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Methods of Cloning and Cloning of Dolly

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ABSTRACT: Dolly, female Finn Dorset sheep that lived from 1996 to 2003, the first clone of an adult mammal, produced by British developmental biologist Ian Wilmut and colleagues of the Roslin Institute, near Edinburgh, Scotland. The announcement in February 1997 of Dolly's birth marked a milestone in science, dispelling decades of presumption that adult mammals could not be cloned and igniting a debate concerning the many possible uses and misuses of mammalian cloning technology.

The concept of mammalian clones, even humans, was not new at the time of Dolly's birth. Among mammals, naturally occurring genetic clones, or individuals genetically identical to one another, had long been recognized in the form of monozygotic (identical) twins. Unlike Dolly, however, such clones are derived from a single zygote, or fertilized egg, and thus they are clones of one another, rather than clones of another individual. Moreover, clones had been generated previously in the laboratory, but only from embryonic cells that were either undifferentiated or only partially differentiated. In animals, the production of clones from fully differentiated (adult) cells (e.g., skin or muscle cells) had been carried out successfully only in lower species, such as frogs.

KEYWORDS: Dolly, sheep, cloning, methods, scientists, genetics

I.INTRODUCTION

For decades, scientists had tried and failed to clone mammals from existing adults. The repeated failures led scientists to speculate about the significance of the timing and process of cell differentiation in the developing mammalian embryo. Of particular interest were changes that occurred to DNA during an animal's development, whereby patterns in gene expression were altered as cells became increasingly specialized in function. It was realized that, through the process of differentiation, adult mammalian cells lose totipotency—the ability to become any of the different cell types required for making a complete and viable animal. It was presumed that the process was irreversible. The successful production of Dolly, however, proved otherwise.[1,2,3]

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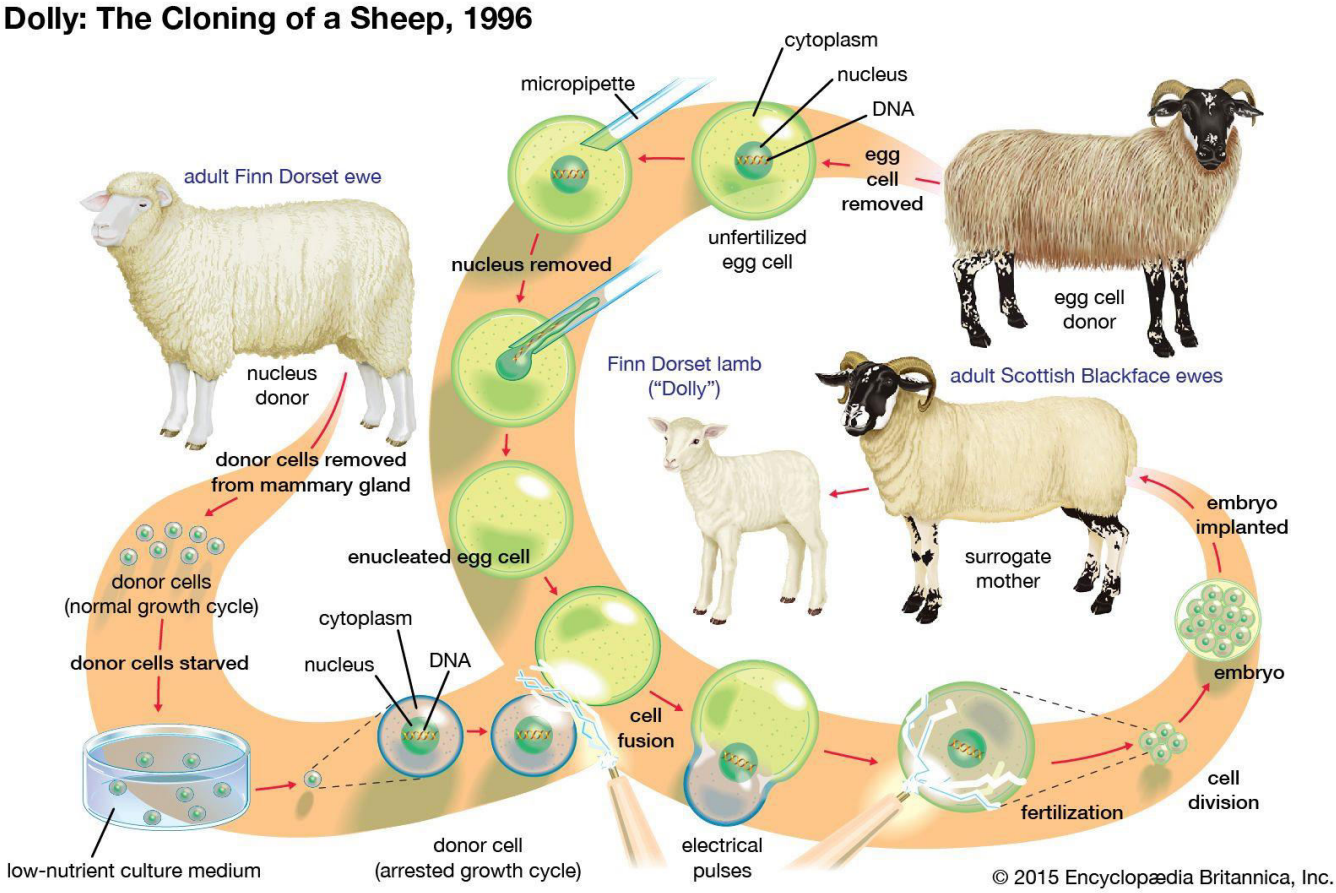

Dolly the sheep; cloning

Dolly the sheep was successfully cloned in 1996 by fusing the nucleus from a mammary-gland cell of a Finn Dorset ewe into an enucleated egg cell taken from a Scottish Blackface ewe. Carried to term in the womb of another Scottish Blackface ewe, Dolly was a genetic copy of the Finn Dorset ewe.(more)

Dolly was cloned from a mammary gland cell taken from an adult Finn Dorset ewe. Wilmut and his team of researchers at Roslin created her by using electrical pulses to fuse the mammary cell with an unfertilized egg cell, the nucleus of which had been removed. The fusion process resulted in the transfer of the mammary cell nucleus into the egg cell, which then began to divide. In order for the mammary cell nucleus to be accepted and functional within the host egg, the cell first had to be induced to abandon the normal cycle of growth and division and enter a quiescent stage. To accomplish that, researchers deliberately withheld nutrients from the cells. The importance of the step had been determined experimentally, though an explanation for its necessity was lacking. Nevertheless, starting with a collection of mammary cell nuclei and host egg cytoplasms derived from Scottish Blackface ewes, a number of fused couplets successfully formed embryos. The reconstructed embryos were transferred to surrogate Scottish Blackface ewes. Of 13 recipient ewes, one became pregnant, and 148 days later, which is essentially normal gestation for a sheep, Dolly was born.[4,5,6]

Dolly remained alive and well long after her birth, with a functional heart, liver, brain, and other organs, all derived genetically from the nuclear DNA of an adult mammary gland cell. The technique used to produce her later became known as somatic cell nuclear transfer (SCNT). SCNT has since been used to generate a wide variety of mammalian clones, from different types of adult cells; its success in producing clones of primates, however, has been notably limited.

On February 14, 2003, Dolly was euthanized by veterinarians after being found to suffer from progressive lung disease.

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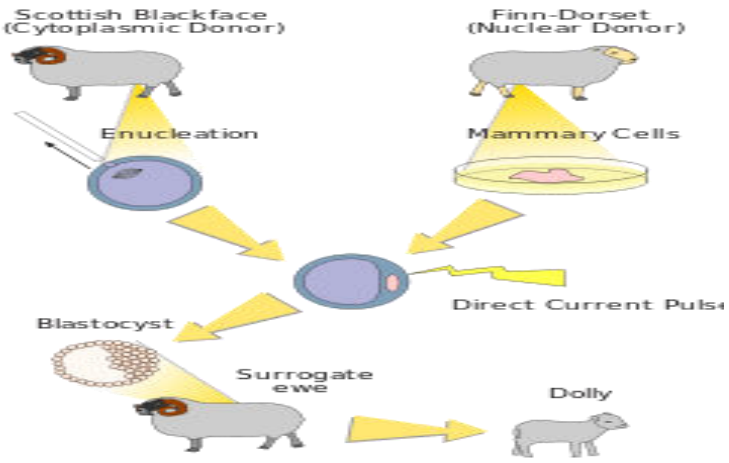
Her body was preserved and displayed at the National Museum of Scotland in Edinburgh.

Dolly (5 July 1996 – 14 February 2003) was a female Finn-Dorset sheep and the first mammal that was cloned from an adult somatic cell. She was cloned by associates of the Roslin Institute in Scotland, using the process of nuclear transfer from a cell taken from a mammary gland. Her cloning proved that a cloned organism could be produced from a mature cell from a specific body part.[2] Contrary to popular belief, she was not the first animal to be cloned.[3]
The employment of adult somatic cells in lieu of embryonic stem cells for cloning emerged from the foundational work of John Gurdon, who cloned African clawed frogs in 1958 with this approach. The successful cloning of Dolly led to widespread advancements within stem cell research, including the discovery of induced pluripotent stem cells.[4]
Dolly lived at the Roslin Institute throughout her life and produced several lambs.[5] She was euthanized at the age of six years due to a progressive lung disease. No cause which linked the disease to her cloning was found.[6]
Dolly's body was preserved and donated by the Roslin Institute in Scotland to the National Museum of Scotland, where it has been regularly exhibited since 2003.

Dolly was cloned by Keith Campbell, Ian Wilmut and colleagues at the Roslin Institute, part of the University of Edinburgh, Scotland, and the biotechnology company PPL Therapeutics, based near Edinburgh. The funding for Dolly's cloning was provided by PPL Therapeutics and the Ministry of Agriculture.[7] She was born on 5 July 1996 and died on 14 February 2003 from a progressive lung disease that was considered unrelated to her being a clone.[5] She has been called "the world's most famous sheep" by sources including BBC News and Scientific American.[8][9]
The cell used as the donor for the cloning of Dolly was taken from a mammary gland, and the production of a healthy clone, therefore, proved that a cell taken from a specific part of the body could recreate a whole individual. On Dolly's name, Wilmut stated "Dolly is derived from a mammary gland cell and we couldn't think of a more impressive pair of glands than Dolly Parton's
Dolly was born on 5 July 1996 and had three mothers: one provided the egg, another the DNA, and a third carried the cloned embryo to term.[10] She was created using the technique of somatic cell nuclear transfer, where the cell nucleus from an adult cell is transferred into an unfertilized oocyte (developing egg cell) that has had its cell nucleus removed. The hybrid cell is then stimulated to divide by an electric shock, and when it develops into a blastocyst it is implanted in a surrogate mother.[11] Dolly was the first clone produced from a cell taken from an adult mammal.[12][13] The production of Dolly showed that genes in the nucleus of such a mature differentiated somatic cell are still capable of reverting to an embryonic totipotent state, creating a cell that can then go on to develop into any part of an animal.[2]

Dolly's existence was announced to the public on 22 February 1997.[1] It gained much attention in the media. A commercial with Scottish scientists playing with sheep was aired on TV, and a special report in Time magazine featured Dolly.[7] Science featured Dolly as the breakthrough of the year. Even though Dolly was not the first animal cloned, she received media attention because she was the first cloned from an adult cell.[14]

Life



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The cloning process that produced Dolly

Dolly lived her entire life at the Roslin Institute in Midlothian.[15] There she was bred with a Welsh Mountain ram and produced six lambs in total. Her first lamb, named Bonnie, was born in April 1998.[5] The next year Dolly produced twin lambs Sally and Rosie, and she gave birth to triplets Lucy, Darcy and Cotton in 2000.[16] In late 2001, at the age of four, Dolly developed arthritis and began to walk stiffly. This was treated with anti-inflammatory drugs.[17]

Death

On 14 February 2003, Dolly was euthanised because she had a progressive lung disease and severe arthritis.[6] A Finn Dorset such as Dolly has a life expectancy of around 11 to 12 years, but Dolly lived 6.5 years. A post-mortem examination showed she had a form of lung cancer called ovine pulmonary adenocarcinoma, also known as Jaagsiekte,[18] which is a fairly common disease of sheep and is caused by the retrovirus JSRV.[19] Roslin scientists stated that they did not think there was a connection with Dolly being a clone, and that other sheep in the same flock had died of the same disease.[6] Such lung diseases are a particular danger for sheep kept indoors, and Dolly had to sleep inside for security reasons.[20]

Some in the press speculated that a contributing factor to Dolly's death was that she could have been born with a genetic age of six years, the same age as the sheep from which she was cloned.[21] One basis for this idea was the finding that Dolly's telomeres were short, which is typically a result of the aging process.[22][23] The Roslin Institute stated that intensive health screening did not reveal any abnormalities in Dolly that could have come from advanced aging.[21]

In 2016, scientists reported no defects in thirteen cloned sheep, including four from the same cell line as Dolly. The first study to review the long-term health outcomes of cloning, the authors found no evidence of late-onset, non-communicable diseases other than some minor examples of osteoarthritis and concluded "We could find no evidence, therefore, of a detrimental long-term effect of cloning by SCNT on the health of aged offspring among our cohort."[24][25]

After her death Dolly's body was preserved via taxidermy and is currently on display at the National Museum of Scotland in Edinburgh.[26]

Legacy

After cloning was successfully demonstrated through the production of Dolly, many other large mammals were cloned, including pigs,[27][28] deer,[29] horses[30] and bulls.[31] The attempt to clone argali (mountain sheep) did not produce viable embryos. The attempt to clone a banteng bull was more successful, as were the attempts to clone mouflon (a form of wild sheep), both resulting in viable offspring.[32] The reprogramming process that cells need to go through during cloning is not perfect and embryos produced by nuclear transfer often show abnormal development.[33][34] Making cloned mammals was highly inefficient – in 1996 Dolly was the only lamb that survived to adulthood from 277 attempts. By 2014 Chinese scientists were reported to have 70–80% success rates cloning pigs,[28] and in 2016, a Korean company, Sooam Biotech, was producing 500 cloned embryos a day.[35] Wilmut, who led the team that created Dolly, announced in 2007 that the nuclear transfer technique may never be sufficiently efficient for use in humans.[36]

Cloning may have uses in preserving endangered species, and may become a viable tool for reviving extinct species.[37] In January 2009, scientists from the Centre of Food Technology and Research of Aragon in northern Spain announced the cloning of the Pyrenean ibex, a form of wild mountain goat, which was officially declared extinct in 2000. Although the newborn ibex died shortly after birth due to physical defects in its lungs, it is the first time an extinct animal has been cloned, and may open doors for saving endangered and newly extinct species by resurrecting them from frozen tissue.[38][39]

In July 2016, four identical clones of Dolly (Daisy, Debbie, Dianna, and Denise) were alive and healthy at nine years old.[40][41]

Scientific American concluded in 2016 that the main legacy of Dolly has not been cloning of animals but in advances into stem cell research.[42] After Dolly, researchers realised that ordinary cells could be reprogrammed to induced pluripotent stem cells, which can be grown into any tissue.[43]

The first successful cloning of a primate species was reported in January 2018, using the same method which produced Dolly. Two identical clones of a macaque monkey, Zhong Zhong and Hua Hua, were created by researchers in China and were born in late 2017.[44][45][46][47]

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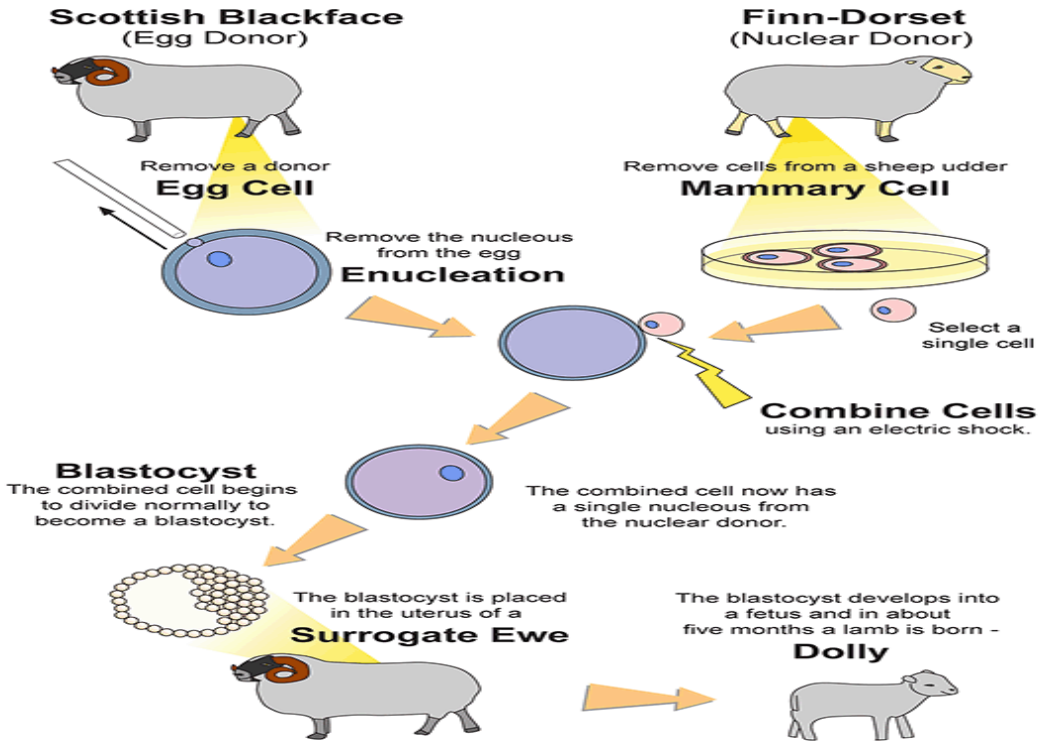
In January 2019, scientists in China reported the creation of five identical cloned gene-edited monkeys, again using this method, and the gene-editing CRISPR-Cas9 technique allegedly used by He Jiankui in creating the first ever gene-modified human babies Lulu and Nana. The monkey clones were made in order to study several medical diseases[7,8,9]

II.DISCUSSION

Cloning is the production of an exact copy of a cell, any other living part, or a complete organism. Cloning of an animal was successfully performed for the first time by Ian Wilmut and his colleagues at the Roslin Institute in Edinburgh, Scotland. They cloned successfully a sheep named Dolly.

During the process of cloning Dolly, a cell was collected from the mammary gland of a female Finn Dorsett sheep. Simultaneously, an egg was obtained from a Scottish blackface ewe. The nucleus was removed from the egg. Then, the nucleus of the mammary gland cell from the Finn Dorsett sheep was inserted into the egg of the Scottish blackface ewe whose nucleus had been removed. The egg thus produced was implanted into the Scottish blackface ewe. Development of this egg followed normally and finally Dolly was born. Though Dolly was given birth by the Scottish blackface ewe, it was found to be absolutely identical to the Finn Dorsett sheep from which the nucleus was taken. Since the nucleus from the egg of the Scottish blackface ewe was removed, Dolly did not show any character of the Scottish blackface ewe.
Dolly is the name of a sheep that has the honor of being the first mammal to be cloned by a group of scientists in Scotland. Dolly was born July 5th, 1996 and she passed away in 2003. She lived for 6 and a half years, as a normal, active ewe. She was not that normal though, she was a clone after all.
There are several breeds of sheep in the world. The sheep we focus on here are easy to remember though. One breed of sheep is the Scottish Blackface and the other is a Finn Dorset. What is easy about those names is that the Blackface actually has a black face. The Finn Dorset is all white. You know that the genetic material in our cells determines how we look on the outside. The black face of the Blackface sheep is determined by its genetic material, which means you can check to see who has that genetic material by just looking at their face! Very easy![10,11,12]

How Dolly Was Made



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Steps for making Dolly the sheep

In Dolly's case, the nucleus of an egg from a black faced sheep was removed. The nucleus from a white faced sheep was inserted into that egg. Now, they had just one cell with the nucleus of a white faced sheep, but the rest of the cell containing items from a black faced sheep. That cell was placed into the black faced sheep who's egg it started out as. The cell eventually grew up and made Dolly. When Dolly was born, her face was white!

Scottish embryologist, Iam Wilmut, feeding his cloned sheep Dolly. The first mammal to be cloned.[10,11,12]

Black faced sheep never have white faced sheep. Dolly's face being white was good proof that the experiment was successful, but scientists double checked Dolly's genetic material. They found that Dolly did have the exact same genetic material as the white faced sheep that donated the nucleus. Dolly did not have any genetic material from the black faced sheep who was her mother.

The story was first published as a short report in a scientific journal called Nature, Volume 385, 1997, pages 810-812. Since that time, attempts at cloning at least 10 species of mammals have been made. Only seven attempts have been successful; cows, sheep, goats, mice, pigs, cats, and rabbits. The rate of success is very low.

Several questions are brought up by Dolly's life and death. Scientists were interested to see if she aged faster because the genetic material was older than that from a fertilized egg. It was thought that she was aging faster, but no one could be sure. Dolly's cells looked a little older in a region in the genetic material, but is that enough to cause premature aging? Dolly got arthritis, a disease that mainly affects older individuals, but again, no one could be sure if it was because she was a clone. Some animals get arthritis early in life. Dolly even gave birth to a daughter, who was completely normal in all ways. Dolly led an active, healthy, sheep life until she passed away.

Dolly got severely sick with a lung infection that affects many sheep. Scientists decided to put her down, euthanize her, to end her suffering. Dolly passed away when she was six and a half years old. That is half the average lifespan for a Finn Dorset sheep. She is now stuffed and on display in Edinborough, Scotland. We learned a lot from Dolly while she was alive and when she had passed away, but she might have left many more questions than answers.[13,14,15]

III.RESULTS

One major obstacle in realizing the potential behind human embryonic stem cells (hESC) is the availability of efficient and reliable engineering methods. Such methods require cloning technologies that can be applied to a variety of platforms and can serve multiple functions. In the last two decades cloning technologies have become more efficient, widening the bottleneck in creating engineered hESC lines. Using TOPO(®) TA cloning kits, genes can be efficiently amplified and inserted into target vectors with minimal manipulation and purification. For more complex cloning procedures we introduce the Multisite Gateway(®) system. This is a cloning platform based on integrase technology that allows for the generation of complex multicistronic gene configurations that can transverse a variety of platforms with ease. These technologies allow the end user to quickly and efficiently select clones, as well as combine multiple genetic elements of

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interest between platform technologies in a high-throughput manner, providing scientists with a toolbox to create tools to dissect stem cell biology.

Cloning is the process of producing individual organisms with identical genomes, either by natural or artificial means. In nature, some organisms produce clones through asexual reproduction; this reproduction of an organism by itself without a mate is known as parthenogenesis. In the field of biotechnology, cloning is the process of creating cloned organisms of cells and of DNA fragments.

The artificial cloning of organisms, sometimes known as reproductive cloning, is often accomplished via somatic-cell nuclear transfer (SCNT), a cloning method in which a viable embryo is created from a somatic cell and an egg cell. In 1996, Dolly the sheep achieved notoriety for being the first mammal cloned from a somatic cell. Another example of artificial cloning is molecular cloning, a technique in molecular biology in which a single living cell is used to clone a large population of cells that contain identical DNA molecules.[16,17]

In bioethics, there are a variety of ethical positions regarding the practice and possibilities of cloning. The use of embryonic stem cells, which can be produced through SCNT, in some stem cell research has attracted controversy. Cloning has been proposed as a means of reviving extinct species. In popular culture, the concept of cloning—particularly human cloning—is often depicted in science fiction; depictions commonly involve themes related to identity, the recreation of historical figures or extinct species, or cloning for exploitation (i.e. cloning soldiers for warfare).

Etymology

Coined by Herbert J. Webber, the term clone derives from the Ancient Greek word κλών (klōn), twig, which is the process whereby a new plant is created from a twig. In botany, the term lusus was used.[1] In horticulture, the spelling clon was used until the early twentieth century; the final e came into use to indicate the vowel is a "long o" instead of a "short o".[2][3] Since the term entered the popular lexicon in a more general context, the spelling clone has been used exclusively.

Natural cloning

Natural cloning is the production of clones without the involvement of genetic engineering techniques.[4] It may occur accidentally in the case of identical twins, which are formed when a fertilized egg splits, creating two or more embryos that carry almost identical DNA. It may also be part of asexual reproduction, which is a process where a single parent organism produces genetically identical offspring by itself.[5][6]

Cloning is a natural form of reproduction that has allowed life forms to spread for hundreds of millions of years. It is a reproduction method used by plants, fungi, and bacteria, and is also the way that clonal colonies reproduce themselves.[7][8] Examples of these organisms include blueberry plants, Hazel trees, the Pando trees,[9][10] the Kentucky coffeetree, Myrica, and the American sweetgum.

If artificial cloning and natural cloning both lead to the same result, which is the formation of a clone, that is, an organism with identical or nearly identical genes to another organism, then the plight of This creation is very different between the two creatures. The main difference between the two is that natural cloning does not involve any human intervention, whereas artificial cloning is a genetic engineering technique. Natural cloning occurs through a variety of natural mechanisms, from single-celled organisms to complex multicellular organisms. Some of the mechanisms are explored and used into plants and animals as binary fission, Budding, Fragmentation, parthenogenesis.[11]

Molecular cloning

Molecular cloning refers to the process of making multiple molecules. Cloning is commonly used to amplify DNA fragments containing whole genes, but it can also be used to amplify any DNA sequence such as promoters, non-coding sequences and randomly fragmented DNA. It is used in a wide array of biological experiments and practical applications ranging from genetic fingerprinting to large scale protein production. Occasionally, the term cloning is misleadingly used to refer to the identification of the chromosomal location of a gene associated with a particular phenotype of interest, such as in positional cloning. In practice, localization of the gene to a chromosome or genomic region does not necessarily enable one to isolate or amplify the relevant genomic sequence. To amplify any DNA sequence

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in a living organism, that sequence must be linked to an origin of replication, which is a sequence of DNA capable of directing the propagation of itself and any linked sequence. However, a number of other features are needed, and a variety of specialised cloning vectors (small piece of DNA into which a foreign DNA fragment can be inserted) exist that allow protein production, affinity tagging, single-stranded RNA or DNA production and a host of other molecular biology tools.

Cloning of any DNA fragment essentially involves four steps[12]

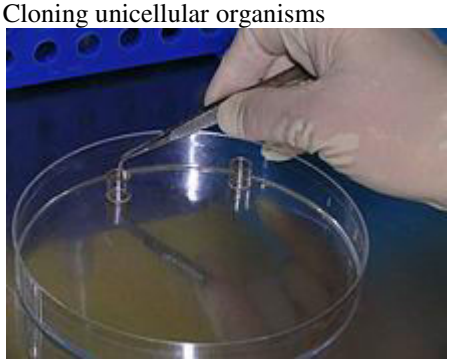
1. fragmentation - breaking apart a strand of DNA
2. ligation – gluing together pieces of DNA in a desired sequence
3. transfection – inserting the newly formed pieces of DNA into cells
4. screening/selection – selecting out the cells that were successfully transfected with the new DNA

Although these steps are invariable among cloning procedures a number of alternative routes can be selected; these are summarized as a cloning strategy.

Initially, the DNA of interest needs to be isolated to provide a DNA segment of suitable size. Subsequently, a ligation procedure is used where the amplified fragment is inserted into a vector (piece of DNA). The vector (which is frequently circular) is linearised using restriction enzymes, and incubated with the fragment of interest under appropriate conditions with an enzyme called DNA ligase. Following ligation, the vector with the insert of interest is transfected into cells. A number of alternative techniques are available, such as chemical sensitisation of cells, electroporation, optical injection and biolistics. Finally, the transfected cells are cultured. As the aforementioned procedures are of particularly low efficiency, there is a need to identify the cells that have been successfully transfected with the vector construct containing the desired insertion sequence in the required orientation. Modern cloning vectors include selectable antibiotic resistance markers, which allow only cells in which the vector has been transfected, to grow. Additionally, the cloning vectors may contain colour selection markers, which provide blue/white screening (alpha-factor complementation) on X-gal medium. Nevertheless, these selection steps do not absolutely guarantee that the DNA insert is present in the cells obtained. Further investigation of the resulting colonies must be required to confirm that cloning was successful. This may be accomplished by means of PCR, restriction fragment analysis and/or DNA sequencing.[18,19]

Cell cloning

Cloning unicellular organisms



Cloning cell-line colonies using cloning rings

Cloning a cell means to derive a population of cells from a single cell. In the case of unicellular organisms such as bacteria and yeast, this process is remarkably simple and essentially only requires the inoculation of the appropriate medium. However, in the case of cell cultures from multi-cellular organisms, cell cloning is an arduous task as these cells will not readily grow in standard media.

A useful tissue culture technique used to clone distinct lineages of cell lines involves the use of cloning rings (cylinders).[13] In this technique a single-cell suspension of cells that have been exposed to a mutagenic agent or drug used to drive selection is plated at high dilution to create isolated colonies, each arising from a single and potentially clonal distinct cell. At an early growth stage when colonies consist of only a few cells, sterile polystyrene rings (cloning rings), which have been dipped in grease, are placed over an individual colony and a small amount of trypsin is added. Cloned cells are collected from inside the ring and transferred to a new vessel for further growth.

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Cloning stem cells

Somatic-cell nuclear transfer, popularly known as SCNT, can also be used to create embryos for research or therapeutic purposes. The most likely purpose for this is to produce embryos for use in stem cell research. This process is also called "research cloning" or "therapeutic cloning". The goal is not to create cloned human beings (called "reproductive cloning"), but rather to harvest stem cells that can be used to study human development and to potentially treat disease. While a clonal human blastocyst has been created, stem cell lines are yet to be isolated from a clonal source.[14]

Therapeutic cloning is achieved by creating embryonic stem cells in the hopes of treating diseases such as diabetes and Alzheimer's. The process begins by removing the nucleus (containing the DNA) from an egg cell and inserting a nucleus from the adult cell to be cloned.[15] In the case of someone with Alzheimer's disease, the nucleus from a skin cell of that patient is placed into an empty egg. The reprogrammed cell begins to develop into an embryo because the egg reacts with the transferred nucleus. The embryo will become genetically identical to the patient.[15] The embryo will then form a blastocyst which has the potential to form/become any cell in the body.[16]

The reason why SCNT is used for cloning is because somatic cells can be easily acquired and cultured in the lab. This process can either add or delete specific genomes of farm animals. A key point to remember is that cloning is achieved when the oocyte maintains its normal functions and instead of using sperm and egg genomes to replicate, the donor's somatic cell nucleus is inserted into the oocyte.[17] The oocyte will react to the somatic cell nucleus, the same way it would to a sperm cell's nucleus.[17]

The process of cloning a particular farm animal using SCNT is relatively the same for all animals. The first step is to collect the somatic cells from the animal that will be cloned. The somatic cells could be used immediately or stored in the laboratory for later use.[17] The hardest part of SCNT is removing maternal DNA from an oocyte at metaphase II. Once this has been done, the somatic nucleus can be inserted into an egg cytoplasm.[17] This creates a one-cell embryo. The grouped somatic cell and egg cytoplasm are then introduced to an electrical current.[17] This energy will hopefully allow the cloned embryo to begin development. The successfully developed embryos are then placed in surrogate recipients, such as a cow or sheep in the case of farm animals.[17]

SCNT is seen as a good method for producing agriculture animals for food consumption. It successfully cloned sheep, cattle, goats, and pigs. Another benefit is SCNT is seen as a solution to clone endangered species that are on the verge of going extinct.[17] However, stresses placed on both the egg cell and the introduced nucleus can be enormous, which led to a high loss in resulting cells in early research. For example, the cloned sheep Dolly was born after 277 eggs were used for SCNT, which created 29 viable embryos. Only three of these embryos survived until birth, and only one survived to adulthood.[18] As the procedure could not be automated, and had to be performed manually under a microscope, SCNT was very resource intensive. The biochemistry involved in reprogramming the differentiated somatic cell nucleus and activating the recipient egg was also far from being well understood. However, by 2014 researchers were reporting cloning success rates of seven to eight out of ten[19] and in 2016, a Korean Company Sooam Biotech was reported to be producing 500 cloned embryos per day.[20]

In SCNT, not all of the donor cell's genetic information is transferred, as the donor cell's mitochondria that contain their own mitochondrial DNA are left behind. The resulting hybrid cells retain those mitochondrial structures which originally belonged to the egg. As a consequence, clones such as Dolly that are born from SCNT are not perfect copies of the donor of the nucleus.

Organism cloning

Organism cloning (also called reproductive cloning) refers to the procedure of creating a new multicellular organism, genetically identical to another. In essence this form of cloning is an asexual method of reproduction, where fertilization or inter-gamete contact does not take place. Asexual reproduction is a naturally occurring phenomenon in many species, including most plants and some insects. Scientists have made some major achievements with cloning, including the asexual reproduction of sheep and cows. There is a lot of ethical debate over whether or not cloning should be used. However, cloning, or asexual propagation,[21] has been common practice in the horticultural world for hundreds of years.

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Horticultural

Propagating plants from cuttings, such as grape vines, is an ancient form of cloning.

The term clone is used in horticulture to refer to descendants of a single plant which were produced by vegetative reproduction or apomixis. Many horticultural plant cultivars are clones, having been derived from a single individual, multiplied by some process other than sexual reproduction.[22] As an example, some European cultivars of grapes represent clones that have been propagated for over two millennia. Other examples are potato and banana.[23]

Grafting can be regarded as cloning, since all the shoots and branches coming from the graft are genetically a clone of a single individual, but this particular kind of cloning has not come under ethical scrutiny and is generally treated as an entirely different kind of operation.

Many trees, shrubs, vines, ferns and other herbaceous perennials form clonal colonies naturally. Parts of an individual plant may become detached by fragmentation and grow on to become separate clonal individuals. A common example is in the vegetative reproduction of moss and liverwort gametophyte clones by means of gemmae. Some vascular plants e.g. dandelion and certain viviparous grasses also form seeds asexually, termed apomixis, resulting in clonal populations of genetically identical individuals.

Parthenogenesis

Clonal derivation exists in nature in some animal species and is referred to as parthenogenesis (reproduction of an organism by itself without a mate). This is an asexual form of reproduction that is only found in females of some insects, crustaceans, nematodes,[24] fish (for example the hammerhead shark[25]), Cape honeybees,[26] and lizards including the Komodo dragon[25] and several whiptails. The growth and development occurs without fertilization by a male. In plants, parthenogenesis means the development of an embryo from an unfertilized egg cell, and is a component process of apomixis. In species that use the XY sex-determination system, the offspring will always be female. An example is the little fire ant (Wasmannia auropunctata), which is native to Central and South America but has spread throughout many tropical environments.

Artificial cloning of organisms

Artificial cloning of organisms may also be called reproductive cloning.

First steps

Hans Spemann, a German embryologist was awarded a Nobel Prize in Physiology or Medicine in 1935 for his discovery of the effect now known as embryonic induction, exercised by various parts of the embryo, that directs the development of groups of cells into particular tissues and organs. In 1924 he and his student, Hilde Mangold, were the first to perform somatic-cell nuclear transfer using amphibian embryos – one of the first steps towards cloning.[27]

Methods

Reproductive cloning generally uses "somatic cell nuclear transfer" (SCNT) to create animals that are genetically identical. This process entails the transfer of a nucleus from a donor adult cell (somatic cell) to an egg from which the nucleus has been removed, or to a cell from a blastocyst from which the nucleus has been removed.[28] If the egg begins to divide normally it is transferred into the uterus of the surrogate mother. Such clones are not strictly identical since the somatic cells may contain mutations in their nuclear DNA. Additionally, the mitochondria in the cytoplasm also contains DNA and during SCNT this mitochondrial DNA is wholly from the cytoplasmic donor's egg, thus the mitochondrial genome is not the same as that of the nucleus donor cell from which it was produced. This may have important implications for cross-species nuclear transfer in which nuclear-mitochondrial incompatibilities may lead to death.

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Artificial embryo splitting or embryo twinning, a technique that creates monozygotic twins from a single embryo, is not considered in the same fashion as other methods of cloning. During that procedure, a donor embryo is split in two distinct embryos, that can then be transferred via embryo transfer. It is optimally performed at the 6- to 8-cell stage, where it can be used as an expansion of IVF to increase the number of available embryos.[29] If both embryos are successful, it gives rise to monozygotic (identical) twins.[19]

IV.CONCLUSION

Dolly the sheep
Dolly, a Finn-Dorset ewe, was the first mammal to have been successfully cloned from an adult somatic cell. Dolly was formed by taking a cell from the udder of her 6-year-old biological mother.[30] Dolly's embryo was created by taking the cell and inserting it into a sheep ovum. It took 435 attempts before an embryo was successful.[31] The embryo was then placed inside a female sheep that went through a normal pregnancy.[32] She was cloned at the Roslin Institute in Scotland by British scientists Sir Ian Wilmut and Keith Campbell and lived there from her birth in 1996 until her death in 2003 when she was six. She was born on 5 July 1996 but not announced to the world until 22 February 1997.[33] Her stuffed remains were placed at Edinburgh's Royal Museum, part of the National Museums of Scotland.[34]

Dolly was publicly significant because the effort showed that genetic material from a specific adult cell, designed to express only a distinct subset of its genes, can be redesigned to grow an entirely new organism. Before this demonstration, it had been shown by John Gurdon that nuclei from differentiated cells could give rise to an entire organism after transplantation into an enucleated egg.[35] However, this concept was not yet demonstrated in a mammalian system.

The first mammalian cloning (resulting in Dolly) had a success rate of 29 embryos per 277 fertilized eggs, which produced three lambs at birth, one of which lived. In a bovine experiment involving 70 cloned calves, one-third of the calves died quite young. The first successfully cloned horse, Prometea, took 814 attempts. Notably, although the first clones were frogs, no adult cloned frog has yet been produced from a somatic adult nucleus donor cell.[36]
There were early claims that Dolly had pathologies resembling accelerated aging. Scientists speculated that Dolly's death in 2003 was related to the shortening of telomeres, DNA-protein complexes that protect the end of linear chromosomes. However, other researchers, including Ian Wilmut who led the team that successfully cloned Dolly, argue that Dolly's early death due to respiratory infection was unrelated to problems with the cloning process. This idea that the nuclei have not irreversibly aged was shown in 2013 to be true for mice.[37]

Dolly was named after performer Dolly Parton because the cells cloned to make her were from a mammary gland cell, and Parton is known for her ample cleavage.[20]

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