Biotechnological Applications of Recombinant DNA

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1.0 November 2024

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I Somatic cloning

1. Objectives

- Understand the principles of somatic cell nuclear transfer (SCNT) its role in biotechnology.
- Examine the applications of somatic cloning in agriculture and medicine.
- Explore recent developments, technological advancements, and challenges faced in the application of somatic cloning in various biotechnological fields.

2. Introduction

Nuclear transfer, a method first developed in 1952 to study frog embryonic development, has played a crucial role in advancing cloning technology. By the 1980s, this technique was adapted for cloning cattle and sheep using cells from early-stage embryos. A significant breakthrough came in 1995 when Ian Wilmut, Keith Campbell, and their team successfully cloned live lambs, Megan and Morag, using cells cultured in vitro for an extended period. This was the first time live animals were cloned from cultured cells, marking a transformative moment in biotechnology. This achievement laid the foundation for the development of precise genetic modifications in livestock, with broad implications for agricultural and medical applications*39*[39].

Cloning?

- **Create identical copies (DNA, cell, animal,...)**
- **Process of producing genetically identical individual of an organism either naturally or artificially.**

3. What is Somatic cell cloning?

Somatic-cell nuclear transfer (SCNT) or Somatic Cell Cloning, is a laboratory technique for creating a clone embryo with a donor nucleus. It can be used in embryonic stem cell research, or, potentially, in regenerative medicine where it is sometimes referred to as “therapeutic cloning”. It can also be used as the first step in the process of reproductive cloning. Because somatic cell cloning is a cloning technique, it is best to outline principals underlying the process of cloning. Cloning is derived from the Greek word “ Klon” which means a twig which can replicate itself and grows eventually into a tree.

To clone is to reproduce asexually or to make a copy or a set of copies of an organism following the fusion or insertion of a diploid nucleus into an ovocyte. A true clone is an individual which has all the components that make up the individual, including nuclear genetic material (genome) and other maternally derived factors that are gained from a single unique embryo as a result of sexual reproduction.

In vivo, cloning in mammals involves replacing the genetic material of an egg with the genetic material of somatic cell from an embryo or adult which will eventually develop into a full organism or being; this is a biological clone; an organism genetically identical to another organism.*40*[40]

***Méthode* : Steps for somatic cell cloning**

Common somatic cloning protocols involve the following major technical steps (Figure below).

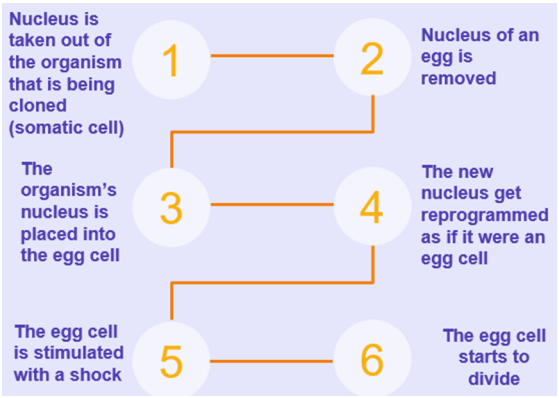
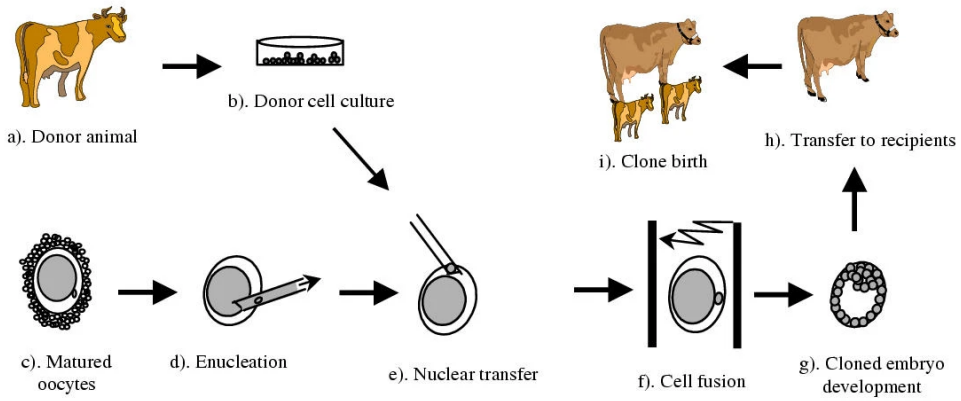


Image 1 Steps for somatic cell cloning



Schematic diagram of the somatic cloning process. Cells are collected from donor (a) and cultured in vitro (b). A matured oocyte (c) is then enucleated (d) and a donor cell is transferred into the enucleated oocyte (e). The somatic cell and the oocyte is then fused (f) and the embryos is allowed to develop to a blastocyst in vitro (g). The blastocyst can then be transferred to a recipient (h) and cloned animals are born after completion of gestation (i).

4. Factors affecting somatic cell cloning

Diverse methodologies have been implemented to enhance the efficacy of nuclear transfer, encompassing modifications to both donor cells and the nuclear transfer procedure. The predominant focus of these actions is directed towards optimizing donor cell characteristics. Noteworthy strategies in this regard involve: a) synchrony of the cell cycle stage of donor cells, as well as synchrony between donor cells and recipient oocytes; b) using somatic cells from donors of various ages, tissue origins, passages and culture conditions; c) transfer of stem cells with low levels of epigenetic marks; and d) modifying epigenetic marks of donor cells with drugs.

Despite marked advancements in enhancing the efficiency of nuclear transfer, with a notable departure from the initial success rate of one live clone per 277 embryo transfers, none of the aforementioned interventions have fully mitigated the inherent challenges associated with nuclear transfer. These outcomes underscore the imperative for further investigations into nuclear reprogramming mechanisms to comprehensively grasp the

underlying processes and substantially refine the capacity of differentiated somatic nuclei to undergo successful reprogramming. The subsequent section elucidates various strategies employed to augment nuclear transfer efficiencies.[41]

4.1. Effect of donor age

Several studies have found that the development rates of somatic cloned embryos remain similar regardless of the donor's age. However, it has observed that clones derived from adult cells often experience abortion during later stages of pregnancy. Moreover, calves that develop to term from adult cells show a higher number of abnormalities compared to those derived from newborn or fetal cells. In addition, results conducted a study in cattle where a large number of cloned embryos were transferred. They concluded that, in general, embryos cloned from fetal cells have higher pregnancy and calving rates than those from adult cells[40].

4.2. Effect of cell culture duration

Studies suggest that cells of higher passages were receptive to nuclear reprogramming. Additional support for this hypothesis comes from a study by Enright et al. ([42]) who showed that cells of later passages contain less epigenetic modifications, i.e., their histones are more acetylated than in earlier passages. This observation agrees with an earlier notion that *in vitro* culture of cells can induce expression of genes that were not expressed before culture. In summary, a greater proportion of late passage cells (passage 18), vs. earlier passage cells (passage 2), were found to be in G0/G1 whether or not they were in serum-starved culture conditions.

4.3. Effect of modification of pre-existing epigenetic marks in donor cells

Epigenetic modifications, such as histone acetylation and DNA methylation, are heritable alterations in chromatin that do not involve changes to the underlying gene sequences. These modifications play a key role in the differentiation of various cell types, despite them having identical genetic material. In natural reproduction, gametes typically exhibit low levels of DNA methylation, which further decreases during early embryonic development. However, in nuclear transfer, the somatic donor nucleus retains the specific epigenetic marks of its original tissue, and these need to be erased during nuclear reprogramming. The presence of these epigenetic modifications in donor cells can affect their ability to be reprogrammed, which in turn influences the success of cloned embryo development both in vitro and in vivo. As a result, pharmacological treatments that reduce epigenetic marks in donor cells before nuclear transfer may enhance their reprogrammability.[40]

5. Cloning competence of various somatic cell types

Many different types of somatic cells have been successfully used for nuclear transfer, including mammary epithelial cells, ovarian cumulus cells, fibroblast cells from skin and internal organs, various internal organ cells, Sertoli cells, macrophage, and blood leukocytes. However, there is still no clear consensus on which somatic cell type is superior for nuclear transfer. This lack of consensus is partly due to the fact that different laboratories use diverse procedures, and cell culture, nuclear transfer, and micromanipulation all require critical technical skills.

To ensure accurate comparisons, the procedures and techniques used, as well as the skill of lab personnel, must be identical for each donor animal and cell type. By looking at the cloning competence of three cell types from the same donor animal, namely ovarian cumulus cells, mammary epithelial cells, and skin fibroblast cells, we can compare the competence of different cell types for reprogramming through cloning. In this case, the

donor animal is a 13-year-old elite diary cow. The following tables summarize the in vitro development of cloned embryos and embryo transfer and calving from different cell types[40]*.

The type of cell used as a donor can have a significant impact on the development of embryos both in the lab and in living organisms. Through various tests and studies, cumulus cells have been found to be the most effective cell type for somatic cloning. This means that when it comes to creating cloned organisms, cumulus cells have shown the best results in terms of both in vitro development and full-term survival. Research conducted on mice has supported these findings when comparing the efficiency of nuclear transfer from different cell types such as neuronal, Sertoli, and cumulus cells, cumulus cell-derived cloned embryos had the highest live birth rate. Additionally, it was observed that cumulus cell-derived cloned mice did not show widespread dysregulation of imprinting, which is important for normal development.

Further studies have compared different cell types for nuclear donors. Results have consistently shown that cumulus and oviduct epithelial cells are the most suitable for nuclear transfer. In cattle, large-scale embryo transfer experiments have also demonstrated the superiority of cumulus cells. Calving rates were highest with cumulus cells, followed by fetal genital ridge cells, and fibroblast cells. Adult fibroblast cells had the lowest calving rate.

In summary, among the various somatic cell types tested, cumulus cells have been identified as the best choice for cloning. They offer the highest cloning efficiency and result in the least number of abnormalities in cloned animals.

Cell type	No. embryo Transferred	No. recipients		No. (%) calves born	Alive to adulthood
		Total	Pregnant		
Cumulus	109	58	10	6 (5.5)	4
Fibroblast	57	29	8	4 (7.0)	0
Epithelium	34	24	1	0	0

Image 2 Summary of embryo transfer and calving of cloned embryos from different cell types

Cell types	No. reconstructed embryos	Embryo development (%)	
		Cleavage	Blastocyst
Cumulus	92	65	57
Fibroblast	110	63	34
Epithelium	96	66	23

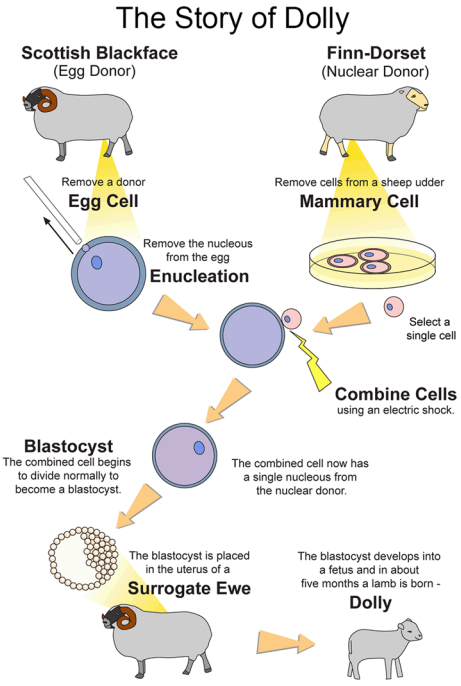
Image 3 Summary of in vitro development of cloned embryos from different cell types

6. Example: Dolly ‘’ same to her mother”

Dolly was the first mammal cloned from a cell from an adult animal. She was created using cells taken from the udder of a 6-year old Finn Dorset ewe. These cells were then cultured in a laboratory for several weeks. The next step involved fusing individual cells with unfertilised eggs, from which the genetic material had been removed. A total of 277 of these "reconstructed eggs" were then cultured for 6 days in temporary recipients. Out of these reconstructed eggs, 29 appeared to have developed normally to the blastocyst

stage. These 29 eggs were then implanted into surrogate Scottish Blackface ewes. After 148 days, one of these implanted eggs successfully gave rise to a live lamb - Dolly. The cloning is passed through these steps:

1. Scientists collected udder cells from Dolly's DNA mother. They allowed the cells to multiply until they reached a sufficient number.
2. A different sheep provided an egg cell, from which the nucleus was removed.
3. Using electricity, the scientists combined a udder cell with the egg cell that had no nucleus. As a result, the egg cell contained all the DNA from the udder cell.
4. The combined cell divided and developed into an embryo, which is the early stage of an animal before it is born or hatched. The embryo was then transferred into another sheep. After five months, this sheep gave birth to Dolly.



Dolly '' same to her mother"

7. Somatic cell cloning Applications

While somatic cell cloning has numerous applications, it is important to consider the ethical and societal implications associated with this technology. Cloning raises ethical concerns regarding the manipulation of life and the potential for misuse. Therefore, it is crucial for scientists, policymakers, and society as a whole to engage in thoughtful discussions to establish guidelines and regulations that ensure responsible use of somatic cell cloning.

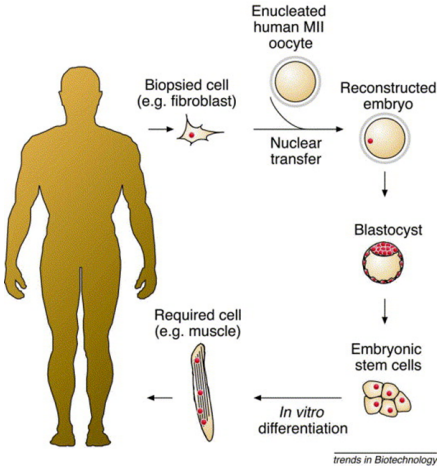
In fact, Scientists tried to clone in cows and pigs, interestingly, the cloning was successful and the progeny was born BUT due to some unknown reason these progeny were more susceptible to infections and they died.

One important application of somatic cell cloning is in agriculture. Through cloning, scientists can reproduce genetically superior plants and animals, ensuring desirable traits are passed on without the need for traditional breeding methods. This can result in increased crop yields, improved disease resistance, and higher quality meat or dairy products. Additionally, somatic cell cloning allows for the preservation of endangered or rare species, helping to maintain biodiversity*43*[43]*.

Somatic cell cloning also has significant implications in the field of medicine. It can be used to produce large quantities of specific cells or tissues for transplantation, such as skin for burn victims or organs for patients in need of organ transplants. This technique offers hope for those on long waiting lists for organ donations, potentially saving countless lives. Furthermore, somatic cell cloning enables the development of animal models for studying diseases and testing potential treatments. By cloning animals with certain genetic traits, scientists can better understand the underlying mechanisms of diseases and work towards finding effective therapies.

7.1. Therapeutic cloning

- Therapeutically cloning is almost identical to reproductive except for the cloned embryo is never implanted in uterus. Instead the embryo is cloned for the sole purpose of extracting stem cells.
- Stem cells have the incredible ability to turn to any other cell in the human body, which means it is crucial for developing new treatment for diseases and have the potential to repair or regenerate tissues or organs.



Therapeutic cloning

7.2. Induced Pluripotent stem cell

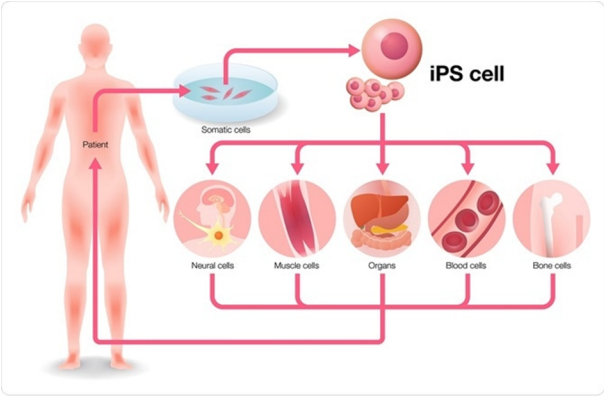
- PSCs are non specialized cells able to differentiate into most cell types when they are placed in certain environment, which can form (bone, limbs, ...).
- PSCs are harvested from embryos in the blastocyst stage (**unethical**).

iPSC

- Allows to convert any mitotic cell to a stem cell.
- Mitotic cells are induced to revert back to a state where it can specialized in two different cell types.
- Take a mitotic cell from a patient body (skin cells) and treated the cells with a specific transcription factors Oct4 (Octamer-binding transcription factor 4) , Sox2 (Sex-determining region Y-box 2), Klf4 (Kruppel-like factor 4), and c-Myc (Myc proto-oncogene).
- These transcription factors enter in competition with the native ones in the cell and took control how the cell behave by changing the packing of DNA and the level of expression of different genes.
- This cause to mitotic cell to revert back to its pluripotent state.

✎*Remarque* : **Update note**

Recent advances with gene-editing technologies — in particular the CRISPR/Cas9 technology — are enabling the rapid generation of genetically defined human iPSC-based disease models.



Induced Pluripotent stem cell

8. Exercice

[solution n°1 p. 11]

Which of the following are important factors affecting the efficiency of somatic cell nuclear transfer?

somatic cloning

- ☐ Synchrony between donor cells and recipient oocytes
- ☐ Modifying epigenetic marks of donor cells
- ☐ The species of the surrogate mother
- ☐ The size of the animal being cloned

Which reagent is commonly used to alter epigenetic modifications in somatic cells before nuclear transfer?

Epigenetic modifications

- ○ Oct4
- ○ Trichostatin A (TSA)
- ○ Sox2

Which cells are best for cloning?

SMC

How many reconstructed eggs were implanted to produce Dolly?

Dolly

What does iPSC stand for?

iPSC

How many days did it take for Dolly to be born after implantation?

Dolly

Solutions des exercices

Solution n°1

[exercice p. 9]

Which of the following are important factors affecting the efficiency of somatic cell nuclear transfer?

somatic cloning

- [x] Synchrony between donor cells and recipient oocytes
- [x] Modifying epigenetic marks of donor cells
- [ ] The species of the surrogate mother
- [ ] The size of the animal being cloned

Which reagent is commonly used to alter epigenetic modifications in somatic cells before nuclear transfer?

Epigenetic modifications

- ○ Oct4
- ◉ Trichostatin A (TSA)
- ○ Sox2

Which cells are best for cloning?

SMC

Cumulus cell types.

How many reconstructed eggs were implanted to produce Dolly?

Dolly

29 eggs

What does iPSC stand for?

iPSC

Induced Pluripotent stem cell

How many days did it take for Dolly to be born after implantation?

Dolly

148 days

Glossaire

Polymerase chain reaction (PCR)

A method widely used to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to amplify a very small sample of DNA (or a part of it) sufficiently to enable detailed study.

Abréviations

DNA : Deoxyribonucleic acid ; is the molecule that carries genetic information for the development and functioning of an organism.

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