Nuclear Reprogramming in Cells

ARTICLE *in* SCIENCE · JANUARY 2009
Impact Factor: 33.61 · DOI: 10.1126/science.1160810 · Source: PubMed

John B Gurdon
University of Cambridge

Nuclear Reprogramming in Cells

J. B. Gurdon[1] and D. A. Melton[2]

Nuclear reprogramming describes a switch in gene expression of one kind of cell to 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 ectopic gene expression, and direct reprogramming. Through these methods it becomes possible to derive one kind of specialized cell (such as a brain cell) from another, more accessible, tissue (such as skin) in the same individual. This has potential applications for cell replacement without the immunosuppression treatments that are required when cells are transferred 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 one-way process, and they become increasingly, and normally irreversibly, committed to their fate. A skin cell does not normally turn into a brain cell, nor does an intestine cell generate a heart cell. Nevertheless, there are certain experimental procedures that enable just these kinds of changes to take place. They entail nuclear reprogramming, a term that describes a switch in nuclear gene expression of one kind of cell to that of secondary or other cell type. This process is of interest for 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. Eventually, it may be possible to derive replacement heart, pancreas, 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 lines of cells from diseased tissues, and hence allows us to analyze the nature of the disease and to screen for therapeutic drugs. We review these procedures, discuss the mechanisms that may be involved, and comment on prospects in this field.

Nuclear Transfer to Eggs and Oocytes

The earliest evidence for the experimental reversal of cell differentiation came from the transplantation of a viable cell nucleus into an enucleated frog egg. Briggs and King (1) first succeeded in producing normal swimming tadpoles of *Rana pipiens* by transplanting the nuclei of embryo (blastula) cells. They found, however, that the transfer of nuclei from slightly older (gastrula) embryos resulted only in abnormal development and concluded that cell differentiation was likely to involve irreversible nuclear changes (2). Soon after this, similar experiments were carried out with eggs of the South African frog *Xenopus laevis* (3). In this case, it was found that even when *Xenopus* nuclei were transplanted from fully differentiated cells, in this case from the intestinal epithelium of feeding tadpoles, entirely normal and fertile male and female frogs were obtained (4). These results led to the conclusion that the process of cell differentiation can be fully reversed and does not require irreversible nuclear changes but involves changes in nuclear gene expression but not in gene content. Therefore, although cells become stably and functionally very different from each other during development, their genome stays the same in all cells (with the exception of antibody-producing cells) and therefore retains the potential to form any cell type.

The next major advance in this field came with the production of a normal adult sheep (Dolly) by transplanting the nuclei of cultured mammary gland cells derived from an adult sheep to enucleated sheep eggs (5). This and later work (6) showed that it is possible to completely reverse the process of mammalian cell differentiation using nuclei from an adult mammal, 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 proliferative and differentiation-competent cells were derived from blastocysts grown after transplanting nuclei from adult monkey cells to enucleated monkey eggs (7). It is therefore likely that human eggs contain the components required to reverse the differentiation of adult human somatic cells.

Efficiency

The gold standard for the completeness of reprogramming by eggs has been described as the formation of a fertile adult animal containing functional cells of every kind (termed totipotency). However, as far as therapy is concerned, we do not need totipotency or even pluripotency (the formation of many but not all cell types) (Fig. 1A) as a necessary attribute. It would not, for example, be therapeutically useful to supply a patient 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 transplanted nucleus of an entirely unrelated cell type. It has been shown that the success of nuclear reprogramming decreases as donor cells become more differentiated (3, 8) (Fig. 2). The frog experiments include the results of serial nuclear transfers (transplanting nuclei from a nuclear transplant embryo to another set of enucleated eggs) and grafts (transplanting nuclei from a nuclear transplant embryo to host embryos raised from fertilized eggs) to produce the conclusion that about 30% of intestinal epithelium cell nuclei can generate functional muscle and nerve cells (9). In mammals, cells of a nuclear transplant blastocyst can be used to derive ES cells, whose differentiation capacity is tested by transplanting these cells to normal host embryos. 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 30% from embryo nuclei (10).

Because of the ethical concerns about obtaining human unfertilized eggs, animal eggs such as those of cows, mice, or rabbits might be used to generate ES cells from transplanted human somatic nuclei. Nuclear transfer between different strains or subspecies are just as successful as those within a species; however, eggs produced by transfers between very different species such as human and mouse, cow, or pig generally die before the 32-cell stage (11). So far, there is no confirmed evidence that proliferating ES cells can be obtained from such donor combinations, including human nuclei in monkey cytoplasm.

Mechanism

An appeal of using eggs to reprogram nuclei is that eggs have the natural ability to reprogram highly specialized sperm 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 discover the mechanisms involved and ask, how is successful reprogramming achieved, and what makes the process frequently unsuccessful even when eggs are used?

The mechanism of nuclear reprogramming by eggs (in second meiotic metaphase) has been explored by the use of oocytes (female germline cells in their meiotic prophase and immediate progenitors of eggs). Multiple mammalian somatic nuclei transplanted to the germinal vesicle of an oocyte are directly reprogrammed to transcribe stem-cell marker genes, including *Oct4*, *Nanog*, and *Sox2* (Fig. 1B) (12). Nuclear reprogramming by oocytes does not yield new cells but, in

---

[1] Wellcome Trust/Cancer Research UK Gurdon Institute and Department of Zoology, University of Cambridge, Cambridge CB2 1QN, UK. [2] Harvard Stem Cell Institute and Howard Hughes Medical Institute, Harvard University, Cambridge, MA 02138, USA.

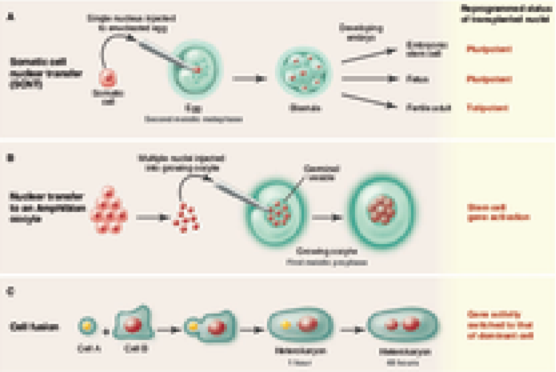

Fig. 1. Design of nuclear transfer experiments (A) to unfertilized eggs (second meiotic metaphase) of frogs or mammals or (B) to first meiotic frog oocytes. (A) and (B) show the transfer of somatic cell nuclei. (C) Design of cell fusion experiments.

content in eggs takes place without cell division and does not need protein synthesis. Mechanisms accompanying this reprogramming include (i) a massive volume increase of 50 times in transplanted nuclei and chromatin decondensation (Fig. 3, A and B), due in part to an oocyte histone chaperone nucleoplasmin (11, 12); (ii) the removal of differentiation marks, such as DNA methylation (13) and histone modifications; and (iii) chromatin protein exchange, especially of the somatic-specific linker histone H1 by the oocyte-specific histone variant B4 or H1foo (14). The general principle that seems to be that, during their formation, oocytes (and hence eggs) acquire 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 recovery after photobleaching experiments (15)), 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 referred to above applies to all those components of an egg that normally reprogram sperm nuclei after fertilization, there would be time in frogs, and even more in mammals, for 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 epigenetic memory of their gene expression in their donor cells. For example, nuclei taken from muscle cells sometimes continue to strongly express muscle genes in neural and other nonmuscle cells of an embryo obtained by nuclear transfer. This may be caused by the incorporation of an abundant egg histone variant (H3.3) into the chromatin of daughters of transplanted nuclei (16). The incorporation of the H3.3 histone is thought to prevent reprogramming and so to preserve a memory of previous gene expression.

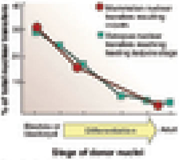

Fig. 2. Nuclear transfer success decreases as donor cells differentiate (3, 4).

Cell Fusion and Cell Extracts

It is possible to fuse two somatic cells and to use a cell-division 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 multinucleate muscle cell (17, 18). If enucleated cytoplasm of one kind of somatic cell (cytoplasts) are fused to another cell, they also impose gene expression of their original cell type on the incoming nucleus. However, these fused cells do not proliferate well, and therefore are not likely to be of therapeutic value.

Some important conclusions can be drawn from these experiments (19, 20). One is that reprogrammed gene expression is commonly preceded by nuclear swelling and chromatin decondensation, such as in nuclear transfer to eggs and oocytes (Fig. 3). Another is that new gene expression does not depend on the activation of donor cell–specific gene expression, nor on cell division; therefore, neither of these is a necessary part of reprogramming. The third conclusion is that differentiated cells (as well as embryo cells) contain regulatory molecules that can redirect gene expression in the nuclei of other cells. When the recipient cell is very large, such as an egg or oocyte (1000 or so muscle cells fused into one large syncytial cell), it is understandable that in vivo reprogramming molecules can override a much smaller supply of regulatory molecules introduced by the incoming nucleus or 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 types; in other words, cells seem to continually self-reprogram themselves and their daughters to remain in the same lineage.

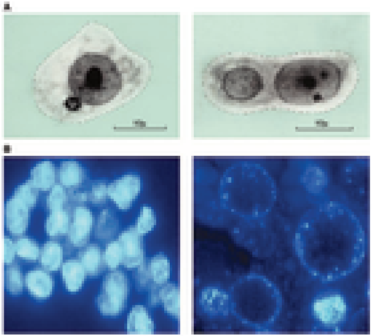

Fig. 3. Nuclear enlarge and chromatin decondensation during nuclear reprogramming. (**A**) A chick erythrocyte 1 hour (left) and 2 days (right) after fusion to a human HeLa cell (12). The dotted lines indicate the outside of the fused hybrid cells. The smaller nucleus is that of the chick erythrocyte. [Adapted with permission from (12)] (**B**) Mouse ES cell nuclei immediately (left) and 2 days (right) after injection into an amphibian oocyte germinal vesicle (18). The injected nuclei have enlarged about 30 times in volume.

Induced Pluripotency

A spectacular advance in this field came when Takahashi and Yamanaka (21) discovered that viral transduction of four genes (Oct3/4, Sox2, c-Myc, and Klf4) into an adult mouse fibroblast population can lead to the appearance of some cells with the characteristics of ES cells. After further selection for the expression of *Nanog*, in addition to the first four genes, the resulting stem cells were shown to enter all cell lineages when transplanted to immunodeficient host embryos; hence, they are pluripotent and termed induced pluripotent cells, or iPS cells. iPS cells from human somatic cells require the same set of factors used in mice (above) (22) or the combination of Oct4, Sox2, Nanog, and Lin28 (23). These procedures have now been confirmed and extended. iPS cells have been obtained from differentiated stomach and liver cells (Fig. 5, arrow B) (24) and can be obtained even if Myc, which can induce cancer, is omitted (25, 26). The resulting stem cells do not appear to be substantially 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, but only after methods are developed to eliminate the concerns of genome integration by the associated viral vectors. Recent work provides a step in this direction by showing that stable viral integration is not required to generate iPS cells when nonintegrating adenoviruses or plasmids are used (27, 28, 29).

The mechanism by which iPS cells arise after the introduction of transcription factors to a differentiated somatic cell is not clear. Because in the first experiments these cells arose at such a low rate (10^{-3} to 10^{-4} of the transfected cell population), and because the treated cell population needs to proliferate in the continuing presence of the factors for nearly 2 weeks, the provenance of the occasional iPS cell is difficult to analyze. In some cases, the pluripotent state may need to be stabilized by the suppression of differentiation processes. Possible mechanisms have been reviewed (10, 11).

Lineage Switching

The possibility of redirecting cell differentiation by overexpression of genes was suggested many years ago by Weintraub with the identification of the "master gene," MyoD (32). The overexpression of this one gene, which encoded a muscle-specific transcription factor, was sufficient to make a range of nonmuscle cell types switch into muscle. However, in other muscle-unrelated cells, the myogenic conversion was temporary, or not observed. Selection for MyoD expression is needed for a number of cell cycles before a muscle phenotype is established. When it has been, MyoD autoactivates its own continuing transcription, and exogenous overexpression of MyoD is no longer required.

Switches in cell type have also been successfully achieved with several other cell types, notably the blood-forming cell lineage, by overexpressing key transcription factors, the balance of which can activate or repress genes determining cell fate (33, 34). In these cases (Fig. 5, arrow C), the process may possibly involve a reversion to a less differentiated state, a kind of dedifferentiation, before the new cell type is formed. As with MyoD, overexpressing cells are selected in culture for many cell divisions before the new cell type is established.

A recent development in this area is the direct conversion of exocrine cells of the pancreas into endocrine β cells (Fig. 5, arrow D) (35). In this case, three transcription factors normally required for β-pancreatic differentiation, namely Pdx1, Ngn3, and MafA, are provided by adenovirus transduction, and up to 20% of the transfected exocrine cells switch to insulin-producing β cells. The adenoviruses carrying the overexpressed genes do not need to be integrated into the recipient cell genomes, and gene overexpression is needed only temporarily. Moreover, this lineage switch does not appear to require cell division. This direct lineage switching, and the iPS formation pioneered by Yamanaka, provide a general strategy for changing cell fates, whereby one can aim to discover the set of transcription factors that can turn one cell type into another.

Protein-DNA Interactions and Binding Access

Two basic characteristics of cell differentiation influence our understanding of nuclear reprogramming. One is that every cell seems to express

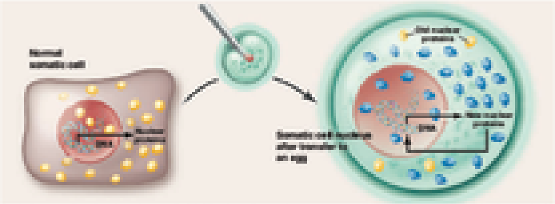

Fig. 4. Chromatin and protein exchange in somatic cell (**left**) nucleus after nuclear transfer to an egg or oocyte (**right**). Yellow indicates donor cell nuclear proteins that maintain gene expression. Blue indicates egg nuclear proteins that replace somatic proteins lost by dilution and that induce new gene expression.

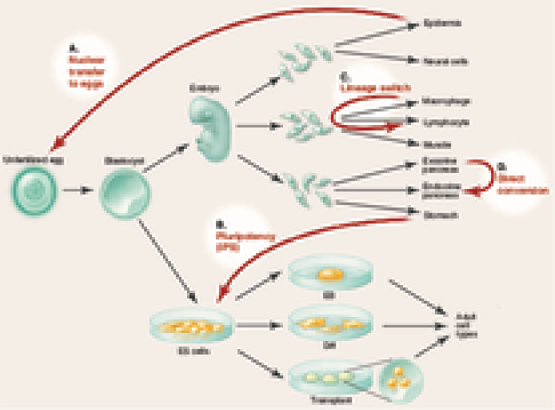

Fig. 5. Four experimental routes for nuclear reprogramming. Blue components represent the normal process of cell differentiation during development from a fertilized egg to adult cells or tissues. Red arrows represent nuclear reprogramming (**A**) by nuclear transfer to eggs, (**B**) by induced pluripotency (iPS), (**C**) by lineage switching back to a branch point and out again in a different direction, and (**D**) by direct conversion. The lower part of the figure shows reprogramming by the generation of ES cells; these can be aggregated into an embryoid body (EB), made to differentiate in culture (Dif), or transplanted to a blastocyst. In such cases, various types of adult cells can be formed.

those genes whose products determine its state of differentiation, a conclusion especially clear from cell-fusion experiments (19, 20). Thus, a muscle cell will maintain by autoactivation a high enough content of MyoD, for example, to continually program itself to be a muscle cell (Fig. 4). The larger the cell, and/or the more embryonic it is, the greater abundance it will have of self-reprogramming molecules. Therefore, eggs will be particularly effective without added factors.

A second characteristic of all nuclear reprogramming experiments is that the experimental resetting of gene expression becomes increasingly difficult as cells become more differentiated (Fig. 2). The differentiated state becomes more firmly established as cells embark on their terminal pathways and shut down inappropriate lineages. To understand the basis of this is a major challenge in this field, and much informative work has already been done on DNA and histone modifications (35). A general hypothesis is the idea of "fleeting access." We propose that combinations of DNA-binding or chromosomal proteins become increasingly tightly associated with the regulatory regions of inactive genes. Even though most proteins are thought to dissociate from DNA at frequent intervals of seconds or a few minutes (15) and in a few instances for longer (36), a multicomponent complex as a whole may have a very long dwell time on inactive genes. It will be a very rare event for a sufficient number of individual proteins in a complex to dissociate from a chromosome at the same time for a gene region to be accessible to reprogramming factors. In embryonic cells, most genes (and in differentiated cells, the active genes) will be in a decondensed configuration with relatively short dwell times for multicomponent complexes.

According to this view, the probability of reprogramming taking place in nuclear transfer, cell fusion, iPS, and lineage-switching experiments would depend on the statistical access frequency of gene regulatory regions together with the duration and concentration of transcription or other regulatory factors. Large cells such as eggs or oocytes with a high content of factors could be especially successful at reprogramming, as would any cell with an experimentally enhanced content of factors. A major advance in the future will be to understand why the nuclei of differentiated cells are reprogrammed so much less well than those of embryonic cells. This will probably require an explanation of chromatin decondensation.

The Future

Will the mechanism of reprogramming be the same in nuclear transfer to eggs, iPS experiments, and lineage switching? Probably not. The concept of fleeting access will be the same, but the actual reprogramming molecules will be different. We already know that eggs have very high concentrations of certain molecules such as nucleoplasmin and histones B4 and H1.X. The eventual identification of egg-reprogramming molecules may well be able to enhance the efficiency of the iPS and lineage-switching routes for adult cells.

The future value of reprogrammed cells is of two kinds. One is to create long-lasting cell lines from patients with genetic diseases, in order to test potentially useful drugs or other treatments (37, 38). The other is to provide replacement cells for patients. To be therapeutically beneficial, replacement cells will probably need (i) to be provided in sufficient numbers, (ii) to carry out their function, even though they are not normally integrated into host tissues, and (iii) to be able to produce the correct amount of their product.

A human adult has about 10^{14} cells, and the liver contains about 10^{11} cells. To create this number of cells starting from a 10^{-5} success rate of deriving iPS cells from skin would require an enormous number of cell divisions in culture, although the prolonged culture of ES-like cells provides a valuable amplification step. However, many parts of the human body need a far smaller number of cells to improve function. An example is the human eye retina, in which only 10^{4} cells could be of therapeutic benefit.

Will introduced cells be useful even if not "properly" integrated into the host? Most organs consist of a complex arrangement of several different cell types. The pancreas, for example, contains exocrine (acinar) cells, ductal cells, and at least four kinds of hormone-secreting cells in the endocrine islet. Replacement endocrine cells can provide useful therapeutic benefit even if not incorporated into the normal complex pancreas cell configuration (39). In some cases, introduced cells can have a functionally beneficial effect, even if indirectly (38, 40). It is not yet clear whether introduced cells will be correctly regulated to produce the desired amount of product.

Looking ahead, alternative routes to cell replacement may emerge. One is to avoid the need to transfer genes into cells if the right combinations of small molecules that can easily enter cells can be found (41). It may also be increasingly fruitful to find populations of naturally dividing cells in adult organs so that these cells in their naturally less-specialized state can be expanded and differentiated in culture before implantation. A future objective, in our view, is to aim for multipotency and oligopotency (the generation of only one or a few cell types) rather than pluripotency (the potential to differentiate into any of the three germ layers) and certainly not totipotency (the potential to differentiate into all embryonic and extra-embryonic cell types) (Fig. 5). Likewise, we would much prefer to be able to create new cells by switching mature cells from a closely related lineage than by going back to totipotency and then narrowing down the differentiation options from a wider range. For replacement therapy, totipotency and germline transmission are not desirable criteria or objectives. An oligopotent state with limited differentiation potential is likely to be much safer and more useful from a therapeutic point of view.

References and Notes

1. R. Briggs, T. J. King, Proc. Natl. Acad. Sci. U.S.A. 38, 455 (1952).
2. R. Briggs, T. J. King, J. Morphol. 100, 269 (1957).
3. J. B. Gurdon, J. Embryol. Exp. Morphol. 10, 622 (1962).
4. J. B. Gurdon, V. Uehlinger, Nature 210, 1240 (1966).
5. I. Wilmut et al., Nature 385, 810 (1997).
6. K. Eggan et al., Nature 428, 44 (2004).
7. [illegible]
8. [illegible]
9. J. B. Gurdon, Annu. Rev. Cell Dev. Biol. 22, 1 (2006).
10. [illegible]
11. [illegible]
12. [illegible]
13. [illegible]
14. [illegible]
15. [illegible]
16. [illegible]
17. [illegible]
18. [illegible]
19. [illegible]
20. [illegible]
21. [illegible]
22. [illegible]
23. [illegible]
24. [illegible]
25. [illegible]
26. [illegible]
27. [illegible]
28. [illegible]
29. [illegible]
30. [illegible]
31. [illegible]
32. [illegible]
33. [illegible]
34. [illegible]
35. [illegible]
36. [illegible]
37. [illegible]
38. [illegible]
39. [illegible]
40. [illegible]
41. [illegible]
42. [illegible]

10.1126/science.[illegible]