Mammalian Cloning

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On March 31st, 1978, J.B. Lippincott, a reputable New York publisher, rushed into print a remarkable and unique book. Entitled "In His Image", this book was authored by a 34 year-old freelance science writer named David Rorvik, and provided the disturbing details of how he had helped an eccentric multimillionaire clone himself. The book centred round the desires of a very wealthy individual (codenamed "Max"), who enlisted the author's help to recruit a respected scientist (codename "Darwin") in his quest to create his own double. In top secret conditions at a remote and unnamed island near Hawaii, experiments were performed in a state-of-the-art medical facility that the millionaire had provided. At this facility, a doctor (codename "Mary") ran a gynaecological service to provide Darwin with egg donors (young women who were seeking tubal ligation, and who were injected with drugs for superovulation). The book also describes Max's attempt at finding the perfect woman to bear his clone. In this respect, several hundred women were interviewed before a candidate was finally found (codename "Sparrow"). Eventually, the cloning experiment took place, Sparrow became pregnant, and during the end of the last trimester, the pair flew to the USA for the birth.

When the book was released on April 1st, it caused a major furor worldwide that preyed on every conceivable public fear. Here was the developmental science's worst PR nightmare, laid out in clear concise prose. However, because of its publishing date, it was not completely clear whether it was a true work of non-fiction or whether it was a clever but fictional commentary on science. Since it had unsettled the scientific and public community to such an extent, the U.S. congress held a hearing on May 31st in an attempt to get to the bottom of it. Unfortunately, Rorvik failed to appear, but countless prominent scientists testified that the concept and possibility of mammalian cloning was simply not possible and likely never would be. In the end, the dispute was only settled four years later due to a defamation charge against a prominent rabbit cloning researcher who work was linked to the book. It was at this time when the publisher finally admitted to it being a hoax.

The debate

It is interesting to note that in recent history, the debate behind mammalian cloning, and in particular human cloning, has been relatively quiet. This is primarily because of the overwhelmingly strong scientific conviction that it was simply not feasible.

> *"The cloning of mammals, by simple nuclear transfer, is biologically impossible."*
>
> *– James McGrath and Dvor Solter*

In retrospect, this is why July 6th, 1996 is considered to be a monumental date in the history of science. This was when Dr. Ian Wilmut announced the successful cloning of a sheep named Dolly, not from the union of a sperm and an egg, but from genetic material derived from an adult cell, a mammary cell from the udder to be more precise. In reality the procedures for this achievement are remarkably straightforward, but in order to fully appreciate the accomplishment, it is first desirable to go over some of the nuances behind the theory.

Cloning theory

To begin with, one must be familiar with the concepts of differentiation in the development of an organism. Basically, this is simply a term that describes how a single cell has the ability and potential to develop into a specific type of cell, whether it is the cells responsible for light uptake in your eyes, or the cells that guide electrical signals in your central nervous

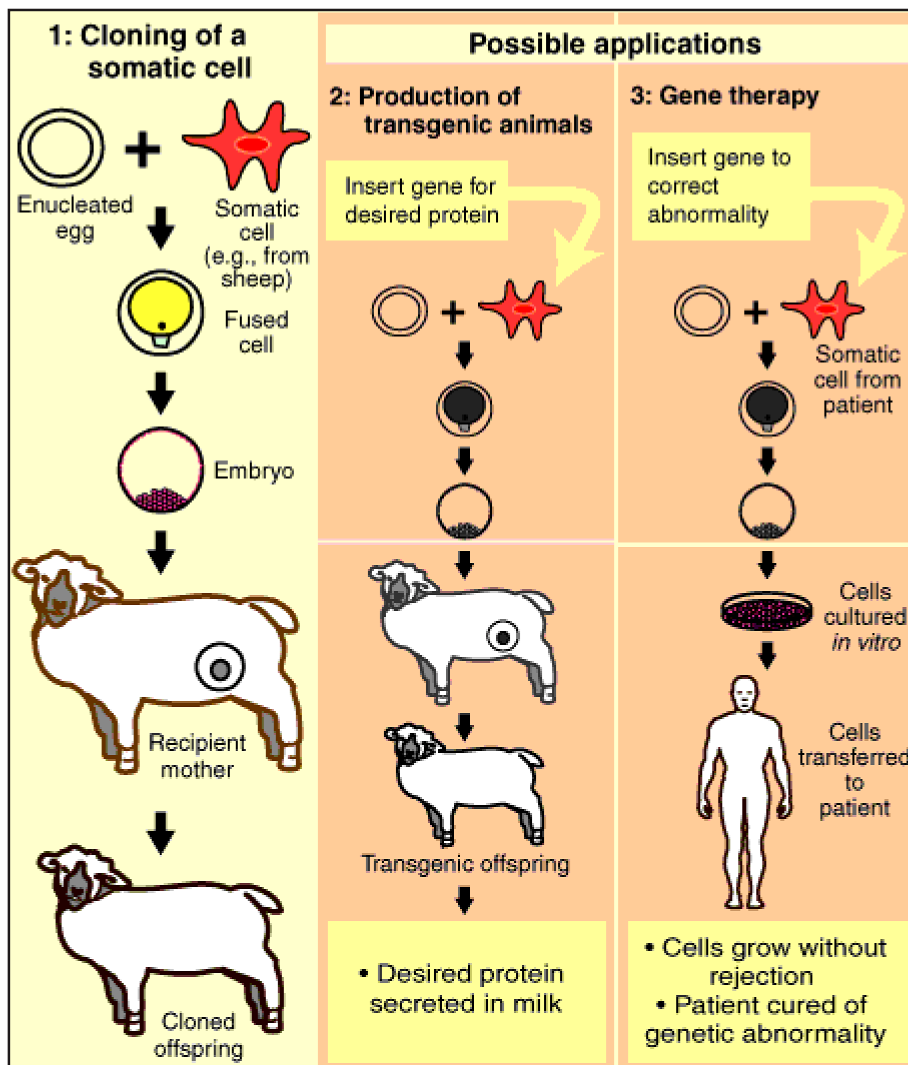

figure 1. Cloning of a somatic cell and possible applications of cloning.

system. Amazingly, it is also important to realize that every cell (except for a very few special exceptions) in a mammal has the same DNA code or genome. This means that technically, the code or blueprint in the eyeball and in a nerve cell, for example, are identical. However, various cell types do not need or use all of this DNA at any given moment – a particular cell only uses the genes required for its specific function. Consequently, as differentiation to more specific cell types proceeds, something happens to the DNA such that only specific parts of the code are utilized. Scientists often refer to this as the DNA in the cell becoming committed or differentiated. It's this differentiation that makes cloning difficult. In order to make a clone, you need to get a genome in pristine condition, and more importantly, this DNA needs to be in a state that has the potential to become any other type of cell. In other words, you do not want this material to be differentiated in any manner whatsoever. Consequently, the idea of cloning an entire mammal becomes technically tricky and needs to be achieved by following one of two routes:

(1) The first route simply entails the use of cells that have not been committed to any developmental pathway. These cells are often called totipotent or pluripotent, and include the zygote (that first cell!), and a few divisions after that (i.e. for a mouse, it's about 3 divisions). A more trendy term that is discussed elsewhere is stem cells. The problem with using stem cells is that using cells at these early embryonic stages is extremely difficult, in that these

cells are not easy to find or work with. This avenue has actually been feasible for a while, and was not actually the cause for the extensive media coverage.

(2) The second route is what caused all the excitement and furor. This alternative route relies on the ability to take differentiated DNA and somehow reset it to a totipotent/pluripotent state. And until 1996, this route was considered to be biologically impossible.

Why was this resetting process deemed impossible? The short answer is that life is complicated. People often forget that a living cell is not a simple bubble with a nucleus inside it. DNA is not just a perfect chain of A's, T's, C's and G's; it is an incredibly complicated structure containing a multitude of different components, molecules, and chemicals. At any given moment, the DNA that you want for cloning purposes is arranged in a myriad of different shapes, altered in a variety of chemical ways and interacting with millions of molecules. We need to forget about the simple ATCG nucleotide double strand and look at the genome in a much larger context.

First of all, DNA is packaged in a very specific manner that is guided by certain key proteins. This combination of DNA and proteins is often referred to as chromatin structure. Nature has evolved a very elaborate system of compacting the DNA molecules using proteins such that the huge chains can fit neatly within the confines of the small nucleus. This packing is thought to play an important role in defining what sequences are used by any given cell type. Proteins that are responsible for this consist of the histones which generally exist in groups of four (H3, H2A, H2B and H4), which control a first order of packing. The addition of other proteins such as the histone H1 creates a second order of packing. Furthermore, there includes an almost endless list of Scaffold proteins that result in even higher order structuring, and a list of high mobility group proteins, which interact to cause structural kinks or bends in the chain.

On top of all this, the DNA (and also the proteins) themselves can be chemically altered to affect function. The two best examples of this are the methylation of cytosine nucleotides, and the acetylation of histone proteins. The idea is pretty straightforward in that the addition of a methyl (-CH3), or acetyl (-C2H5) group somehow changes the way DNA and proteins behave. In other words, methylation may make it easier or more difficult for histones to come on board, or acetylation may make histones more or less likely to compact the DNA structure.

Overall, the common theme in the discussion above is that depending on what happens and when and where it happens, the look and feel of chromatin structure along the genome is altered. This is a particularly relevant fact, because chromatin is often divided into two forms: an open conformation known as euchromatin, and a closed conformation known as heterochromatin. The current hypothesis suggests that in order for genes to be expressed or used, they need to be accessible to the transcription and translation machinery that is ultimately responsible for making the specific proteins for each type of cell. In order for this to occur, accessibility appears to correspond to the open (euchromatin) conformation. Likewise, the areas that are packaged (heterochromatin) are essentially "closed" for business. Currently, most of the data suggests that acetylation of histone proteins plays a key role in opening up chromatin structure. Conversely, methylation of cytosine nucleotides may have a silencing effect (that is, rather than affect the structure per se, it simply blocks access to key transcription proteins). In truth, both topics are still very loosely defined, and the role of methylation is particularly controversial.

At the end of the day, all of this information leads to one inescapable fact: DNA is not just plain old DNA, it can be incredibly variable from tissue cell to tissue cell, in different developmental stages, and at different stages of its own cell cycle. Add to this that these differences spans a huge region (for example, the human genome is a good 3.3 billion nucleotides in length), and it becomes apparent that even the mental exercise of imagining a reversion to a particular specific state is a difficult thing to control. Yet this is what needs to be done to use an adult DNA source for cloning. You need to take that DNA genome, keep it intact and actually get all the histones, proteins, methylation, acetylation events to revert back to the totipotent state. This was simply considered an impossible task to achieve.

Cloning in practice

How is mammalian cloning actually done? What does the researcher need to keep in mind? One of the obvious considerations to think about is the choice of nuclear donor. This is the DNA that you plan to use as your genetic blueprint – that is, the actual instructions to make your clone, and the actual DNA that will be delivered into an enucleated cell (a cell whose original nucleus is removed). There are a number of special criteria to consider, including the donor's tissue type and cell life stage (or cell cycle stage), and even which species of organism will be donate its DNA. Intelligent choices regarding a number of these details contributed to the success of Dr. Ian Wilmut's sheep cloning. For instance, sheep tissue is apparently delayed in cell commitment steps in comparison to other organisms such as mice (sheep begin transcribing genes at the 8-16 cell stage, whereas mice start as early as the 2-cell stage). However, Dolly's success was primarily due to taking DNA at a certain stage of the cell cycle. More specifically the DNA was taken from cells that were deliberately starved to induce a quiescent or sleeplike state. In technical terms, this is referred to as G0 ('G zero') in the cell cycle. Basically, it appears that under these inactive conditions the DNA is less likely to be in use (for transcription or even replication), and the likelihood of differentiation or even errors during replication is far lower.

Another technical consideration is the tissue type of the nuclear source. This is important for the successful reprogramming of the differentiated DNA into that zero-level totipotent "I can be anything" state. In this respect, the type of tissue cell is a key factor since different tissue types have different degrees of chemical modifications and different types of chromatin structure which ultimately can be easier or harder to reprogram. There is also the distinct possibility that the starvation and use of G0 DNA can prolong the ability of the nuclear material to reset itself. This prolonged reprogramming can be so central to the success of the clone that often researchers will attempt what is known as a serial nuclear transfer technique. This is just a fancy term for taking the DNA out, and putting it in a fresh young cell (like an unfertilized egg). After one round of replication, take out the DNA again, and once again transfer it into a fresh young cell. This gives the DNA an opportunity to be in a totipotent-like cytoplasmic environment for two rounds instead of the usual one, and it appears to significantly increase the likelihood of success.

However, this particular success will more or less fall on the skills of the individual taking DNA in and out of very small cells. The technology involved in this act is quite advanced but still relies on the very steady hands (no coffee!) of a practiced operator. However, the recipient cell, which is simply a developmentally very young cell that will receive the DNA, must have its own DNA removed (enucleation). This part of the procedure is especially challenging and relies on the use of microinjection machines. These machines guide specially prepared needles that puncture the zona pellucida (outermost layer) of an egg, without actually puncturing into the cytoplasm. At this point, they attempt to pull out the existing nuclear material as a small stretched out bubble of intact cytoplasm, which will eventually fall into itself and form a structure known as a karyoplast (a small bubble of cytoplasm and nuclear material). A good analogy is to take a balloon and use a vacuum hose to pull a small portion of it out! Despite the technical difficulty, you end up with a cell that to all intents and purposes is fresh or totipotent, but is lacking DNA.

You then supply the DNA using a microinjector. Generally, you can do this by injecting the DNA directly into the cytoplasm of the enucleated cell OR you can inject a karyoplast of material next to the cytoplasm, and then through viral or electrical stimulation, cause the karyoplast and enucleated cytoplasm to fuse together.

In closing

The bottom line is that this is a very tricky procedure, which is probably best exemplified by its low success rate. As an indication of this feature, it is pertinent to inform people that Dolly the sheep was one successful attempt out of about 300, which is approximately equivalent to a 0.3% fruition rate.

Currently, this low rate of success is probably the most compelling reason to not clone humans. With this small chance of success, should an experimenter be willing to attempt this procedure on a person? What are the ethical considerations, should the experiment go wrong? Interestingly, the vast majority of people (scientists or otherwise) appear to agree that the act

of human cloning is repulsive or, at the very least, unsettling. However, the reasons for this are surprisingly personal rather than based on objective observation. This reaction has even been called the "ick factor" in past editorials. Many proponents including those belonging to the human cloning foundation believe that this animosity is simply a natural progression and is similar to those experienced when in vitro fertilization techniques first surfaced. However, this particular topic has appeared to excite public opinion, especially with the activities of biotech firms like Genetics Savings and Clones (who offer gene banking and future pet cloning services), and fertilization specialists seeking notoriety (like Dr. Angatanio who claimed to have cloned the first human in April 2002). It will be interesting to see how this particular storyline unfolds.

As a final note, it should be stressed that the science behind this type of technology has enormous medical potential in the fields of organ transplantation and tissue regeneration. In other words, one has to be very careful at how this technology is regulated. It is for this reason that the U.S. and, more recently, Canada, have passed laws making human cloning illegal except for therapeutic purposes.

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