The somatic cell nuclear transfer

A method of cloning

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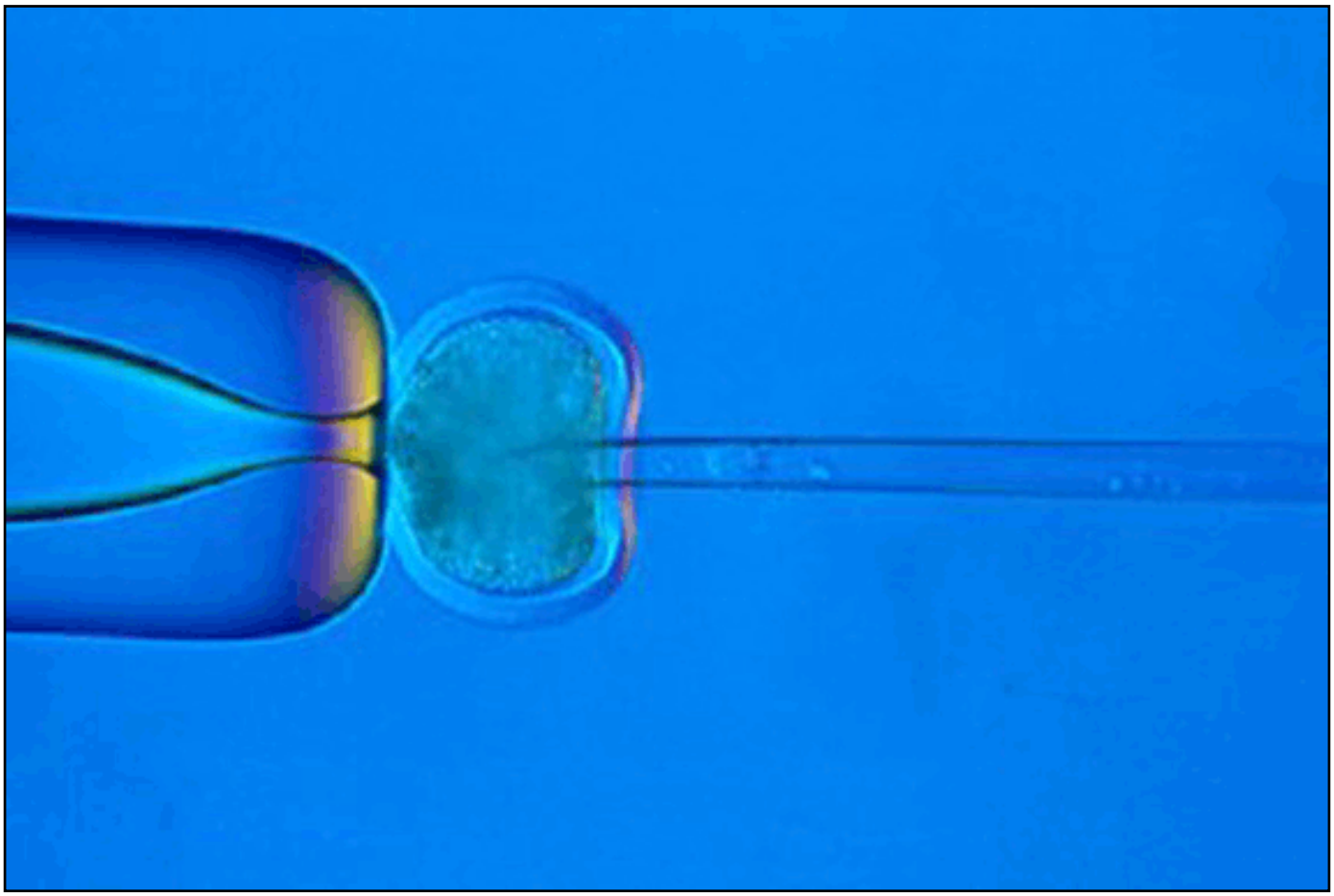


Enucleating of a cell

Content

Preface

Have you ever wished to have an exact copy of yourself? Or dreamt of being able to go to two different events at the same time, or to be able to tidy up your room, while having a cold drink in your garden?
As the following pages will explain cloning isn't making an exact copy of someone but rather making a copy of an individual's genome. Today, scientists are working on several different cloning methods.

When our biology teacher, Mr. Ruggle, told us, that we had to write a paper on "Genetic Engineering", we immediately decided to write a paper about cloning.
Everybody has a general idea of what cloning is, but very few people can tell you straight away how it is done and what for. Therefore we decided to discover more about the process of cloning and its recent achievements.
After some research into the topic we decided to further examine the process of somatic cell nuclear transfer. One of the first and best known experiments, which were done in this field and that was successful, was the cloning of "Dolly" the sheep in 1996.
In short, the following was done: The nucleus of an adult fin-Dorset sheep's mammary gland cell was taken and fused into a Scottish- Blackface unfertilized oocyte. This was implanted in a surrogate mother sheep and carried to term. A cloned version of the Fin-Dorset was born and named "Dolly". It lived for 6 years.
This experiment fascinated us and we decided to dig deeper into the topic of cloning.
Our research began and we started to collect articles from different experiments. Through a colleague we got in touch with our interview partner Cem Sievers. He is a professor at the ETH in Zürich. He showed us some experiments in the lab, which were very interesting.
In conclusion, the aspect we find most fascinating about cloning is the vast amount of possibilities provided by this technique.

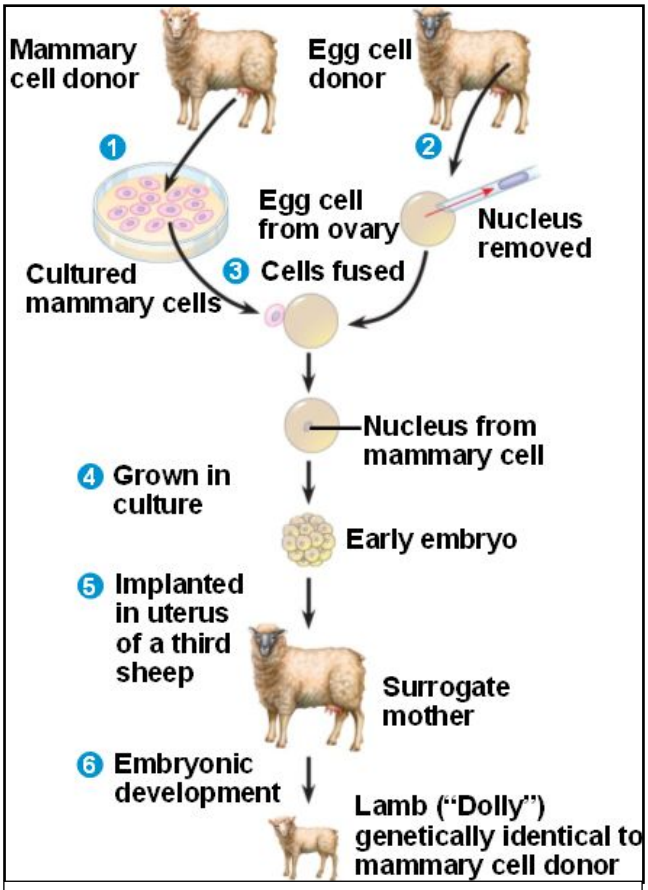
Questions:

1. What exactly is the somatic cell nuclear transfer (SCNT)?
2. What factors could improve SCNT?
3. What are the advantages and disadvantages of cloning?

The applied Technique:

This paper looks at a technique called the somatic cell nuclear transfer, short SCNT.
As the name already suggests, this technique uses adult body cells (Greek soma= body) as nucleus donors from which the nucleus is transferred into another, previously enucleated cell.
These are the steps:
Two cells are harvested; a somatic cell that acts as the nucleus donor and an unfertilized oocyte that acts as the nucleus recipient. In order to enucleate a cell, the nucleus has to be detached from the surrounding cytoplasm holding everything in place. This is achieved by an electric current causing the structure of the cytoplasm to be loosened. Only then it is possible to extract the nucleus by inserting a needle which is just large enough to contain the nucleus. In doing so as little cytoplasm as possible is extracted[1].
This step is done in both the donor, as well as in the recipient cell.
The oocyte is then ready to receive the nucleus from the somatic cell. As the nucleus is inserted it has to be activated and reprogrammed in order for the cell to divide and develop

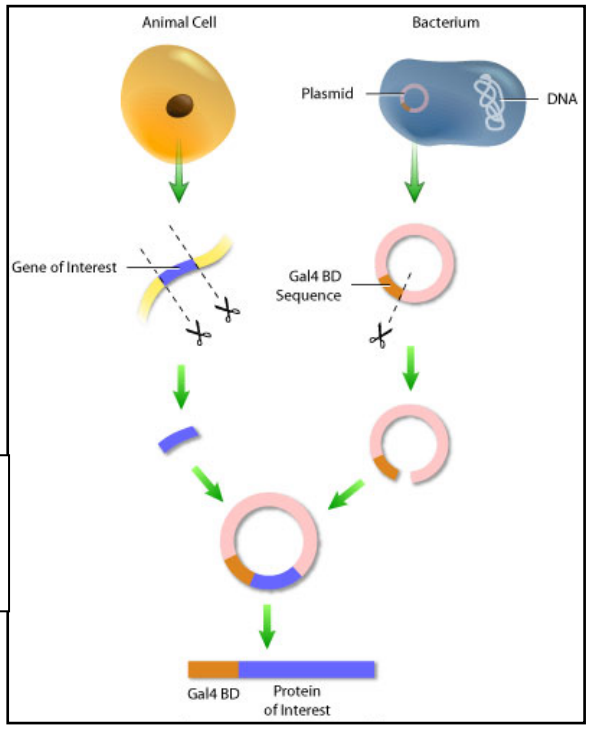
[1] *Link to see this step in reality: http://learn.genetics.utah.edu/content/tech/cloning/whatiscloning/*



All the steps are here shown on the example of the sheep "Dolly" that has been cloned in 1996

into a fully functional individual. Again, an electric impulse is used to stimulate the nucleus, causing it to stabilize inside the new cell and start normal division.
But to develop all necessary traits as an embryo, the genetic material of a highly differentiated somatic cell has to be to some extent pluripotent (this means that the cell is able to differentiate into any of the three germ layers). The nucleus therefore has to be reprogrammed to act pluripotent and this is achieved by inducing the expression of transcription and epigenetic factors as well as of certain genes.
As soon as pluripotency is achieved normal cell division can start while the zygote is grown in-vitro.
When the zygote has become a blastula it is transferred to a surrogate mother who carries the embryo to term. This is the so called clone of the nucleus donor and the two share the exact same genes.

But this technique isn't the only one when talking about cloning. A more frequently used technique in laboratories is molecular cloning. The difference of molecular cloning to SCNT is that not the whole nucleus is transferred into another organism but only one specific gene of the DNA. When the desired part of DNA is cut, it is inserted into a vector, which is circular DNA in a plasmid. The plasmid is then engrafted in the cell of an organism where it multiplies. The information it transports is later expressed in the phenotype.



The process of cutting the DNA, inserting it in a vector and putting the circular DNA inside a plasmid which then multiplies inside an organism (the last step is not shown here).

Introduction and recent History

The technique of somatic cell nuclear transfer (short: SCNT) is a common method of cloning. It has greatly evolved since one of the first experiments with the sheep Dolly and has become more and more specific over time. It is a branch of genetics which researchers are focusing on and are constantly experimenting with. In the context of life today, benefits of cloning humans are unclear. There are also several serious moral and ethical aspects to take into consideration with regards to this process. The moral and ethical issues with cloning cattle and live stock are obviously different. Here SCNT can be useful for producing cattle that grows faster, that has more

muscle mass as well as more essential substances to us. Moreover, cloning could reduce or even stop the extinction of animals, since endangered species could be cloned. An enormously wide range of experiments is being done on this topic and in the following paragraphs we picked out some of the very interesting publications from 2005 to 2013 and shortly summarized them.
It has been observed that by using SCNT, only a few clones survive. There are also often neonatal deaths and organ abnormalities in clones.
In February 2005 a group of researchers in Beijing, China, published their work about trying to define the genetic cause for these abnormalities. They examined eight important genes involved in foetal development. In this project the researchers tried to determine the influence of the age of the donor cell on the gene expression. They found derivations of genes in the clones but most of the gene derivations were common in the clones' derived from the adult and from the foetal fibroblasts. Still, there were some genes that were deregulated either in one of the fibroblast ages or in the other. The heart was the organ that was most affected by deregulations, it was suggested that the neonatal deaths might be due to gene deregulations. *Source: PMID: 15240423*
In the same year German scientists published a fairly interesting paper suggesting and supporting the theory that neonatal deaths and generally speaking the low efficiency of cloning might be due to mitochondrial DNA recombination errors. *Source: PMID:15745633*

In 2006 an American group of researchers published a paper concerning cloning that has improved the tool quite a bit. One of the reasons why SCNT has such a low efficiency in mammals is the weak fusion between the small somatic donor cell and the big egg cell. In this experiment the researchers treated one group of bovines with PHA (phytohemagglutinin). They discovered that this protein improved the fusion efficiency of SCNT in cattle as well as the development of cloned blastocysts. *Source: PMID: 16045975*

In 2007 the FDA (Food and Drug Administration) gave its permission to produce clones of cattle, goats and sheep and they legalized the sale of these animals for consumption. Their report stated that to eat clones or their progeny was as safe as eating the animals they were cloned from. *Source: PMID: 17055042*

In 2008, a paper from workers in South Korea was written, examining the effects of amino acids believed to be nonessential in SCNT. Their experiments suggested that those amino acids in fact have a positive aspect on the yield and quality of cloned pigs, also increasing their total cell number. *Source: PMID: 17886265*

In 2009, American scientists in Michigan discovered that bovine SCNT- derived embryos could be activated by PLCZ, a sperm-specific enzyme which could also improve some aspects of nuclear reprogramming.
Source: PMID: 19074500

In 2010, a study was conducted in China; the gained insights might in future save thousands of lives. This study suggests that through SCNT autologous histocompatible stem cells could possibly be produced. This would mean that in case of a heart attack for example dead cells could be replaced by a clone's stem cells. This would eradicate the problem of incompatibility with donor organs. They stated: "The nuclear-transferred embryonic stem cells with chitosan hydrogel have been proved to possess therapeutic potential to improve the function of infarcted heart. Thus the method of in situ injectable tissue engineering is promising clinically." *Source: PMID: 19905874*

Experiments have been done to try and use SCNT for producing interspecies. In the study of Chinese researchers in Baotou, 2011, foetal goat fibroblasts were transferred into an in-vitro matured sheep egg cell. They divided the procedure of cloning into two steps, which significantly improved blastocyst rate of a goat-sheep interspecies cloned embryo.
Source: PMID:21082282

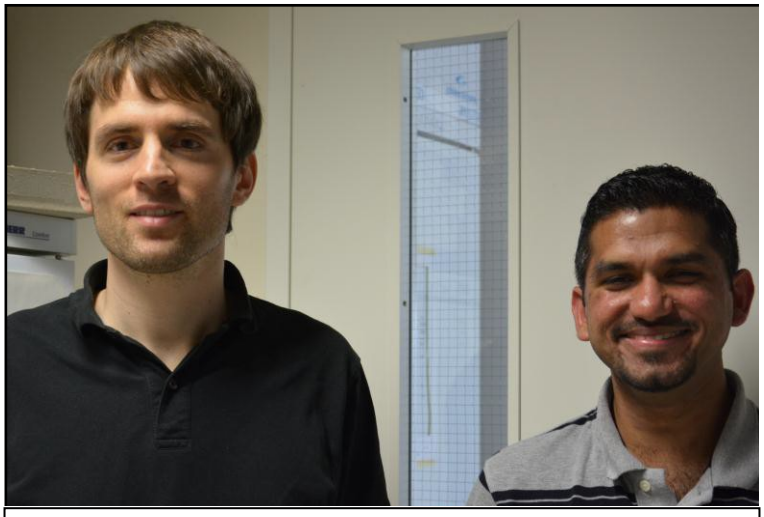
American and Chinese scientists worked together to publish a very interesting paper in April 2012. They found out that the treatment of somatic cells with Xenopus egg extract had a very positive influence on the development of the embryo. It reduced DNA methylation (: adding a methyl group to adenine or cytosine in the DNA) and increased the total cell number in blastocysts. *Source: PMID: 23515109*

On the 20th of March 2013, PloS One published a Chinese work. Myostatin is a regulator that inhibits skeletal muscle development and growth. The researchers using RNA expression targeting Myostatin managed to significantly reduce MSTN and in doing so produced sheep that had a tendency to increase in their body weight faster than control sheep. These findings mean that it could be possible to indirectly regulate the muscle mass of a sheep. *PMID: 23526994*

All these experiments demonstrate that cloning is possible, and has been possible for multiple years but that its efficiency is rather low. One of the biggest problems that scientists are trying to eradicate is the occurrence of errors in the step of reprogramming. Researchers are trying to find all kind of factors that would optimize the survival of the somatic cell nuclear transferred embryos. Others are focussing on the fact that through SCNT, one could optimize muscle mass of cattle. Another breath-taking aspect is the one concerning possible therapeutically uses of SCNT.
To the question whether there are alternative treatments, SCNT is in a way a kind of alternative treatment itself to artificial insemination or natural fertilisation. However the basis is different, since in both other situations there are at some stage haploid cells, whereas during cloning you always have diploid cells. SCNT can also be looked at as an alternative to molecular cloning or to IPSC (induced pluripotent stem cells). Nevertheless, there still are multiple differences to the techniques, methods and the fields of use. Which one of these alternatives is used depends on what one aims to do.

Interview and visit of the lab:

Interview Questions with Cem Sievers Professor f. Computational Biology (D-BSSE)
and Allwyn Pereira Department of Biosystems Science and Engineering (D-BSSE) PHD-student
For reasons of simplicity we chose not to differentiate between their answers. The interview was more like a discussion and both experts were talking and completing each other.



Cem Sievers and Allwyn Pereira in the lab

1. **A commonly known experiment with Dolly the sheep was done in**

1996. How much did the efficiency of cloning develop since then?
Dolly the sheep was only one example of numerous experiments. Although it wasn't the first experiment in this field, it was one of the most important. Nowadays you could have a clone of your pet, if you wanted. There are actually some companies who offer that.
Japanese scientists have done extensive studies comparing the efficiency of SCNT to IVF (in-vitro fertilization). Calves for example are much more likely to survive by IVF than by SCNT. When I say survive, I mean the process of getting through the first years of life. Scientists observed that there is a lot of fetal loss during pregnancy of the SCNT derived embryos.
Coming to your question of efficiency, we can say that the efficiency has increased since 1996. But SCNT is a very inefficient method of cloning, because it depends on many other factors. In nature there will always be factors that can't be controlled.

2. **What are the most recent advances and achievements concerning the somatic cell nuclear transfer?**
A really interesting thing that came up in the last years is that you can take a very specialized cell, for example a B-cell (which is a cell that produces antibodies) and you can still produce a cloned body. This is special, since it was believed that specialized cells wouldn't be able to obtain pluripotency and give rise to an organism.
What is already possible with SCNT is to save animals from extinction. Scientists have regenerated African wild dogs by this cloning process.

3. **Are the silent and expressed genes in two clones identical?**
That is something that is very difficult to say, actually what should be identical are the genes, but there can still be changes in the ones being silenced or expressed. When DNA is replicated, the DNA polymerase will always make some errors and mistakes. Therefore the clones have the same genetic information, but the expressed and silent genes can change from clone to clone which is why for example two clones might not look exactly the same.

4. **What is the difference between the embryonic stem cells and your own body stem cells?**
In principle the embryonic stem cells are much more potent, so they could give rise to any tissue within the body, whereas the adult stem cells are further differentiated. Great therapeutically aspects would for example be to grow entire tissues with embryonic stem cells. This is why scientists distinguish between pluripotence and totipotence. The whole advantage of having a clone is that the body doesn't reject the new body cells, because they have the same genetic material as your own cells.

5. **Are there any projects about the cloning of humans? If so, what is being done and which technique is applied?**
Most of the people would agree that cloning of humans is unethical. I don't know about any projects, but I would not be surprised to hear one day that people have abused knowledge and actually cloned a human.

6. **What is cloning used for today?**
Today, in most of the labs they use molecular cloning more than SCNT.
At the time SCNT is still in development and used as a means to

understand development whereas molecular cloning is commonly used for medical purposes.

7. **Which diseases do you think will be treated with cloning in the future?**
   They are working on a method to heal cancer with molecular cloning. They would inject a piece of DNA in the tumor cells and those surrounding the tumor. This piece of DNA would recognize the cancerous cells and kill them from the inside, whereas the healthy cells would survive. There were attempts on humans where this method worked. However this works best when the tumor is still in a very early stage.

8. **What are the dangers of cloning?**
   The major danger of putting clones into nature is the unpredictable environmental factors affecting the growth. There is a good example with golden rice. Scientists have introduced vitamin A into the rice, and planted it. The major danger of planting cloned rice is that you also don't know the effects of it on the other organisms. What would happen if birds ate it, or how is the ecological balance going to react to it? Nature has evolved over millions of years, so the wild type of rice is more likely to survive the rather harsh conditions set in nature than a man-made type of rice. It is very unlikely that humans will be able to regenerate something superior to what nature has created.

9. **Are you or were you involved in a project with cloning? If yes, what was it about? What did you find out?**

Drosophila melanogaster, the flies used in the experiment

The experiment I can show you is an example of molecular cloning. We have taken out specific DNA segments from flies, cloned them, tagged them with EGFP (green fluorescent protein) and inserted them back through vectors. By this process we could visualize the location of the inserted DNA segment.
Allwyn Pereira also showed us an experiment where they mutated extracted genes and inserted them through vectors giving rise to flies with curled wings, white eyes and other characteristics shown in figure 2.

Figure 2: *Poster showing the different mutations in flies*

Discussion

What factors improve SCNT?
Scientists have discovered that certain substances improved the embryonic development and increased the total cell number of the clones produced by SCNT. Through SCNT it is also possible to accelerate muscle growth in cattle. This possibility is used for agricultural purposes and has therefore very profitable aspects for the economy. From the experiments we looked at before we derive that a lot of research concerning SCNT has been done allowing an improvement in the techniques and the survival of clones.
An advantage of cloned animals and crops is that they can be used in agriculture because of the economical profits of favourable traits in cloned cattle or crops.
However there are still a lot of discussions concerning the dangers that cloned crops would bring along. Researchers are not sure whether it would be safe to put such crops into the wildness, because of possible interactions they would have with the surroundings. The scientists learned from previous unfortunate experiences that putting something into nature without being absolutely sure to know how it affects the environment can have fatal issues. Moreover, even if we think to have taken into consideration all the possible aspects, nature will always be a step ahead. Would it be worth the risk to put the natural balance in danger just to have crops that have more essential nutrients, that grow faster or that are more resistant to certain factors? Although this question might sound simple, economic advantages of cloned crops weigh quite heavy in the balance.
All over the world a lot of research is being done about cloning, but a lot of laboratories have only one goal: to enlarge our knowledge. However one can never know whether this knowledge will not be abused by others in order to chase personal goals and achievements. SCNT is a tool that has to be worked with cautiously and with a certain overview.

Therapeutically, a further, very interesting aspect is the possibility of using molecular cloning in order to control cell death of tumor cells in the case of an internal cancer. Molecular cloning is the usual process used to create transgenic organisms. In our interview with our experts we also talked about the dream of eradicating genetic diseases by using the process of molecular cloning relying on vectors, with the help of which we could change the genotype that is responsible for the disease. Nevertheless, the huge problem is that it would be imperative to do this in each one of the patient's body cells which is impossible.

What would be the Advantages and disadvantages of human cloning?
The moral and ethical aspects of human cloning are fairly controversial discussed among scientists. One of the first aspects that come to mind when thinking of cloning is the fright of not being unique and having to share your genome and identity. On the other hand it could provide a new means to reproduce and have children, for example in cases where one partner in the relationship cannot have children. However, cloned individuals are more prone to have genetically conditioned deformations and diseases, due to reprogramming errors.

Probably the most controversial aspect is the cloning of humans in order to harvest their organs when needed. This would eradicate the problem of rejection in organ transplantation. But wouldn't we go one step too far bringing a human into life only serving as organ donor for another human being?

During our interview we had a very interesting discussion with our experts about the possibilities that embryonic stem cells provide and the differences between stem cells of

early embryos and those that are in specific parts of the adult body.
The most important aspect to us is the one dealing with the possibility to replace a partially destroyed organ. Stem cells (totipotent) derived from your clone in its early embryonic stage could be given specific functions in order to grow tissues that would be able to replace the destroyed area. This would have a revolutionary effect on patients having organs with partially dead tissue since the transplanted tissue wouldn't be rejected. On the other hand to produce these totipotent stem cells, it is necessary to create human life.
But isn't it killing a human being, when these stem cells are taken out and the embryo dies?
The great moral and ethical question is whether "normal" human beings are superior to cloned ones. Can mankind create humans just in order to exploit them?

Summary

Shortly, cloning is a branch of genetic engineering that is being explored more and more. The molecular cloning is already widely used today. SCNT, somatic cell nuclear transfer, is more of a means to understand human development and a hope of a source for embryonic stem cells that are totipotent and could have a therapeutical future. During the last couple of years the methods, possibilities and efficiency of SCNT have greatly improved and led to successful achievements, for example "Dolly" and many others since then. Sometimes the improvement was only due to adding a specific protein. When talking about cloning, one of the first things that come to mind is human cloning; it is a fairly delicate subject. Most people would agree to say that it is unethical. However, cloning animals for human benefits is a different story. The controversy of cloning is already one of the hottest subjects among scientists and will be further discussed in the future.

Sources

http://biotech.about.com/od/cloning/tp/DNAcloning.htm 07.04.13
http://learn.genetics.utah.edu/content/tech/cloning/whatiscloning/ 07.04.13
http://highschoolbiology.otago.ac.nz/index.php?option=com_content&view=article&id=106
http://en.wikipedia.org/wiki/Cell_potency#Pluripotency 14.04.2013

Books

- Principles of cloning written by Jose Cibelli, Robert P. Lanza, Keith H.S Campbell and Michael D. West
- Gene cloning an manipulation written by Christopher Howe
- Grosses Handbuch Genetik, Grundwissen und Gesetze written by Juliette Irmer and Ulrike Siedel
- Genetik 50 Schlüsselideen written by Mark Henderson
- Gentechnologie für Einsteiger written by T.A Brown
- Dolly der Aufbruch ins Biotechnische Zeitalter written by Ian Wilmuth, Keith Campbell and Colin Tudge

Else

- Pubmed, PMID means Pubmed ID