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Nuclear Reprogramming in Cells

J. A. Smith¹ and J. A. Smith²

Nuclear reprogramming describes a switch in gene expression of one kind of cells that of another unrelated cell type. Early studies in frog cloning provided some of the first experimental evidence for reprogramming. Subsequent procedures included mammalian somatic cell nuclear transfer, cell fusion, induction of pluripotency by simple gene expression, and direct reprogramming. Through these methods it became possible to derive one kind of specialized cell from a fully differentiated somatic, non-stem cell. These cells existed in the same individual. This has provided applications for cell replacement without the immunorejection treatments that are required when cells are transplanted between genetically different individuals. This article provides some background to this field, a discussion of mechanisms and efficiency, and comments on prospects for future nuclear reprogramming research.

As a fertilized egg develops into an adult organism, specialized cells are formed by a complex process, and then become increasingly, and normally irreversibly, committed to their fate. It does not occur automatically that one, single cell, or a finite cell set that can generate all possible cell types, that is, totipotency. Therefore, there are certain experimental procedures that enable such large-scale changes to take place. They avoid nuclear reprogramming to some that describe a switch in nuclear gene expression of a subset of cells that of another or other cell type. This process is to induce the three reasons. First, identifying how reprogramming takes place can help us understand how cell differentiation and specialized gene expression are normally maintained. Second, nuclear reprogramming represents a first major step in cell replacement therapy, in which defective cells are replaced by normal cells of the same or a related kind but derived from a different cell type. Thirdly, it may be possible to derive replacement stem, progenitor, or other types of cells from the skin of the same individual, thereby avoiding the need for immunosuppression. Third, nuclear reprogramming enables the culture of stem cells from diseased tissues, and hence allows one to analyze the nature of the disease and its causes for therapeutic drugs. We review these procedures, discuss the mechanisms that may be involved, and comment on prospects for the field.

Nuclear Transfer in Eggs and Sperm

The earliest evidence for the experimental reversal of cell differentiation came from the transfer of a single cell nuclear into an enucleated egg. This, Hwang and King (1) first succeeded in producing normal, non-neoplastic offspring of these systems by introducing the nuclei of mature differentiated cells. They found, however, that the number of nuclei from slightly older organisms

could be needed only to observed development and concluded that cell differentiation in body is involve irreversible nuclear changes (2). Some other this, similar experiments were carried out with eggs of the South African lungfish *Lepidoscyphus* (3). In this case, it was found that even when foreign nuclei were transferred from fully differentiated cells, in the case from the intestinal epithelium of feeding supports, entirely normal and fertile male and female frogs were obtained. These results led to the conclusion that processes of cell differentiation can be fully reversed and does not require irreversible nuclear changes. It is not a change in nuclear gene expression but not a gene control function, although cells become fully and functionally very different from each other during development, they generate from the same in all cells with the exception of antibody-producing cells and function remain the potential to form any cell type.

The next major advance in this field came with the production of normal adult sheep (Clonal) by transferring the nuclei of cultured embryonic gland cells derived from an adult sheep to an enucleated sheep egg (4). This and later work (5) showed that it is possible to completely reverse the process of mammalian cell differentiation using nuclei from an adult organism and this suggests that this same procedure might work with humans. An important step in this direction has recently been taken by the generation of monkey embryonic stem (ES) cells from the nuclei of adult monkey cells. These pluripotential and differentiation-capable cells are derived from ES cells in germline after transplanting nuclei from adult monkey cells to enucleated monkey eggs (6). It is therefore likely that human eggs contain the components required to reverse the differentiation of adult human somatic cells.

Overview

The gold standard for the completeness of reprogramming by egg has been described as the formation of a fully adult animal containing functional cells of every kind (normal embryonic). However, as far as things is concerned, no in-

terrupted organism is even pluripotential (the formation of every kind of cell type) (Fig. 1A) as a necessary condition. It would not, for example, be theoretically useful to supply a person with spinal cord injury with replacement cells of every kind. In the case of somatic cell nuclear transfer, it is important to determine the efficiency of obtaining a particular differentiated cell type by using the reprogrammed nucleus of an entirely unrelated cell type. It has been shown that the success of nuclear reprogramming decreases as donor cells become more differentiated (7, 8) (Fig. 2). In the frog experiments, nuclei from nuclei of somatic tissues (transferring nuclei from a nuclear transfer cell) to an other set of enucleated eggs and grafts (transferring nuclei from a nuclear transfer cell) to an other set of enucleated eggs to produce the conclusion that about 50% of successful applications cell nuclei can generate functional nuclei and some cells (9). In mammals, cells of a nuclear transfer (transferring) can be used to derive ES cells, whose differentiation capacity is tested by transplanting these cells to nontransferring animals. The frequency with which a normal adult is obtained from the nucleus of a specialized cell is usually 1% to 2%, as compared with about 50% from somatic nuclei (10).

Because of the ethical concerns about obtaining human cell nuclei, animal cell nuclei are those of mice, rats, or which might be used to generate ES cells from transplanted human or mouse nuclei. Nuclear transfer between different species is successful as just as successful as those within a species, however, egg produced by transfer between very different species such as human and mouse, can, in principle, be followed by ES cell (11) (Fig. 3). In fact, there is no confirmed evidence that pluripotential ES cells can be obtained from such distant combinations, including human nuclei in monkey cytoplasm.

Reproduction

The appeal of using eggs to reprogram nuclei in the egg has been the natural ability to generate highly specialized germ-line nuclei with 100% efficiency. Another advantage of this procedure is that it does not require a permanent genetic change in the transplanted nucleus or in the resulting reprogrammed cells. Therefore, it is important to determine the mechanisms involved and why have a successful reprogramming system, and what makes the process frequently unsuccessful even when eggs are used?

The mechanisms of nuclear reprogramming by egg (in normal somatic reprogramming) has been explained by the use of specific chromatin proteins in the somatic genome and immediate progression of eggs. Multiple mammalian somatic nuclei reprogrammed in the germ-line nuclei of an embryo are directly reprogrammed in their nuclei (non-ES) nuclear genes, including *Oct4*, *Sox2*, and *Oct4* (Fig. 4B). Nuclear reprogramming by egg (non-ES) nuclear genes, but, in

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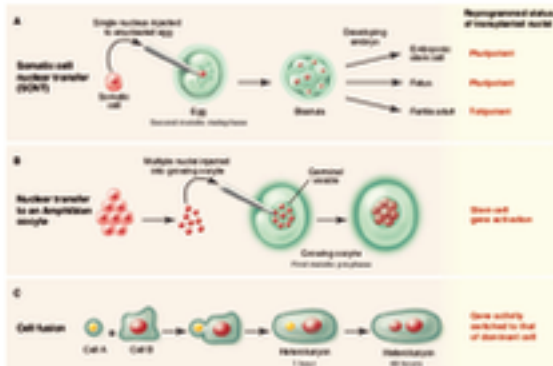


Fig. 1. Diagrams of nuclear transfer experiments (A) to enucleated egg (second nuclear metaphase) of frogs or mammals or (B) to first nuclear frog oocyte. (B) and (C) show the transfer of somatic cell nuclei. (C) Diagram of cell fusion experiments.

contrast to eggs, when placed without cell division and does not need protein synthesis. Mitochondria accompanying the reprogramming include (i) a mature α -globin molecule of 161 base to form fetal hemoglobin and (ii) a mature β -globin molecule of 146 base to form adult hemoglobin (Fig. 3, A and B), due to just to an overall histone clearance (methylation) (17, 22), (iii) the removal of differentiation marks such as DNA methylation (17) and histone modifications, and (iv) chromatin protein exchange, especially of the

very-specific linker histone H1 by the more-specific histone variants (H1a, H1b, H1c). The general principle here seems to be that, during their formation, erythrocytes have expressed very high concentrations of certain proteins that are responsible for the above effects. If egg proteins can be exchanged in seconds or minutes for those in transplanted somatic nuclei as suggested by recent fluorescence microscopy after photochemical experiments (17), complete reprogramming should always take place.

This concept of rapid exchange does not, however, agree with the fact that eggs are often unsuccessful in fully reprogramming somatic nuclei. If the rapid exchange of chromosomal proteins outlined to above applies to all those components of an egg that normally reprogram somatic nuclei after fertilization, then nuclei should fuse in frogs, and even more in mammals, the transplanted somatic nuclei to be fully reprogrammed before the first egg division (20 hours in mammals). This often does not happen. One reason may be that transplanted nuclei carry an opposite memory of their gene expression in their donor cells. For example, nuclei taken from muscle cells sometimes continue to strongly express muscle genes in novel and other somatic cells of an embryo obtained by nuclear

transfer. This may be caused by the incorporation of an *enhancerless* hetero source (23, 24) into the chromatin of daughter of transplanted nuclei (25). The incorporation of the 3'UTR is thought to prevent reprogramming and so to preserve a memory of previous gene expression.

Cell fusion and cell hybrids

It is possible to fuse two somatic cells and to use a cell fusion inhibitor to ensure that the two nuclei remain separate (Fig. 1C). In these heterokaryons, the dominant cell, usually the larger and more actively dividing partner, imposes its own pattern of gene expression on the other partner. Examples include the fusion of an erythrocyte with a growing cultured cell or of a human liver cell with a growing bone muscle cell (17, 26). If successful cytoplasmic of one kind of somatic cell (cytoplasm) can direct another cell, this also implies gene re-expression of their original cell type in the receiving nucleus. However, these fused cells do not produce cells and therefore are not likely to be of therapeutic value.

Similar important conclusions can be drawn from these experiments (17, 26). One is that reprogrammed gene expression is immediately provided by nuclear enuclei and chromatin

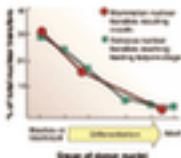


Fig. 2. Nuclear transfer success decreases as donor cells differentiate (2, 6).

downstream, such as to nuclear translocation of myoD and myogenin (Fig. 1). Another is that gene expression does not depend on the activation of tissue cell-specific gene expression, but on cell division, therefore, neither of these is a necessary part of myogenesis. The third conclusion is that differentiated cells as well as embryonic cells contain regulatory molecules that can induce gene expression in the nuclei of other cells. When the myogenin cell is very large, such as an egg or myotube (300 μ m or more), cells fused into one large myotubular cell, it is understandable that *in vivo* myogenesis induction can involve a much multiplicity of regulatory

cell population can lead to the appearance of some cells with the characteristics of ES cells. After further selection for the expression of MyoD, in addition to the first few genes, the resulting stem cells were chosen to consist of cell lineage which transcribed to nonmuscle-specific genes, hence, they are pluripotent and cannot induce myogenesis with *myoD* cells. ES cells from human embryos acknowledge the same set of factors used to make induced iPS as the combination of Oct4, Sox2, Klf4, and c-Myc (2). These procedures have been confirmed and extended. ES cells have been obtained from differentiated somatic and liver cells (Fig. 5).

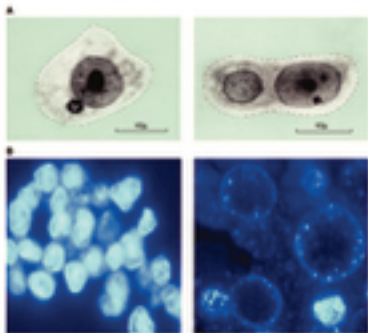


Fig. 3. Nuclear envelope and chromatin decondensation during nuclear reprogramming. (A) A chick erythrocyte (left) and 2 days (right) after fusion to a human fibroblast cell (2). The dashed lines indicate the nuclei of the fused hybrid cells. The smaller nucleus is that of the chick erythrocyte. (B) Fused cells 4 days after fusion (left) and 7 days (right) after fusion into an amphibian-type germinal vesicle (3). The hybrid nuclei have enlarged about 30 times in volume.

molecules introduced by the incoming nucleus in cell (Fig. 4). These molecules probably have a role in normal non-nuclear transfer conditions by ensuring that cells and their daughters do not escape from their lineage or change cell type in other words, cells seem to continuously self-regulate themselves and their daughters to remain in the same lineage.

Nuclear Reprograming

A spectacular advance in this field came when Takahashi and Yamanaka (2) discovered that viral transfection of four genes (Oct4, Sox2, c-Myc, and Klf4) into an adult mouse fibro-

cyte (Fig. 5) and can be obtained even if they, which can induce cancer, is omitted (2). The resulting stem cells do not appear to be cells markedly different from ES cells and may eventually provide a suitable source of different cell types for patient-specific cell replacement therapy in humans and of disease-specific cell lines to test potential therapeutic agents. Recently, other methods are developed to eliminate the concern of genomic integration by the associated viral vectors. Recent work provides a way in this direction by showing that nuclei viral integration is not required to generate iPS cells or for subsequent subsequent pluripotency (3,24,25).

The mechanism by which iPS cells achieve after the introduction of transcription factors is a differentiated somatic cell is not clear. However, in the first experiments these cells arise at such a low rate (10^{-7} to 10^{-8}) of the transfected cell population, and because the total cell population made is prohibitive in the continuing process of the factors for nearly 3 weeks, the persistence of the nontransfected cell is difficult to analyze. In some cases, the progenitor stem may need to be selected by the expression of differentiation processes. Possible mechanisms have been suggested (26,27).

Gene Switching

The possibility of redefining cell differentiation by reorganization of genes was suggested many years ago by Wessells with the introduction of the "mouse gene" (28)(29). The reorganization of the *myo* gene, which encoded a muscle-specific transcription factor, was sufficient to make a range of nonmuscle cell types exhibit some muscle. However, in other muscle-associated cells, the myogenic conversion was temporary, as an external inhibitor for *MyoD* expression is needed for a number of cell cycles before a muscle phenotype is established. When it has been, *MyoD* transcription is then continuing transcription, and complete transcription of *MyoD* is no longer required.

Another in cell type have also been successfully achieved with several other cell types, usually, the blood-forming cell lineage, by over-expressing key transcription factors, the factors of which can activate myogenic genes downstream cell (30,31). In fibroblast cells (Fig. 5, arrow 1), the process may possibly involve a conversion to a less differentiated state, a kind of dedifferentiation, before the cell type is changed. In cell MyoD, transcription of cells are selected in culture for many cell divisions before the new cell type is established.

In recent development in this area is the direct conversion of somatic cells of the genome into embryonic (3 cells) (Fig. 5, arrow 2) (32). In this case, four transcription factors normally required for *Escherichia coli* differentiation, namely, *Pax6*, *Ngn2*, and *Wnt3*, are provided by adenovirus transfection, and up to 30% of the transfected somatic cells are found to be producing (3 cells). The adenovirus carrying the transcriptional genes do not need to be integrated into the somatic cell genome, and gene reorganization is needed only temporarily. Moreover, this lineage switch does not appear to require cell division. Thus direct lineage switching, and the iPS cell system generated by Wessells, provide a gene switch strategy for changing cell fates, whereby one can use the same set of transcription factors that generate one cell type can another.

Protein-DNA Interactions and Signaling Events

Two basic characteristics of cell differentiation influence our understanding of nuclear reprogramming. One is the very cell lines to express

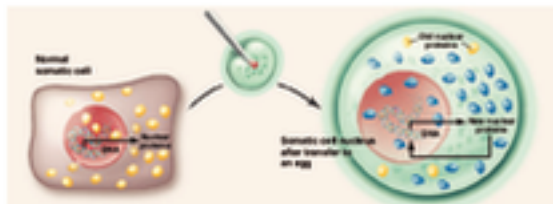


Fig. 4. Chromatin epigenetic reprogramming is essential for full reprogramming after nuclear transfer to an egg cytoplasm (right). Yellow indicates donor cell nuclear proteins that maintain gene expression. Blue indicates egg nuclear proteins that replace somatic proteins lost by dilution and that induce new gene expression.



Fig. 5. Four experimental routes for nuclear reprogramming. The comparison represents the normal process of cell differentiation during development from a fertilized egg to adult cells or tissues. Red arrows represent nuclear reprogramming. (A) by nuclear transfer to eggs, (B) by nuclear pluripotency (iPS), (C) by transgene switch back to a branch point

and not again in a different direction, and (D) by direct transduction. The lower part of the figure shows reprogramming by the generation of iPS cells; these can be aggregated into an embryonic body (EB), made to differentiate to various (iPS), or transplanted to a blastocyst. In each case, various types of adult cells can be formed.

