research Branch of the Library of Parliament works exclusively for Parliament, conducting research and providing information for Committees and Members of the Senate and the House of Commons. This service is extended without partisan bias in such forms as Reports, Background Papers and Issue Reviews. Research Officers in the Branch are also available for personal consultation in their respective fields of expertise.

N.B. Any substantive changes in this publication which have been made since the preceding issue are indicated in **bold print**.

CE DOCUMENT EST AUSSI PUBLIÉ EN FRANÇAIS

CANADA

LIBRARY OF PARLIAMENT
BIBLIOTHÈQUE DU PARLEMENT

THE DOLLY PHENOMENON: "CLONING" IN MAMMALS

INTRODUCTION

On 27 February 1997, a research paper was published in the journal *Nature*. The senior author is Dr. Ian Wilmut of the Roslin Institute, in Roslin, Scotland, near Edinburgh. Two of Wilmut's colleagues are employed with the private firm PPL Therapeutics, also located in Roslin. The paper describes how a healthy lamb was produced using an adult mammalian cell, the first time this has been achieved. The news that scientists had apparently had "cloned" a sheep, to produce a Finn Dorset ewe named "Dolly," generally dominated the coverage in all news media for the next several days. The dominant aspect of much of the reporting - apart from an avalanche of mostly bad puns - was that the "cloning" of humans might soon be possible.

GENETICS AND INHERITANCE

Genetics is the study of heredity and variations in organisms. Until well into the present century, this aspect of biological science was conducted at the "macro" level, through the observation and selective breeding of animals and plants. In the 1860s, Gregor Mendel had formulated a concept of the *gene* as the unit of inheritance, although that term was not coined until the early twentieth century when Mendel's studies of inheritance in plants were re-discovered, repeated and extended. Since the 1950s, the science of genetics has expanded exponentially. With the elucidation of the structure of *deoxyribonucleic acid* (DNA) in 1953 by James Watson and Francis Crick a seminal event in what is now known as *molecular genetics*, the groundwork was laid for the very rapid expansion of the understanding, and manipulation of inheritance that has taken place in the past four decades.

The functional unit in living organisms is the *cell*, a microscopic entity on which is based the many structures and activities of an organism. The organism's hereditary material, DNA, is found within the cell. In higher organisms, most, but not all, of the hereditary material is contained in a discrete cellular body, the *nucleus*. The DNA in the nucleus is organized into *chromosomes*, the number of which varies with the species. In animals (and plants), DNA also is found in highly specialized bodies in the cell, outside of the nucleus, known as *mitochondria*. The mitochondria function to combine oxygen with nutrients to release useable chemical energy for essentially all the functions of life. In animals, mitochondria are inherited only from the mother through the egg cell; sperm cells do not contain mitochondria. It is known that mitochondrial DNA can influence development. The DNA in mitochondria also has been linked to certain genetic diseases that afflict humans.

Most of the cells in the body are called *somatic* cells. In these cells, which are in the *diploid* state, the chromosomes are paired in the nucleus: human cells, for example, contain 46 chromosomes in 23 pairs. A second specialized category of cell includes the sex, or *germ*, cells - the eggs and the sperm. These cells contain only one of each chromosome and are referred to as *haploid* - human germ cells, therefore, contain 23 chromosomes. When, during reproduction, fertilization takes place, a sperm and egg cell fuse to produce a diploid cell with two sets of chromosomes, one set from each of the parents. This newly created diploid cell - the *zygote* - divides and forms an embryo; after many more cell divisions, the embryo becomes a fetus, and so on.

In the very early stages after fertilization, each of the cells of the embryo has the capacity to develop into all of the specialized cells that make up an adult animal; such cells are described as being *totipotent*. In contrast, adult cells - nerve cells, liver cells, etc. - have become specialized, or *differentiated*, to carry out a specific task or function. Although these cells carry the full complement of genetic information from the zygote, most of the genes have been "switched off." Until very recently, it was believed that adult cells could not be reprogrammed to act as embryonic cells.

THE ROSLIN EXPERIMENTS

The experiments carried out at the Roslin Institute built upon information and technologies that had been developed by many scientists over many years. The production of identical individual animals through human intervention is at least two decades old. In the 1970s, identical copies of mice, sheep and cattle were produced by physically splitting embryos at a very early stage into individual cells and implanting those cells into surrogate mothers. The number of identical individuals that could be produced in this way was very limited because only the totipotent cells from the very early embryo could be used.

In 1986, the technique of *nuclear transfer* in livestock was developed and this also permitted a number of identical individual animals to be produced. In one application of this technique, the nuclei were removed from sheep eggs and these *enucleated* eggs were fused with totipotent cells harvested from a second sheep's embryo. The fused cells were implanted in surrogate ewes and carried to term. The resulting sheep were essentially identical to each other and directly related to the parent sheep that had produced the embryo from which the totipotent cells were taken.

In 1995, the scientists from the Roslin Institute advanced the technology further. In a paper published in 1996, the authors described how five lambs were born from individual cells derived from a culture (population) of embryonic cells that had been growing in the laboratory for up to 13 generations and fused with enucleated eggs. Two of the five lambs survived beyond 10 days and grew into apparently normal healthy animals. For the first time, the Roslin scientists were able to bring about the birth of viable offspring after nuclear transfer from embryonic cells that were not fresh but had been growing in culture, and also were showing signs of differentiation. This was achieved, in part, by inducing the embryonic cells to become "quiescent" through nutrient deprivation. Induction of the quiescent state appears to be key in enabling the cell nucleus to be reprogrammed so that it will behave like a totipotent cell.

In the most recent paper from the Roslin Institute, enucleated eggs were fused with individual cells from three populations of older cells: (1) from a 9-day old embryo, (2) from a 26-day old fetus, and (3) from the mammary gland of a pregnant six-year old Finn Dorset ewe. In each case, the cell line used had been induced to become quiescent to facilitate reprogramming. In total, eight live lambs were born, but one died immediately after birth. Of

the seven survivors, the single live birth from the adult mammary gland cell - Dolly - clearly is the most interesting. The Roslin team apparently has developed a technique to induce an adult cell to behave like a totipotent embryonic cell, and to develop into a healthy animal.

RAMIFICATIONS OF THE DOLLY EXPERIMENT

The word "clone," which has been used to describe Dolly, has come to mean something different from its original, scientific definition. In the popular definition, a clone is an individual - person, plant or animal - that copies, or closely resembles, another in appearance or in function. In biological science, a clone is precisely defined as a population of genetically identical organisms, cells, viruses or DNA molecules, derived from the reproduction of a single progenitor, by *asexual* means. Many plants are easily cloned: a cutting or a leaf may be rooted and grow into a mature plant. The cloning of animals is much more difficult.

Shortly after the news about Dolly was released, it was announced that scientists at the Oregon Regional Primate Research Center had "cloned" two monkeys from embryonic cells - *not* from adult cells - the first time that this had been done with a primate species. Scientists at the Center have stated that they do not plan to produce "clones" from adult monkey cells.

Although Dolly represents a brilliant scientific advance, she is not, in precise scientific terms, a "clone" of the Finn Dorset ewe from which the adult cell was obtained. Although the nuclear DNA in "mother" and "daughter" are essentially identical (allowing for the possibility of chance mutations), Dolly carries in her cells the mitochondrial DNA of the Scottish Blackface ewe from which the egg was obtained, *in addition to* the mitochondrial DNA of the Finn Dorset ewe. Therefore, Dolly may be said to be something less (or something more) than a clone.

It has been pointed out that the success rate in the Roslin studies was very low: only one apparently normal embryo resulted from 277 nuclear transfers. The reason(s) for the high failure rate is not known. Although Dolly appears to be normal and healthy, it is not known if she will continue to develop as a normal sheep, and if she will be fecund and able to breed normally. Concerns also have been expressed that her "six-year old chromosomes" may bring about accelerated aging. It is also not certain that the technique that worked with sheep will work with other species. Whatever may be the ultimate fate of Dolly, however, the technology to produce animals from adult cells may well become routine in this century.

Concerns about attempts to "clone" humans from adult cells are appropriate, although governments, and people generally, appear to be unanimous in their opposition to such a procedure. No-one has offered any ethically acceptable reason for cloning in humans. The suggestion that humans might be cloned to provide "spare parts" for their progenitors has been roundly condemned. In Canada, Bill C-47, the Human Reproductive and Genetic Technologies Act, which died on the Order Paper when the 35th Parliament was dissolved in the spring of 1997, would have prohibited the cloning of human beings in this country. While there is no prohibition against cloning animals in Canada, animal-rights groups and others might well view the process as unethical and harmful to the animals involved.

The Roslin researchers have expressed no interest in applying their technology to humans. Their hope is that the production of "cloned" offspring in farm animal species may some day "provide enormous benefits in research, agriculture and biotechnology." One approach would be to develop lines of animals that would secrete large quantities of useful proteins in their milk for the treatment of human diseases. Also, the increased understanding of genetics and embryology that underwrote the production of Dolly from an adult cell offers dividends in the study of human reproduction and of gene-linked diseases such as cancer.