


**Further reading**


**Biography**

David King is Coordinator of Human Genetics Alert, a watchdog group focusing on ethical and social issues raised by human genetics. He was formerly editor of *GenEthics News* and Director of the Genetics Forum. He has a PhD in molecular biology from Edinburgh University and a BA from Christ's College, Cambridge. He is a member of the Ethics Group of the North Cumbria Community Genetics Project. He has written for many publications and is a regular contributor to TV and radio current affairs programmes on genetics.

7 Clones and cloning

New reproductive futures

Sarah Franklin

The issue of cloning attracted increasing public attention in the late 1990s, in large part due to the production of Dolly the sheep at the Roslin Institute in Scotland in 1996 by Ian Wilmut and his team of scientists working in close collaboration with Scotland's leading biotechnology company, PPL Therapeutics. The novel technique used to produce Dolly confirmed the viability of a new kind of biological reproduction by successfully merging an adult cell from one female sheep (a mammary cell) with an egg cell from another sheep to create a fully viable embryo. Before the cloning of mammals in the 1990s such a means of reproduction was considered out of the question because it was assumed that an egg cell could only be fertilised by a sperm and when cloning was achieved it could only materialise through the fusion of a young fetal cell with an enucleated egg cell. Using sophisticated techniques of molecular engineering and micromanipulation, Wilmut's team produced a live, healthy offspring, Dolly, whose viability confirmed the utility of a process known as somatic cell nuclear transfer (CNR).

Somatic cells are distinguished from germ cells because they are the differentiated cells that make up all of the distinct types of tissue in the body. A key principle of biological development that was challenged by the success of the Dolly experiment is the assumption that it is one-way and irreversible. In other words, it was assumed that once a cell committed itself to a particular developmental pathway, by specialising to become a specialised cell type, it could not, as it were, 'go back in time' to provide the complete set of genetic instructions necessary to produce a new embryo. This function was previously believed to belong exclusively to germ cells, that is, only to eggs and sperm.

A key discovery enabling adult, differentiated cells to serve as partner cells to germ cells involved the role of cell cytoplasm. Cytoplasm is often described as the gelatinous 'soup' in which all of the cell's distinct organelles, such as mitochondria, ribosomes and the nucleus, are immersed. The cytoplasm of egg cells is particularly powerful, in part because the egg cell (ovum) is the largest of all mammalian cells — 100 times larger, for example, than the mammmary cell with which it was fused to create Dolly. After slowing down an ovum and a mammmary cell to the G0 phase of their cycles, known as quiescence, and equivalent to cellular sleep, Roslin scientists injected the entire mammmary cell from a Finn
Dorset ewe into an ovum from a Blackface ewe, from which the nucleus, containing its nuclear DNA or genetic blueprint, had been removed. Using an electrical apparatus to restart the cell cycle, jolting the newly united cells out of their sleep phase, a new, reconstructed cell was capacitated, developing to become an embryo, which was carried by two additional surrogate sheep during gestation. The viability of this new technique of cellular merger was confirmed when Dolly was born as a healthy and normal lamb, although in 277 other cases the same technique failed.

According to Ian Wilmut, the cytoplasm of the egg cell is so powerful it can, in effect, ‘reset the clock’ of adult DNA, restoring its original capacities to provide the instructions for any kind of cell – and indeed all of the cells necessary to create a viable offspring. The analogy used by Wilmut is that the egg cell cytoplasm ‘reprogrammes’ adult DNA. It is this capacity to reprogramme one kind of cell to become another that has been seen to offer significant possibilities for growing replacement tissue, such as liver, skin or other organs, which have both medical and commercial potential in the field now known as regenerative medicine.

What has come to be called ‘the Dolly technique’, also known as nuclear transfer technology, is increasingly widely used to create what are described as ‘reconstructed cells’. These cells are in turn used to create cell lines and cell cultures which have a wide range of potential applications. Among these is the potential for an individual’s own cells to be used to produce spare parts in the event of major illness or organ failure. Whereas, in the past, significant problems of tissue rejection have hampered the progress of transplantation, the new techniques offer the possibility of a kind of bespoke medicine which uses a person’s own tissue to create new means of repairing damaged organs. For example, skin tissue could be used to create replacement heart tissue – and in a manner that bypasses the problem of rejection, because the new cells are a perfect tissue match (histocompatible).

Cellular reconstruction techniques, or what is now known as the field of tissue engineering, is complex and at an early stage, so that it is anticipated many of the benefits of this new field will not be available for at least ten, if not fifty, years. Moreover, the field is very controversial, because experimentation requires the use of both adult and embryonic cells (e.g. Lovell-Badge, 2001). For some religious groups, the use of human embryonic tissue is unacceptable under any conditions – even if, for example, the embryos come from in vitro fertilisation programmes, and are available for research because couples have donated the embryos they no longer wish to use or keep in storage (Franklin, 1999). In contrast, many members of the medical and scientific professions have lobbied intensively, and in Britain successfully, to allow limited use of human embryos to explore medical applications of cloning technology in the pursuit of future cures and treatments for disease.

There are thus two main sources of opposition to human cloning. One set of objections surrounds the possibility of using the Dolly technique to clone a baby or adult (Silver, 1999). Although some experts in the field of assisted conception have argued cloning should be available as an additional method to overcome childlessness for those couples who desire to attempt it, other scientists, including Ian Wilmut, strongly oppose such proposals, arguing the technique is at too early a stage in development to be used safely or reliably. Having witnessed the birth of several lambs who have suffered from significant, often fatal, abnormalities as a result of having been created through the Dolly technique, Ian Wilmut argues it is immoral to risk producing a human being by this means (see Chapter 4). Such a person might be born with very serious, untreatable and unprecedented types of congenital malformations.

Many scientists who oppose the use of cloning for human reproduction, however, strongly support the use of cloning for a second, more limited, set of medical purposes – in particular for growing human tissues to be used for a range of therapeutic purposes – in spite of objections to the use of human embryos (reconstructed or otherwise) for experimental purposes. To devise regulatory policy, a distinction has been drawn between reproductive cloning and therapeutic cloning by advisory bodies such as the Human Fertilisation and Embryology Authority, who recommend that reproductive cloning be banned under any circumstances, but that stem cell research using the Dolly technique to create reconstructed embryos be allowed under licence when human embryonic tissue is involved.

Cloning is a somewhat misleading term for all of these developments because its definition is imprecise. Etymologically, cloning derives from the Greek word for twig, referring to the process by which a new plant can often be created from a cutting. Cloning thus, classically, refers to the creation of a new organism from only one parent. Technically, this does not accurately categorise Dolly the sheep, who was created from a merger of two cells from two different animals. Although DNA fingerprinting has definitively established that Dolly’s nuclear genetic material is 100% derived from the adult cell which came from one animal, and although she has all of the familiar characteristics of a Finn Dorset, not a Blackface, sheep, it is confusing to refer to Dolly as a ‘clone’ in the strict sense of the term, because her origins are more complex. What ‘cloning’ means in the case of Dolly is that she has been born via the process of nuclear transfer, and that her consequent nuclear genetic identity with one parent makes her a ‘clone’. However, if one of the main implications of Dolly’s birth is that the relationship between nuclear genetic material and its surrounding cellular environment is much more complex, and interactive, than was previously assumed, then she must be seen as unique. In other words, it is somewhat misleading to describe Dolly as identical to her genetic parent, since this would suggest it is only DNA which creates individuality. Although the association of a person’s DNA with his or her individual uniqueness is often emphasised in accounts of sexual reproduction and biological development, the Dolly technique demonstrates that, as in the case of genetically identical twins, individuality is more than merely genetic.

In the future, cloning will play an increasing role in the production of new living organisms. An expanding toolkit of cellular reconstruction techniques
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will enable an increasingly broad range of recombinant organisms to be produced with increasing molecular precision, for a wide range of potential applications – from the production of improved milk for infants (nutraceuticals) to new drugs, and new cell lines. Dolly was initially created as part of a process of exploring new forms of animal breeding, and her birth represents an intensification of animal domestication. In creating Dolly, Roslin scientists were initially attempting to devise new means of reproducing dairy animals that have had useful human genes added to their nuclear DNA, so that they can produce the specific enzymes that are missing in people who suffer from disabling genetic conditions, such as cystic fibrosis. For example, using in vitro fertilisation techniques, a female sheep embryo can be implanted with a human gene carrying the instructions to make alpha-1-antitrypsin (AAT), and then gestated to become a ewe with the capacity to excrete this valuable enzyme in her milk. Enzymes extracted from the milk of such sheep can thus be used to make new pharmaceutical products aimed at replacing the enzyme that is missing in people who suffer from specific genetic diseases. AAT was the first pharmaceutical of this kind to be licensed in Britain, by PPL Therapeutics in May 2000, for use in the treatment of cystic fibrosis – which is the most common genetic disorder in Western Europe. Animals who produce medicines in this way are known as ‘bio-reactors’, and it was, in part, the attempt to produce such sheep more efficiently and reliably that led to the discovery of the Dolly technique.

In turn, the Dolly technique has been rapidly expanded, so that, in combination with other new and established methods, it now comprises one of the basic procedures for the biotechnology industry. This industry is itself often described as embryonic, with projects such as the human genome map providing a new language in which to understand human health and disease, the ageing process, and the basic mechanisms of biological reproduction. Similarly, the mapping of the genomes of mice, fruit flies, micro-organisms such as yeast, and higher vertebrates such as dogs and sheep is yielding basic information about the nature of what are commonly described as the genetic instructions for the production of all living things. In turn, this information is being combined with an increasingly complex understanding of the protein pathways which condition genetic expression, and the cellular processes with which they interact, to devise new means of harnessing biological reproduction as a means of manufacture.

Cloning, then, is neither new nor necessarily controversial. Techniques of cloning have been used in agriculture for millennia, and methods such as polymerase chain reaction (PCR), which is a form of cloning widely used in genetic engineering, have been standard and non-controversial procedures for decades. What is new about the method of ‘cloning’ known as the Dolly technique is its global prominence as a highly publicised and powerfully symbolic marker of an intensification of molecular engineering at the level of the basic biological pathways of organic reproduction. Dolly’s status as a world-famous ‘clone’ is thus most accurately interpreted as a form of symbolic association, which attaches the highly controversial spectre of cloning (itself a term that has long signified cultural anxiety about man-made life) to a significant development in reproductive biology. This association is fully justified, in that the success of the Dolly technique is indeed a very good example of the power of biotechnology to re-engineer living organisms, and of the desires of scientists and commercial industries to increase their abilities to do so. Thus, despite the fact that it is somewhat misleading to describe Dolly as a clone in the technical sense of the term, this term accurately conveys a significant level of public anxiety about the question of limits to scientific manipulation of life itself. Both the many techniques which come under the broad umbrella of cloning, and the wider biotechnological industry of which they are a part, rely on an increasing ability to rewrite the rules of biological reproduction.

CNR and stem cells

In May 1999 the California-based pharmaceutical corporation Geron purchased Ian Wilmut’s Dolly division at Roslin for 25.7 million dollars (in stock options), and announced a 20 million dollar research programme over the next 6 years to combine the use of nuclear transfer with Geron’s human cell-line technology to create a new market in human tissue replacement therapy, or human therapeutic cloning.

The new company, Geron BioMed, is a wholly owned subsidiary of Geron Corporation, based at Roslin, and is described by its parent company as the world’s ‘premiere research and development consortium in transplantation biology’ (Geron press release, 4 May 1999). The goal of this consortium is to develop novel forms of human cell therapy that combine Geron’s expertise in overcoming the cellular ageing process with Roslin’s ability to reprogramme adult cells to re-differentiate. In other words, the aim is to provide a kind of bespoke tissue therapy for a wide range of human diseases from heart failure to cancer treatment to Parkinson’s disease. This will be accomplished by taking body cells from the afflicted patient, turning them into pluripotent stem cell lines that act like early embryonic tissue, which can then be redirected to produce numerous forms of specialised human tissue suitable for re-implantation back into the patient.

The company’s most widely publicised research is the ability to clone telomerase, the enzyme which controls the mammalian cellular ageing process. In their own words, Geron’s greatest breakthrough has been to ‘discover the key to cellular mortality’. This ‘key’ is Geron’s patented telomerase reactivation technology. Telomeres are described by Geron as the finger-like protrusions at the ends of each chromosome (Geron uses the analogy of shoelaces, with their protected tips). Found in all mammalian cells, telomeres act as the ‘motic clock’ of cellular replication by shortening with each cell division. Telomere shortening eventually causes the cessation of cell division, in other words, cell death. However, telomere shortening can be reversed by means of reactivating the enzyme telomerase, which adds DNA repeats to the telomeres as...
cells divide, thus ‘resetting’ the cellular clock, and making cells more youthful, or, indeed, immortal.

From Geron’s point of view, what is very useful about the Dolly technique is that it enables significant improvements in human cell line technology. Geron’s ability to reprogramme telomerase within specially cultured cell lines made of human embryonic stem cells offers, in their words, the capacity ‘to produce large quantities of perpetually-young and healthy differentiated cells for use in the repair of degenerating organs’ (Geron Corporation, 1997).

In 2001 George Bush announced a ban on the creation of any new stem cell lines in the US. Following a personal consultation with the Vatican, and in response to public pressures to ban the use of human embryonic tissue in the manufacturing of cell lines, Bush’s restrictions created a less favourable research environment in the US, where stem cell research is most advanced. Meanwhile, stem cell research in Europe has been strongly encouraged by the European Commission, leading some countries, most notably Germany, to liberalise their laws on embryo research to allow them to be used for making cell lines. According to the leading EC scientific research bulletin of 2001, ‘Europe seems determined not to miss the stem cell train. The European Union already funds – to the sum of 27.4 million euro – 15 research projects involving 117 laboratories in countries from Finland to Portugal’ (European Commission, 2001, p. 7).

Britain has emerged as the leading European country to use the Dolly technique in conjunction with human embryonic cells to manufacture stem cells, and has produced comprehensive legislative guidelines to regulate this field. According to the British House of Lords Select Committee Report on Stem Cell Research, published in February 2002:

Until recently it has generally been considered that in mammalian cells the process of differentiation is irreversible. However, it has been demonstrated in animals that it is possible to reprogramme (‘dedifferentiate’) the genetic material of a differentiated adult cell by CNR. Following this seminal finding, many studies have also suggested that adult stem cells may have greater ‘plasticity’ than previously suspected: they may be reprogrammed to give rise to cell types to which they normally do not give rise in the body. The potential of specialised cells to differentiate into cell types other than those to which they normally give rise in the body is little short of a revolutionary concept in cell biology. It has significantly increased the possibilities for developing effective stem-cell based therapies.

(House of Lords (HL), 2002, p. 13)

The British House of Lords Report offers a thorough consideration of stem cell research and concludes it should be ‘strongly encouraged by funding bodies and the Government’ in Britain (HL, 2002, p. 48). Research on human embryos is described as ‘necessary, particularly to understand the processes of cell differen-

The report recommends the establishment of a British stem cell bank to be ‘responsible for the custody of stem cell lines, ensuring their purity and provenance’ (p. 50) and concludes that existing mechanisms for regulation of research, and mechanisms for procuring informed consent from donors, are sufficiently robust to accommodate new developments in the area of stem cell research.

Although the House of Lords Committee acknowledges that they were only able to give limited attention to the role of commercial interests in stem cell research, it devotes an entire section of its report to this concern and acknowledges it has ‘been aware throughout that commercial interests could, and to some extent already do, play an important role in the development of such research’ (HL, 2002, p. 32). It is also acknowledged that ‘biotechnology is a growth industry’, citing an Ernst & Young report that by the end of 2000 ‘the total value of Europe’s publicly quoted biotechnology companies stood at 75 billion Euros, compared with 36 billion Euros a year earlier’ (HL, 2002, p. 32).

These references, along with acknowledgement that the United Kingdom ‘has by far the most public biotechnology companies’ in Europe, and that ‘investor interest is considerable and evidently based on the assumption that future profits may be significant’, confirm the extent to which the British government recognises the importance of economic growth in the biotechnology sector as a national priority. This is further underlined by reference in the House of Lords report to China and Singapore, which ‘provide examples that deserve special mention’:

In China the government has encouraged a number of universities to invest heavily in stem cell research. In doing so universities have attracted not only public funds but investment by private companies like the Beijing Stemcell Medengineering Company. Leading Chinese researchers are often US-trained and have links with American laboratories. In Singapore, the Economic Development Board has provided initial finance for the Singapore genetics Programme; it is said that by 2005 some $7 billion dollars will have been invested in relevant research. In both China and Singapore there is concern with ethical issues but also an interest to maintain the competitive advantage gained by light regulation.

(HL, 2002, p. 32)

In March of 2002, shortly following publication of the House of Lords Report, and following the approval of the first licences for stem cell research using human embryos in Britain, one of the world’s leading stem cell scientists, Austin Smith of the Centre for Human Genome Research in Edinburgh, published an article in Nature suggesting that much of the work conducted...
on stem cells may have misidentified their properties. Cells which were thought to have been 'reprogrammed', or 'de-differentiated', may, his study suggested, have simply merged with other cells to produce cell populations with a double set of chromosomes (Terada et al., 2002, and Ying et al., 2002). Smith claims it took the better part of two decades to stabilise and characterise murine cell lines, and that it is likely that human embryonic cell lines may be similarly time-consuming to establish. Such claims, although they counter much of the hype that has surrounded human therapeutic cloning and stem cells, point towards their eventual use, albeit possibly as a more distant option than was once hoped.

**Conclusion**

The development of cloning by nuclear transfer, now known as CNR or 'the Dolly technique', has undergone several transformations since its inaugural use as a form of animal breeding in a livestock research facility in the mid-1990s. Amidst ongoing unconfirmed reports in the early twenty-first century that several women may already be pregnant with cloned offspring, a human has so far not been born who has been scientifically proven to have been cloned. Human reproductive cloning is widely considered to be unethical, due to the high incidence of congenital abnormality in all known cloned vertebrates, and has been denounced by all but a few maverick practitioners, such as the Italian clinician Severino Antinori. Use of the Dolly technique for the production of stem cells, and for what has come to be known as the field of regenerative medicine, has, in contrast, launched one of the first major post-genomic bio-industries. Stem cell research using CNR, which is often described as tissue engineering, has become a major research focus in Europe, and continues to be a high priority area for many US biotechnology companies, such as Associated Cell Technologies, based in Worcester, Massachusetts, which is the leading US competitor to the Geron corporation. China and Singapore also have burgeoning stem cell industries which receive significant public assistance, in what has become one of the most competitive global biotechnology sectors.

It is likely that the future of 'cloning' will lie primarily in the petri dish, although it is certain also to occupy a prominent position in the media, in popular culture and in academic debate. It is claimed by many to be one of the most significant developments in twentieth century biology, and is likely to play a leading role in the formation of health-care strategies in the future, along with pharmacogenetics and genetic screening technologies. It will, in all of these capacities, continue to pose unique challenges at the level of governance and regulation, as well as in the effort to maintain public trust towards the genomic sciences as they permeate ever more deeply into definitions of human identity, health and reproduction.

**Notes**

1. See further at the PPL Therapeutics website, www.ppl-therapeutics.co.uk.
2. The useful feature of early embryonic cells is their ability to retain the capacity to differentiate into any kind of tissue: so-called 'totipotentiality'. Geron distinguishes between their 'pluripotent' cell lines, which can become most, but not all, forms of tissue (for example, they cannot, at present, be directed to become blood cells), and the 'true' totipotential capacities of early embryos.
3. Like early embryonic cells, cancer cells do not lose their telomeres, which is why cancer cells are commonly used to establish immortal cell lines. Initially, Geron aimed for a market in diagnostic kits which would test for telomerase to identify oncogenesis. This is still an important emerging market for Geron, though somewhat displaced by the new avenues made possible through using nuclear transfer to make better immortal ('more immortal') cell lines.
4. A normal cell line made from body tissue has to be mixed with several other ingredients, many of unknown function, in order to activate and become productive. Making a cell line of, for example, the mammary tissue of a Finn Dorset ewe requires a complicated cocktail of ingredients to achieve a small fibroblast, as the resulting scab-like collection of mammary cells in culture has many needs that are not well understood.

**References**


**Further reading**