Nuclear Reprogramming in Cells

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Nuclear Reprogramming in Cells

Nuclear Transfer to Eggs and Oocytes

Efficiency

Mechanism

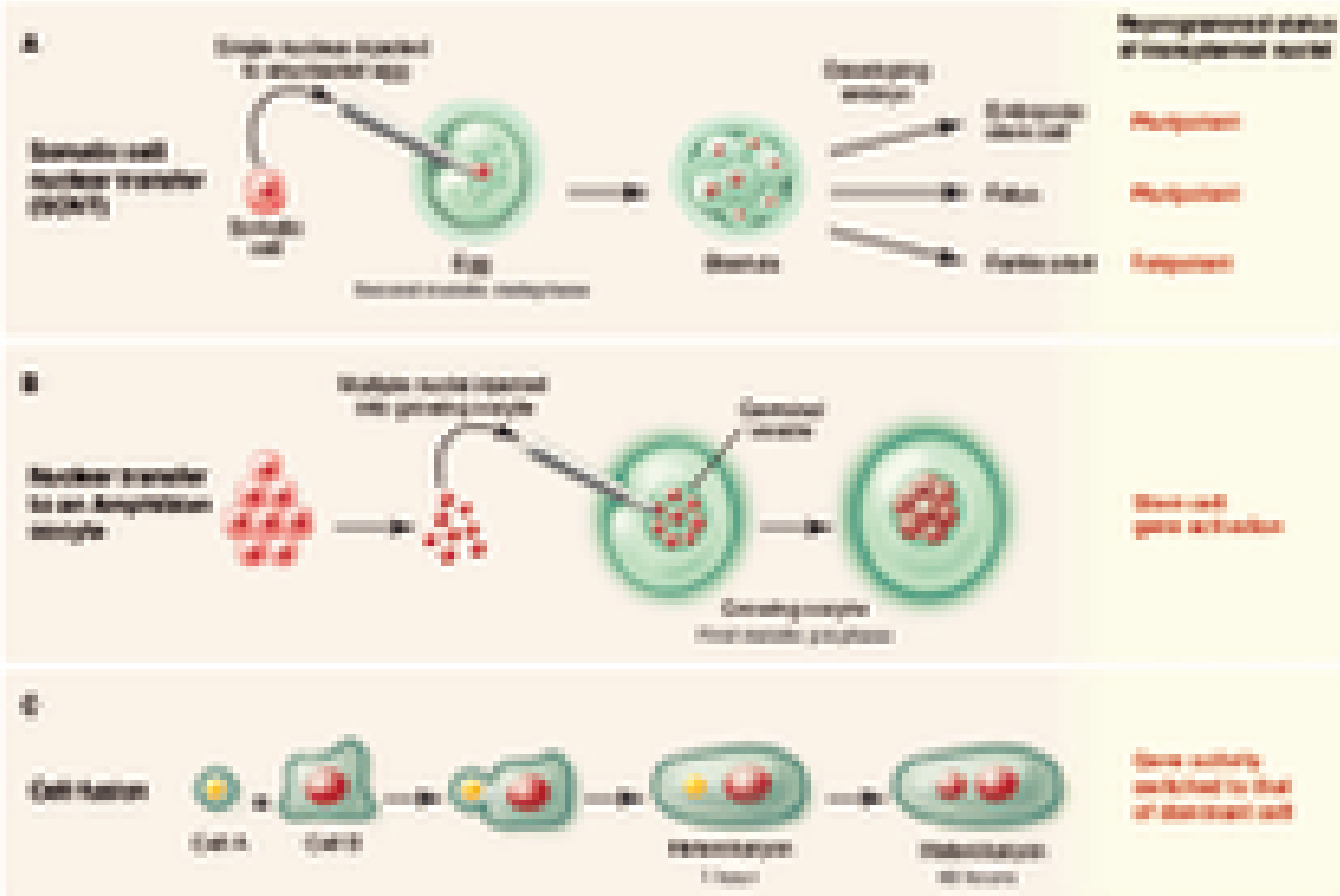

Fig. 1. Designs of nuclear transfer experiments (**A**) to unfertilized eggs (second oocyte metaphase) of frogs or mammals or (**B**) to fast-growing frog oocytes. (**C**) and (**B**) show the transfer of somatic cell nuclei. (**C**) Design of cell fusion experiments.

contrast to eggs, which place reprogramming and stem cell activation at the top of the hierarchy. Mechanisms accompanying this reprogramming include (i) a massive cellular increase of 50 times its diameter and (ii) chromatin decondensation (Fig. 1, A and B), due in part to an excess histone chaperone nucleoplasmin (20, 21); (iii) the removal of differentiation marks such as DNA methylation (22) and histone modifications; and (iv) chromatin protein exchange, especially of the oocyte-specific linker histone H1 by the oocyte-specific histone variants B4 or H1foo (23, 24). The general principle here seems to be that, during their formation, oocytes (and future 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 nonplanted somatic nuclei (as suggested by laser fluorescence recovery after photobleaching experiments (25)), 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, then nuclei in time to stage, and even more so in mammals, the transplanted somatic nuclei to be fully reprogrammed before the first egg division (24 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 tissues with. For example, skeletal muscle nuclei cells sometimes continue to strongly express muscle genes in cloned and after even muscle cells of an embryo cleaved by nuclear transfer. This may be caused by the incorporation of an activated egg histone variant (H1.1) into the chromatin of daughters of transplanted nuclei (26). The incorporation of the H1.1 histone is thought to prevent reprogramming and so to promote a memory of previous gene expression.

Fig. 2. Nuclear transfer success decreases as donor cells differentiate (2, 8).

Cell Nuclei and Cell Extracts

It is possible to fuse two somatic cells and to use a cell-division inhibitor to uncover that the two nuclei remain separate (Fig. 1C). In these heterokaryons, the dominant cell usually the larger and more actively dividing partner, induces its own pattern of gene expression on the other partner. An example includes the fusion of an erythrocyte with a growing cultured cell or of a human liver cell with a mouse liver muscle cell (27, 28). If mechanical compliance of the fused cell remains cell cytoplasm can blend to another cell, they also impose gene expression of their original cell type on the resulting nucleus. Moreover, 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 (29, 30). One is that reprogrammed gene expression is commonly generated by nuclear reading and chromatin

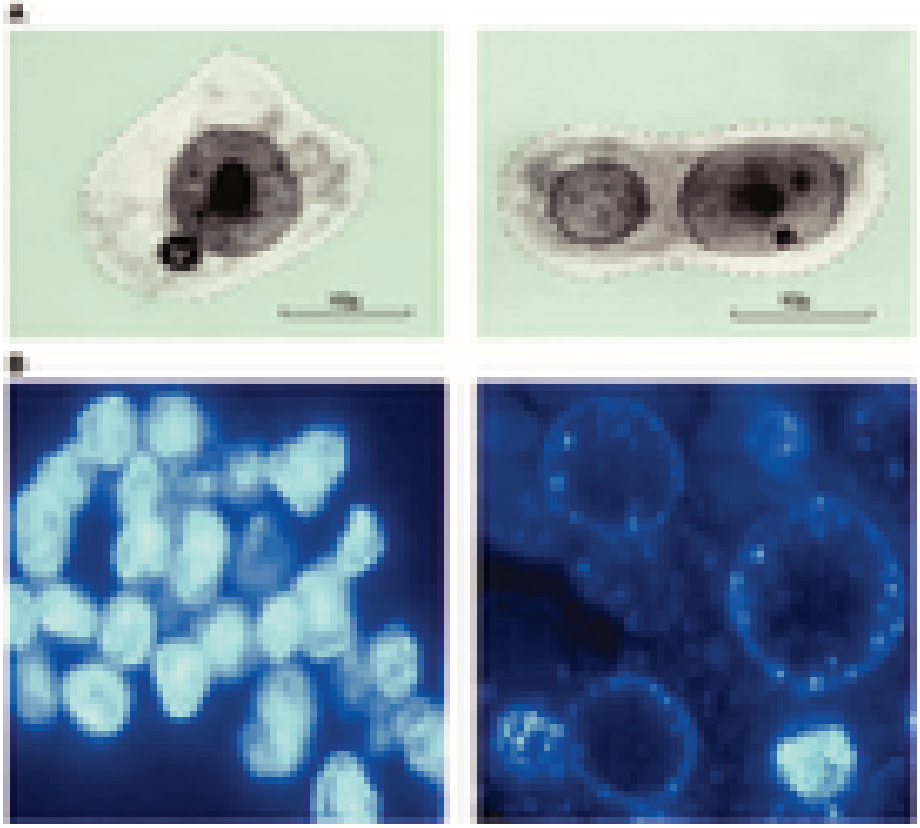

Fig. 1. Nuclear enlarge and chromatin decondensation during nuclear reprogramming. (**A**) A chick erythrocyte (Hoechst 33342 and 5-bromodeoxyuridine) and a human HeLa cell (DAPI) fused with Xenopus egg extract. Chick DNA is found in the nucleus of the chick erythrocyte, but it is also found in the nucleus of the HeLa cell. The smaller nucleus is that of the chick erythrocyte. [Adapted with permission from (*170*) © Nature Publishing Group] (**B**) Mouse myoblasts (C2C12) fused with chicken erythrocytes immediately (left) and 2 days (right) after exposure to a mouse oocyte extract. The nuclei of the chicken erythrocytes have enlarged about 30 times in volume.

nucleotide introduced by the incoming nucleus or cell (Fig. 4). These molecular products have a role in normal host-nuclear reprogramming by ensuring that cells and their daughters do not change from their lineage or lineage-cell type to other ones, cells come to irreversibly self-reprogram themselves and their daughters to remain in the same lineage.

Natural Reprogramming

A spectacular advance in the field came when Takahashi and Yamanaka (*21*) discovered that viral transductions of four genes (*Oct-3/4*, *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 *Fbxo15* in addition to the first four genes, the resulting stem cells were shown to retain all cell lineages when transplanted to immunodeficient host embryonic tissues. They are pluripotent and termed induced pluripotent cells, or iPS cells (iPS cells from human somatic cells express the same set of factors and to more referred (*22*) as the combination of *Oct4*, *Sox2*, *Nanog*, and *Lin28* (*23*). These preconditions have been confirmed and extended. iPS cells have been obtained from different stomach and liver cells (Fig. 1).

The mechanism by which iPS cells arise after the introduction of transcription factors is a still developed concept, and is not clear. Because in the first experiments these cells arose at only a low rate (10^{-4} to 10^{-5} of the transduced cell population), and because the treated cell population took six additional to the proliferating presence of the factors for nearly 3 months, the generation of the reprogrammed 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 (*24*, *25*).

Lineage Switching

The possibility of reinforcing cell differentiation by an overexpression of genes was suggested many years ago by Weintraub with the identification of the "master genes" *MyoD1* (*26*). The overexpression of this one gene, which encoded a muscle-specific transcription factor, was sufficient to make a range of mesenchymal cell types switch into muscle. However, in other muscle-committed cells, the myogenic conversion was temporary, as was observed. Selection for *MyoD* expression is needed for a number of cell cycles before a muscle phenotype is established. When it has been, *MyoD* overexpression no more overriding transcriptional and epigenetic re-engagement 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 overengineering key transcription factors, the balance of which can activate or suppress genes determining cell fate (*27*, *28*). As these cases (Fig. 5, panel C), the genome may possibly become compromised in how differentiated was a type of differentiation. Switch between cell type is achieved via switching, so overengineering cells can selected as cultures 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, panel D) (*29*). In this case, three transcription factors normally required for β-pancreas differentiation, namely *Pdx1*, *Ngn3*, and *MafA*, are provided by adenovirus transfection, and up to 20% of the transduced exocrine cells in vivo switch producing β cells. The adenoviruses carrying the reprogramming genes do not need to be integrated into the exocrine cell genome, 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 technique pioneered by Yamanaka, provide a practical strategy for changing cell fates efficiently and overcome obstacles to the use of transcription factors that can have only cell-type-restrictive.

Protein-DNA Interactions and Packing Issues

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

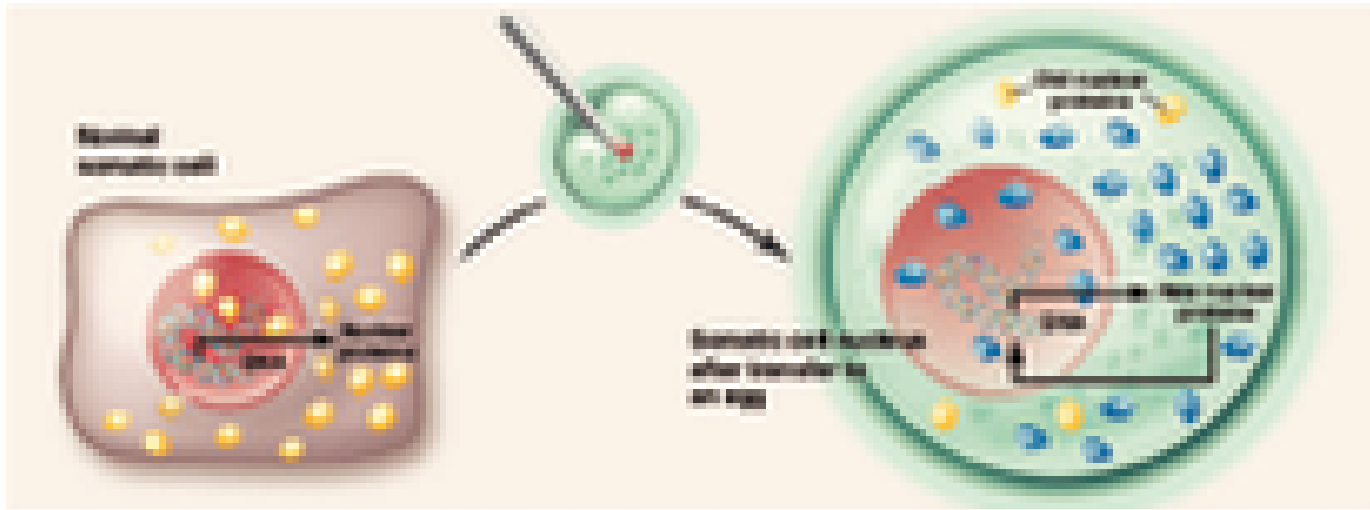

Fig. 4. Chromatin dynamics in cloning. In a variant of **A** (left), a differentiated somatic cell nucleus is transferred to an egg cytoplasm (**right**). Yellow indicates donor cell nuclear proteins that maintain gene expression. Blue indicates egg nuclear proteins that replace somatic proteins lost by dilution and that induce new gene expression.

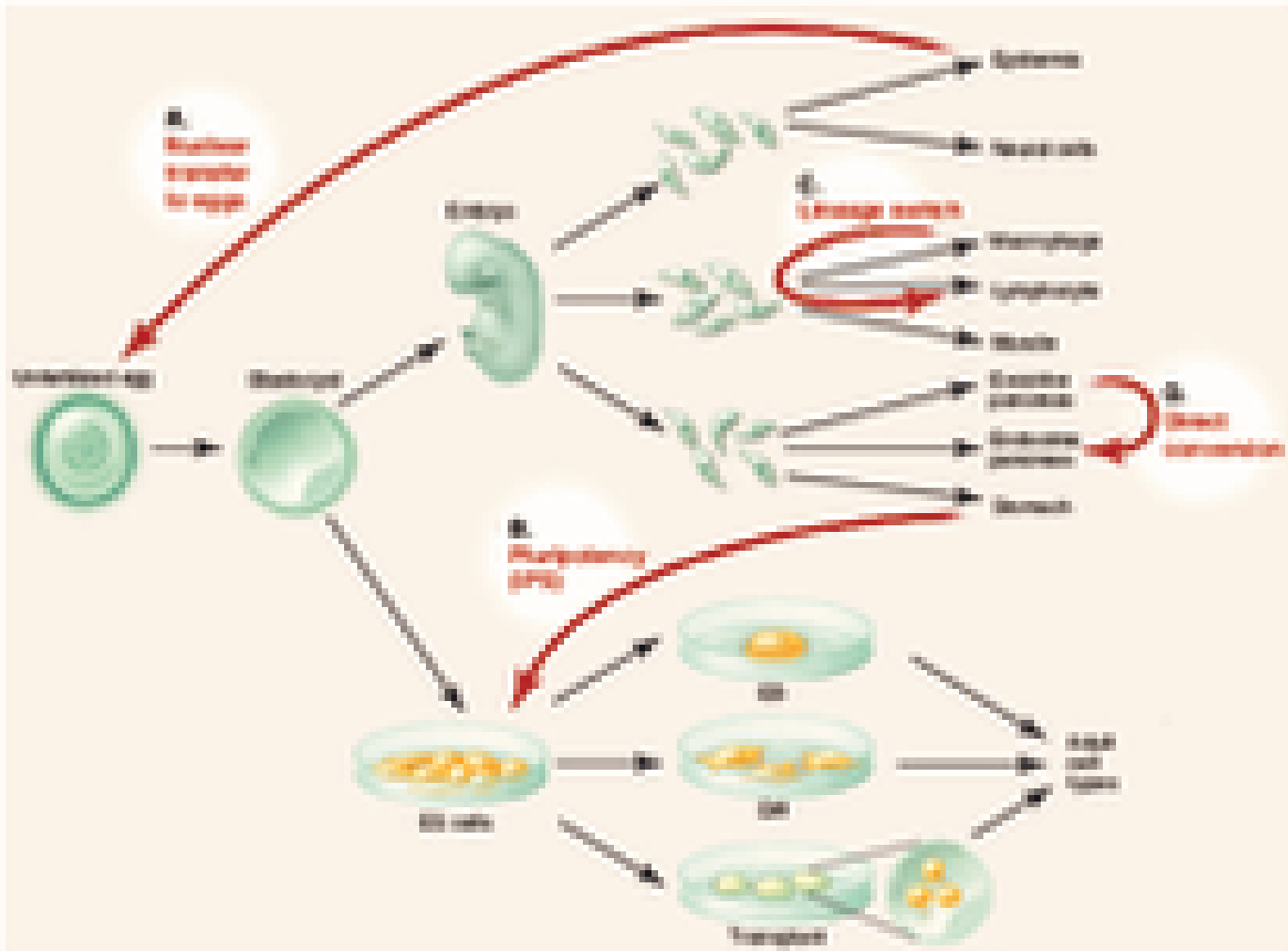

Fig. 5. Four experimental routes for nuclear reprogramming. Blue components represent the normal process of cell differentiation during development from a fertilized egg to adult cells or tissues. Red arrows represent nuclear reprogramming. (**A**) by nuclear transfer to eggs, (**B**) by induced pluripotency (iPS), (**C**) by lineage switching back to a bipotent state and out again to a different direction, and (**D**) by direct conversion. Bottom part of the figure shows reprogramming by the production of iPS cells. Mice can be aggregated into an embryonic body (EB), made to differentiate as cultured EB cells, or transplanted to a blastocyst. In each case, various types of adult cells can be formed.

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The future

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References and Notes

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