What's all the fuss about?

Why did the announcement of the birth of Dolly the sheep in 1997 cause such an interest in the scientific community? Why was there such a huge public interest in this scientific story?

Dolly was the first mammal ever to be cloned from an adult cell. She was produced by transferring the nucleus from an adult differentiated cell into an enucleated fertilised egg by a process called **nuclear transfer**. From a scientific view point her birth represented a remarkable breakthrough because it showed that the nucleus from a specialised cell could lose its specificity and function like the nucleus of a newly fertilised egg.

To the media, she represented the dawn of an era in which human cloning became a possibility. Here we will explore how Dolly came about and the implications of her being.

Background

An adult mammal, such as a sheep or a human, is made up of many trillions of cells. There are about two hundred different types of cells including epithelial cells which line organs, bone cells, muscle cells, white blood cells which are responsible for the immune system and nerve cells. Each of these cell types is derived from a single cell, the fertilised egg or **zygote**.

Initially the zygote divides to form a ball of identical cells. Differences soon arise between various cells as they and their descendants become set along a particular developmental pathway. Once a cell has **differentiated**, for instance into a kidney cell, it remains a kidney cell and does not normally change into another type of cell. Differentiated cells do not normally undergo further division but some, such as melanocytes or cartilage cells, do divide. When this happens, the new cells are of the same type as the parent cell.

Once differentiation has occurred it tends to be stable and to be inherited through a number of cell generations, even when cells are cultured in the laboratory. When certain tissues are wounded the cells that remain may undergo a partial reversal of the processes of differentiation known as **dedifferentiation**. The dedifferentiated cells then divide to form a mass of cells called a **blastema**. These cells then redifferentiate to participate in the regeneration of the tissue. In most cases cells redifferentiate into the same cell type as those that gave rise to the blastema.

As cells differentiate they may gain or lose organelles and change in shape, ultrastructure and behaviour. Some cells synthesise specific substances: for example mammary gland alveolar cells synthesise lactoglobulin and erythroid cells make haemoglobin. The differences in composition and metabolism of tissues mainly result from from different genes being switched on or off in different cells. Regulation of protein synthesis mainly by controlling the synthesis of mRNA is the key to cytodifferentiation and each cell type synthesises mRNA from only a selection of its complement of genes.

Most differentiated cells continue to carry the organism's full genetic code. Dolly could only be produced if a way could be found to completely dedifferentiate an adult cell and make it "forget" its developmental past.

In 1975, a developmental biologist in Cambridge, John Gurdon, produced tadpoles by transferring nuclei from cultured, adult amphibian keratinocytes into enucleated eggs (keratinocytes are epidermal skin cells that make keratin). Although this involved differentiation into complex tissues and organs, the tadpoles did not develop into adults, leaving open the question of whether a differentiated adult nucleus could be fully reprogrammed.

How Dolly was made

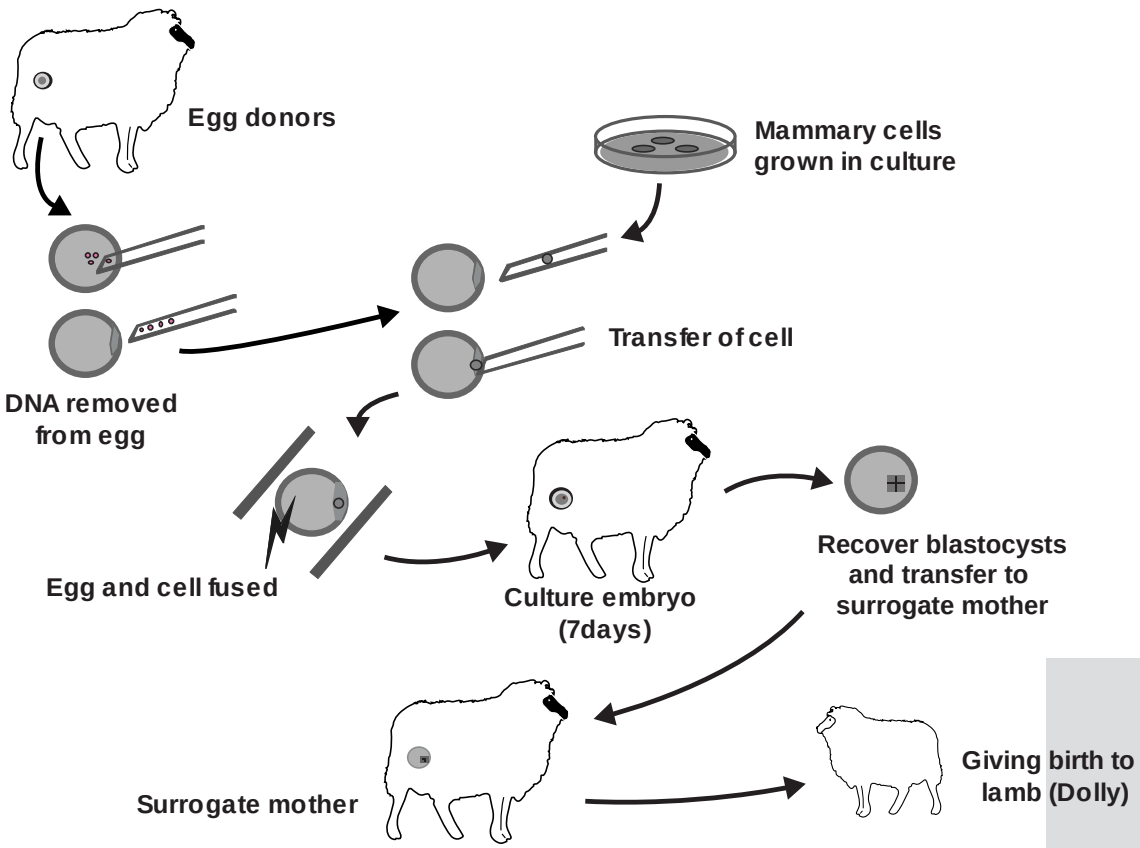
The key to producing Dolly came from understanding the intricacies of the cell cycle and choosing nuclei from cells in the appropriate stage of the cell cycle as donor nuclei.

Cells were taken from the mammary gland of a 6-year-old Finn Dorset ewe and allowed to divide in culture. Starving the cells caused them to exit the growth cycle and arrest in G0. This is a stage in the cell cycle during early interphase before DNA synthesis takes place. Cells in G0 are described as **quiescent**, having entered a "resting" phase during which protein synthesis is reduced to about one fifth its normal level.

The cytoplasm of the unfertilised egg is thought to contain factors that "reprogramme" nuclear DNA. These normally act on sperm DNA but nuclear transfer experiments suggest they can also "reprogramme" DNA from somatic cells. Scientists think that the nuclei of somatic cells that are resting rather than actively dividing are more amenable to being reprogrammed, though they don't know why.

Individual mammary gland cells in G0 were fused with unfertilised, enucleated eggs from Scottish Blackface ewes by electric pulsing, which also activates the egg and starts the development process. Two hundred and seventy seven of these 'reconstructed eggs', each now with a diploid nucleus from an adult animal were generated. Rather than implant each of these into individual ewes, to reduce the number of animals required, the eggs were cultured for 6 days in the oviducts of receptive ewes. Twenty nine of the eggs that appeared to have developed normally to the blastocyst stage were implanted into 13 surrogate Scottish Blackface ewes. One became pregnant and delivered a live lamb, Dolly, 142 days later.

Signals from the cytoplasm of the egg "reprogrammed" the nucleus. It no longer expressed itself as the nucleus of a mammary gland cell, but as a nucleus from a newly formed zygote.



KEY TERMS

nuclear transfer
zygote
differentiation (cytodifferentiation)
dedifferentiation
melanocyte
blastema
enucleated egg
quiescence

1. If a fully differentiated mammary gland cell were to divide, what type of cell would you expect to be produced?

2. Donor cells were "starved", what does this term mean here and why do cell biologists use this technique?

3. Why did the egg containing the donated nucleus develop into a whole new animal rather than a ball of mammary gland cells?

4. Normally penetration of the egg by the sperm cell signals the start of the developmental process. What can replace this signal when no sperm are involved?

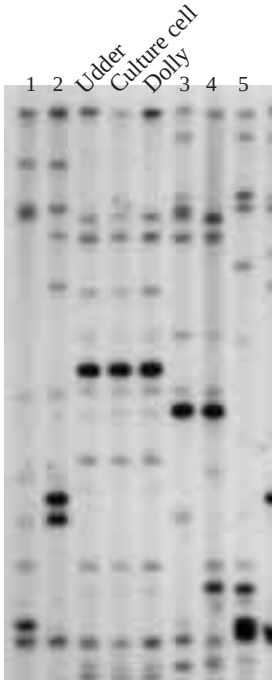
5a. During the set of experiments which resulted in Dolly being born, what percentage of 'reconstructed eggs' resulted in live lambs?

b. Comment on the commercial viability and ethics of this process.

6. Dolly is not an exact genetic copy of the Finn Dorset ewe whose mammary cell was used to create Dolly - explain (hint - mitochondria).

Proving Dolly was what she was claimed to be

Following her birth, there was some controversy over Dolly's authenticity. The donor cell was taken from a culture of mammary cells, which, it was acknowledged, could contain both stem cells as well as terminally differentiated epithelial cells. It was suggested, however, that a culture of embryonic derived cells was used by mistake. Since the donor animal was pregnant when cells were collected there was also a remote possibility that cultures made from mammary tissue might also be contaminated with stray circulating foetal cells. Two approaches, **DNA fingerprinting** and **microsatellite analysis** were taken to quash any further speculation that the donor nucleus might have come from foetal or embryonic tissue.



DNA fingerprint analysis

DNA from Dolly's cells was compared with DNA from the cultured mammary cells used in the nuclear transfer experiments. The results of the fingerprint analysis are shown on the right:

Lanes 1,2,3,4 and 5 contain DNA derived from members of the same flock of Finn Dorset ewes as Dolly's donor.

4 minisatellite probes were used in this **DNA fingerprint analysis**.

Microsatellite analysis

DNA from Dolly, the mammary tissue from which the donor cell was derived and cells from the same culture as the donor cell were all subjected to **microsatellite analysis** using 10 different microsatellite markers. The results are summarised in the table below:

Marker	Number of allelles found in Finn Dorset population	Alleles present in cells from Dolly, mammary tissue and cultured cells (size in base pairs)
TGLA53	7	151/151
SPS115	4	248/248
TGLA126	7	118/126
TGLA122	9	190/190
ETH3	4	104/106
ETH225	4	148/150
FCB11	6	124/126
MAF209	4	109/121
FCB128	5	112/112

KEY TERMS

DNA fingerprinting
microsatellite analysis

Further information in the Student Support Notes may help you answer the following questions

1. Does the DNA fingerprint supports or dispute the claim that Dolly is derived from the nucleus of a Finn Dorset udder cell? Explain.

2. The DNA fingerprint analysis includes fingerprints of Finn Dorset sheep from the same flock as the donor ewe. What do these show and why were they included?

3. Apart from the 3 experimental lanes, a special relationship exists between 2 other animals. Which lanes contain this DNA and can you suggest how these animals are related to each other. Why include these samples?

4. Why are two sizes given for each of the microsatellite probes? What does it mean if both of these are the same?

5. How do the results from the microsatellite analysis dispute the allegation that Dolly was derived from a stray fetal cell?

6. Which is the most polymorphic of the markers used in the microsatellite analysis of Dolly? Why is it better to use markers which are highly polymorphic in this type of investigation?

Dolly has a little lamb

Dolly has produced normal lambs on mating with a Welsh Mountain ram.

Is Dolly ageing prematurely?

One concern raised was that Dolly might age prematurely because she was derived from a somatic cell rather than a germ line cell. This concern comes form the observation that as normal somatic cells age, changes take place in their DNA.

At either end of every strand of DNA from eukaryotic cells there are special sequences of DNA. These are called **telomeres**. In most vertebrates, telomeres are short G-rich sequences: 5'- TTAGGG-3' repeated hundreds or even thousands of times. At each cell division some of these repeated sequences are lost (on average 40 - 200 base pairs are lost per division per cell). Their function is not entirely clear but telomeres are believed to act as "protective caps" on the ends of chromosomes and are important for replication and the stability of the chromosome.

The Hayflick limit states that there is a finite limit to the number of cell divisions a normal somatic cell can go through. Normal human fibroblasts for example, stop dividing after 50 generations *in vitro*. This phenomenon is thought to be due to the erosion of telomeres.

Telomeres are believed to shorten during DNA replication because normal **DNA polymerase** does not completely replicate the ends of linear chromosomes. A special DNA polymerase enzyme called **telomerase** does a more complete job. Unfortunately, telomerase is in short supply in normal somatic cells. Germ cells (cells which produce sperm and eggs) have a much higher level of telomerase activity. This is thought to explain why their telomeres are much longer than those found in somatic cells and may explain why telomere length does not normally change from one generation to the next.

Telomeres usually measure 24 units in the cells of one year old ewes. When she was one year old Dolly's telomeres measured only 19 units, closer to the size expected of a seven year old ewe. In all other respects Dolly functioned and appeared like a normal one year old ewe. Dolly is now 4 years old (year 2000) and has remained healthy.

Potential uses of nuclear transfer

- Animal breeders could use nuclear transfer to rapidly expand numbers of elite animals generated using traditional breeding systems.

- Transgenic sheep and cattle are already used to produce valuable human pharmaceutical proteins in their milk and transgenic pigs are being developed as donors of organs in human transplantation. Until now these animals have been made by adding DNA by injection directly into eggs. This is inefficient because genes are not directly targetted and there is no guarantee they will be expressed by the correct cells. New technologies make it possible to perform much more sophisticated manipulations of DNA in cultured cells so genes are directly targetted. The nuclei from cells with the desired modifications can then be used as donors in nuclear transfer experiments to generate genetically modified cattle, sheep and pigs.

- Cloning could be used in conservation programmes. Blood samples, skin biopsies and other cell types could be collected of endangered breeds of animals, grown in the laboratory, then frozen in liquid nitrogen for long term storage prior to nuclear transfer as required. More information at http://roslin.ac.uk.

Ethical/moral issues and concerns

Points for consideration here include the following:

- Animal welfare - do the experimental procedures used in cloning research cause undue suffering to the animals involved? How do we judge what is 'undue'?
- Religious concerns - is it blasphemous to manipulate DNA and in some cases cross species barriers?
- Unnatural - is modern biotechnology unnatural in that it goes against and interferes with Nature, making it intrinsically wrong? Compare with traditional selective breeding methods.

Several concerns regarding animal biotechnology more generally have been raised. It is important to decide which of these are justified and which aren't. Some of these concerns are explored below:

- Animals engineered to grow faster might produce unexpected and harmful results for those who eat foods derived from such animals. This enhanced growth rate may also have welfare implications for the animals. There is currently controversy over the use of milk and meat produced in the USA by injecting cattle with growth hormone. It could be argued that cloning is the safer option.
- Widespread use of cloning might narrow the gene pool and reduce genetic diversity, so producing livestock which could be vulnerable to new diseases or other environmental threats. On the other hand cloning could be used to amplify numbers of breeds on the brink of extinction.
- Animals engineered in biomedical research to be models of human diseases might escape and introduce genetic disorders on to wild populations. Of course this is not a problem peculiar to animal models produced by genetic modification techniques.
- Organs from genetically modified animals might transmit viral diseases if used in human transplant surgery. Pig organs, for example, contain viral DNA which do not harm the pig, but may affect humans. Others which affect pigs but not humans are also of concern here. Many safety checks must be carried out before these organs are used on patients.
- Genetically modified animals might escape into the environment. Transgenic salmon designed to grow at a faster rate might out compete normal salmon for scarce food if they escape into the wild. Ecological problems can of course be caused by other human activities as well such as burning fossil fuels.

Moral and ethical issues are dealt with fully in the BBSRC publication:
Ethics, Morality and Animal Biotechnology. by R. Straughan (available free of charge from the BBSRC, Polaris House, North Star Avenue, Swindon SN2 1UH

KEY TERMS

somatic cell
germ cell
telomere
DNA polymerase
transgenic
genetic diversity

1. Why was it important to show that Dolly could breed normally?

2. What are "telomeres" and why is it not surprising that when she was one year old Dolly's telomeres were about the same as that expected for a 7 year old ewe?

3. Why are the scientists working on Dolly not overly concerned by the revelations concerning Dolly's telomeres?

4. Why might a farmer using embryos produced by nuclear transfer from cows in elite herds see a much bigger jump in performance of the herd compared with a farmer using artificial insemination?

5. Discuss the moral and ethical issues raised by genetic modification and nuclear transfer techniques.