

Stem Cells

Repr◻grammed

possibilities in nuclear reprogramming

By Xianlin Li



How did the stem cell, so small that it can only be seen through a microscope, become the focus of an international debate engulfing the worlds of science, politics and religion? From the outset, stem cell research has walked a fine line between questions of morality and the lure of medical breakthroughs. Advances in research involving a process called nuclear reprogramming have added another dimension to the stem cell debate. The latest development in nuclear reprogramming research involves the production by scientists at Harvard of new embryonic stem cells by fusing preexisting embryonic stem cells with large numbers of adult cells. Though this research is still in its infancy, the process of creating new stem cells has garnered approval from both the scientific community and the President's Council on Bioethics. Nonetheless, nuclear reprogramming research remains controversial.

The process of cellular differentia-

tion rests on heritable modifications to the DNA sequence of a cell. These modifications include two types of epigenetic changes, or changes that alter the expression of genes while keeping the genetic code intact: histone acetylation and DNA methylation (1). DNA methylation involves the addition of a

methyl group to areas of the genome known as CpG dinucleotides, and is believed to be responsible for maintaining the individual properties of adult cells. Since epigenetic modifications are re-

versible, modifications in adult cells can theoretically be erased by nuclear reprogramming, returning the cells to an embryonic state.

Nuclear reprogramming occurs after the process of nuclear cloning or nuclear transfer, which is the method by which an isolated nucleus from an adult donor cell is transferred into an enucleated egg, or oocyte (Figure 1) (2).

"Nuclear reprogramming occurs after the process of nuclear cloning or nuclear transfer, which is the process by which an isolated nucleus from an adult donor cell is transferred into an enucleated egg, or oocyte (6)."

Reproductive Cloning

In the 1950s and 1960s, the first direct nuclear transfer experiments were performed with amphibians. Nuclei isolated from embryonic cells and transplanted into enucleated eggs were shown to be reprogrammed in their new cytoplasmic environment (3). Experiments using nuclei from differentiated adult cells resulted in a much lower success rate compared to nuclei from larval or embryonic cells (3). These findings led to the conclusion that the difference in success rate was linked to the stage of development of the donor nucleus. Only donor cells with nuclei in the G_0 or G_1 phases of the cell cycle, which involve protein synthesis and cell growth, appeared to be efficient in promoting the development of nuclear transfer embryos (4).

When the embryo resulting from a nuclear transfer experiment is injected into a female uterus, it has the potential to grow into a cloned infant in a process called reproductive cloning (2). Animal clones produced by nuclear transfer successfully demonstrate the ability of the cytoplasm to direct the reprogramming of somatic cells to a totipotent state (5). A totipotent cell has

“Embryonic stem cells produced by nuclear reprogramming are genetically identical to the patient’s cells. This eliminates one of the biggest complications of tissue replacement therapy – immunological incompatibility between hosts and transplanted tissue.”

the capacity to form an entire organism. However, the process of reproductive cloning is extremely inefficient. Only a tiny percentage of manipulated embryos develop normally: typical nuclear transfer experiments using differentiated adult cells produce normal

offspring at a rate of less than 1% (2). Improper epigenetic reprogramming of the somatic donor nucleus is believed to be responsible for the high rate of abnormality (4).

The exact process by which reprogramming occurs is unknown. However, in order for a somatic nucleus to become reprogrammed successfully, a number of critical steps must occur sequentially (6). The transplanted nuclei must become transcriptionally silent; the cellular memory of the adult cell must be erased; the reconstructed “one-cell embryo” needs to be properly activated; and normal embryonic gene expression must occur throughout the later stages of development (6). Since these steps are regulated in part by epigenetic changes, the inability to “reprogram” the epigenetic profile of a somatic donor nucleus to that of a fertilized zygote results in the failure of the nuclear transfer embryo to develop normally (4).

In an attempt to minimize this problem, J. B. Gurdon and other scientists began to investigate the use of unfertilized eggs, or oocytes, as the recipients of transplanted nuclei (Figure 2). The use of oocytes in nuclear transfer experiments was a pivotal breakthrough in the effort to clone mammals successfully. Unlike earlier nuclear transfer experiments, which had been carried out with eggs in the second meiotic metaphase, transplantation of nuclei into oocytes did not initiate DNA synthesis and cell division (3). Experimental evidence from Simonsson and Gurdon strongly supports the existence of enzymatic activity that is capable of demethylating DNA in the absence of DNA replication (7). Transplantation into oocytes was also favored because unlike with somatic cells, the early developmental genes of oocytes did not need to be reprogrammed (4).

In 2001, Rideout *et al.* demonstrated the feasibility of transferring a somatic cell nucleus from an adult cell to an

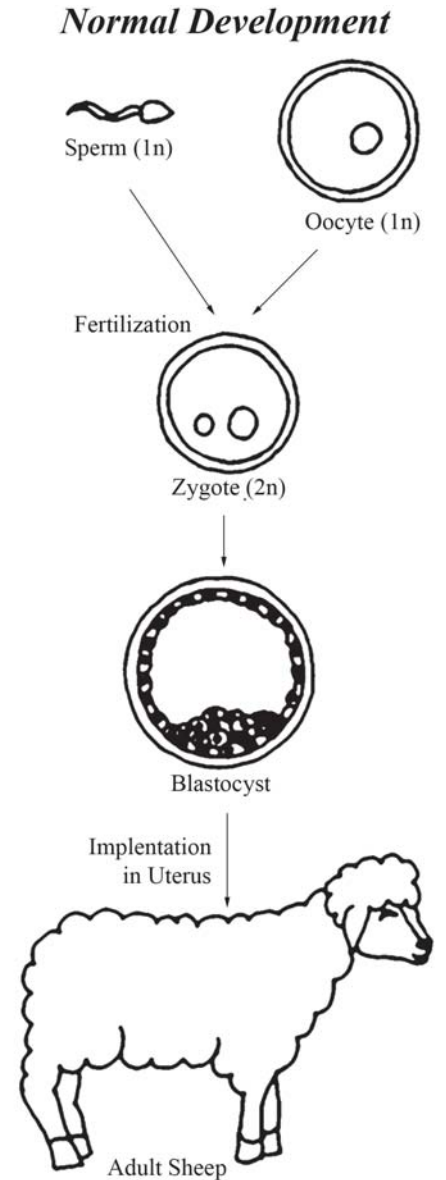


Figure 1. During normal development, a sperm fertilizes an oocyte to produce a zygote. The blastocyst eventually develops into an animal.

enucleated oocyte (1). Since the efficiency of the process remained low and since oocytes are not abundant in mammals, it is important to identify effective donor cells in order to reduce the number of oocytes used in experiments (1,2). Understanding the factors that participate in nuclear reprogramming after nuclear transfer is an important concern because nuclear reprogramming also plays a role in the process of therapeutic cloning.

Therapeutic Cloning

A distinction must be made between reproductive cloning and cloning for therapeutic purposes. In therapeutic cloning, embryos produced by nuclear transfer are grown in culture to generate embryonic stem cells, with the intention of studying or treating disease rather than producing a live organism (Figure 3).

In tissue replacement therapy, embryonic stem cells derived from therapeutic cloning differentiate in vitro to become functional cells (2). Embryonic stem cells produced by nuclear reprogramming can be genetically identical to the patient's cells. This eliminates one of the most significant complications of tissue replacement therapy—immunological incompatibility between the host and the transplanted tissue. Thus, therapeutic cloning can be a method of treating diseases whose current therapies are limited by the availability of compatible tissue transplants (2).

Experiments in animal models have shown that therapeutic cloning combined with gene therapy can be used to treat genetic disorders. Rag2 mutant mice lacking mature B and T cells, which mediate immune responses in mammals, were treated with stem cells derived from the reprogramming of somatic fibroblast cells (2). As only successfully reprogrammed cells are selected for culture, there is a higher degree of efficiency associated with the generation of embryonic stem cells from somatic donor cells compared to the process of reproductive cloning (2).

However, the consequences of nuclear reprogramming are still a source of concern because abnormal expression of some imprinted genes can lead to the eventual onset of dis-

ease. Since genetic imprinting is regulated by epigenetic modifications, more experimentation will be needed to determine whether or not there are any adverse effects associated with the function of somatic cells derived from reprogrammed embryonic stem cells. A better understanding of the process of nuclear reprogramming may offer a method of producing embryonic stem cells for therapeutic purposes while potentially overcoming some of the ethical dilemmas associated with this research.

The Use of Embryonic Stem Cells

Since oocytes are rare and relatively hard to isolate, the use of embryonic stem cells provides a more convenient alternative for studying nuclear reprogramming (1). One of the first approaches to examine whether or not embryonic stem cells can reprogram somatic cell nuclei was performed by Blau and Blakely in 1999. Embryonic stem cells were fused with somatic cells to form heterokaryons, or cells that possess two distinct and intact nuclei (8). In a study done by Tada *et al.* in 2001, mouse embryonic stem cells were fused with T cells. Subsequent analysis of the hybrid cells showed that the somatic nucleus acquired some characteristics of the embryonic stem cells with which it had been fused (1). This

and other experiments demonstrated the ability of genes silenced during development to be reactivated by cytoplasmic factors present in somatic cells without the process of nuclear transfer (8).

In research conducted recently by a team of scientists at Harvard, Cowan *et al.* investigated the effects of fusing

"Cowan et al. investigated the effects of fusing human embryonic stem cells with human somatic fibroblasts, or cells that form connective tissue. Their findings showed that human embryonic cells do indeed have the capacity to reprogram adult somatic cell chromosomes after cell fusion (2)."

and other experiments demonstrated the ability of genes silenced during development to be reactivated by cytoplasmic factors present in somatic cells without the process of nuclear transfer (8).

Reproductive Cloning

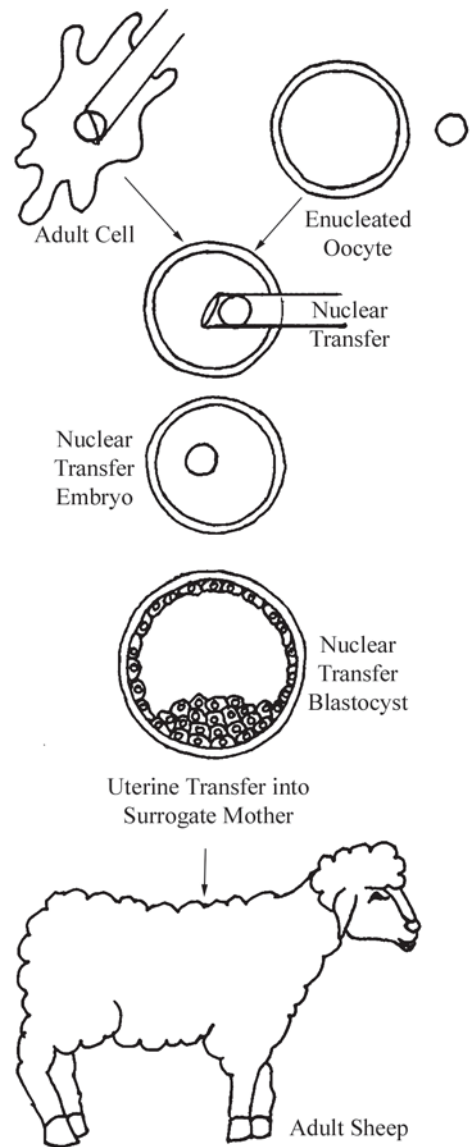


Figure 2. During reproductive cloning, the blastocyst is transferred into a surrogate mother. Some blastocysts will give rise to cloned animals.

human embryonic stem cells with human somatic fibroblasts, or cells that make up connective tissue. Their findings showed that human embryonic cells do indeed have the capacity to reprogram adult somatic cell chromosomes after cell fusion (5). The authors used a method in which human embryonic stem cells and human fibroblasts labeled with drug-resistant markers were fused in the presence of polyethylene glycol and then grown in the presence of antibiotics to select for hybrid cells (5). Roughly one out of

Therapeutic Cloning

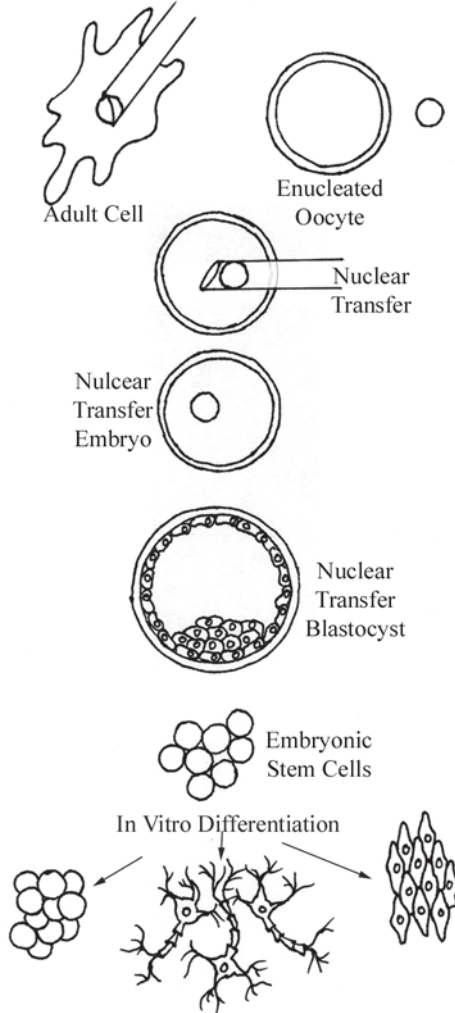


Figure 3. Therapeutic cloning yields embryonic stem cells that can differentiate in vivo into any type of cell to be used for therapeutic purposes.

1,000 of these hybrid cells was reprogrammed to act like an embryonic stem cell (9). The hybrid cells that were reprogrammed exhibited characteristics of human embryonic cells but not of fibroblasts, including the production of transcription factor called OCT3, alkaline phosphatase activity, telomerase activity, and the presence of three embryo-specific antigens (5). The hybrid cells also exhibited dominant embryonic transcription as well as methylation patterns and cell-cycle characteristics similar to those seen in human embryonic stem cells (5).

One complication of Cowan's method is that the hybrid cells produced contained two sets of chromosomes, a condition known as tetraploidy. In

order for the method to become useful for therapeutic purposes, the extra chromosomes of the embryonic stem cell must be eliminated without the loss of any associated reprogramming activity (5). Yuri Verlinsky of

the Reproductive Genetics Institute in Chicago claims to have bypassed this problem by spinning the nucleus out of the stem cell and replacing it with the nucleus of an adult cell (9). His findings have been submitted for publication. In an interview with MSNBC, Harvard researcher Kevin Eggan, who worked with Cowan on the research, said, "We can make a lot of embryonic stem cells, and we can genetically manipulate embryonic stem cells, which to a biologist is really exciting, because now we can get there and we have a system which is manipulatable in different ways than cloning is. It's going to let us have an alternative to eggs as a source of material to look at. This is really cool" (9).

Nuclear Reprogramming and the Political Landscape

Concerns about the use of oocytes from the human body were bypassed to some extent when research showed that oocytes could be produced from existing embryonic stem cell lines (8). However, the larger issue of using embryos generated by in vitro fertilization or by nuclear transfer to produce embryonic stem cells remains controversial. Unlike an embryo derived from in vitro fertilization, a cloned embryo has almost no potential to develop into a normal human being because of epigenetic problems (10). However, disapproval of the use of reproductive cloning is widespread and

the close similarities between therapeutic and reproductive cloning raise many other ethical concerns. These new developments could bypass the ethical dilemmas associated with cloning, but the authors of the study warn that their method should not be considered as a replacement for therapeutic cloning (9).

If introduced, a bill supporting nuclear reprogramming research, among other possibilities, could be passed by the Senate (9). In the future, research related to nuclear reprogramming is expected to involve some researchers trying cell fusion methods to convert adult cells into stem cells, and others continuing to fuse adult cells with components of stem cells to regenerate tissue (9). Whether such research is supported by public or private funds, the field of stem cell research will continue to move forward.

H —Xianlin Li '09 is a Biochemical Sciences concentrator in Canaday Hall.

References

1. Surani, M. A. "Nuclear reprogramming by human embryonic stem cells." *Cell* 122.5 (2005): 653-654.
2. Hochedlinger, K. et al. "Nuclear transplantation, embryonic stem cells, and the potential for cell therapy." *N. Engl. J. Med.* 349 (2003): 275-286.
3. Gurdon, J. B. "Nuclear reprogramming and stem cell creation." *Proc. Natl. Acad. Sci. USA* 10.1 (2003): 11819-11822.
4. Rideout, W. et al. "Nuclear cloning and epigenetic reprogramming of the genome." *Science* 293 (2001): 1093-1097.
5. Cowan, C. et al. "Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells." *Science* 309 (2005): 1369-1373.
6. Dean, W. et al. "Epigenetic reprogramming in early mammalian development and following somatic nuclear transfer." *Seminars in Cell & Developmental Biology* 14 (2003): 93-100.
7. Fujita, N. et al. "Nuclear transfer: epigenetics pays a visit." *Nature Cell Biology* 6.10 (2004): 920-922.
8. Pomerantz, J. et al. "Nuclear reprogramming: a key to stem cell function in regenerative medicine." *Nature Cell Biology* 6.9 (2004): 810-816.
9. Boyle, A. "Stem cell hybrids reveal new path to therapies." *MSNBC*. 22 August 2005. URL: <<http://www.msnbc.msn.com/id/9040770/>>.
10. Jaenisch, R. "Human cloning — the science and ethics of nuclear transplantation." *N. Engl. J. Med.* 351 (2004): 2787-2791.
11. Sprangrude, G. "Stem cells and tissue regeneration: when is a stem cell really a stem cell?" *Bone Marrow Transplantation* (2003): 32, S7-S11.

"The new developments could bypass the ethical dilemmas associated with cloning but the authors of the study warn that their method should not be considered as a replacement for therapeutic cloning (1)."