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CELL BIOLOGY

Cell identity reprogrammed

The discovery that cell differentiation can be reversed challenged theories of how cell identity is determined, laying the foundations for modern methods of reprogramming cell identity and promising new regenerative therapies.

SAMANTHA A. MORRIS

All cells of an organism derive from a single cell. As development progresses, cells become increasingly specialized to perform defined functions, a commitment that is accompanied by a restriction in the range of potential fates of those cells. In the late nineteenth century, a predominant thought was that, when they differentiate, cells retain only those pieces of heritable information required to maintain cell-type identity and function[1]. This led to the theory that differentiation is an irreversible process (Fig.1a). John Gurdon's seminal paper in *Nature* on nuclear reprogramming of cell identity, with Tom Elsdale and Michael Fischberg[2], provided a remarkable challenge to this dogma, and formed the basis for today's cell-reprogramming field.

Gurdon and colleagues' 1958 paper was preceded by the work of Robert Briggs and Thomas King[3]. To investigate the developmental potential of differentiating cells, Briggs and King used a method called nuclear transfer, in which the nucleus is removed from one cell (in this case, an egg) and replaced with an intact nucleus from a different cell. Briggs and King's experiments were a technical feat that had previously been accomplished only in single-celled organisms[4].

Using this method in the more-complex Northern leopard frog (*Rana pipiens*), they were able to produce normal, swimming tadpoles by replacing egg-cell nuclei with nuclei from blastomeres — cells that are made through the splitting of a fertilized egg cell during early development[3]. However, the transfer of nuclei from *R. pipiens* cells at more-advanced stages of differentiation — from when the hollow ball of blastomeres differentiates into a multilayered structure called a gastrula, onwards — did not support the development of normal frogs[5] (Fig. 1b).

Thus, Briggs and King's results demonstrated that the nuclei in blastomeres are not irreversibly changed with differentiation. However, they also indicated that, as development progresses, the potential of transplanted nuclei to support normal development decreases — suggesting that cell differentiation might be irreversible and might involve irreversible genetic changes. Thus, Briggs and King concluded[5] that the nuclei of cells in the late-stage gastrula have an "intrinsic restriction in potentiality for differentiation".

In 1958, Gurdon, Elsdale and Fischberg addressed the questions surrounding the potential of differentiated cells using a different species of frog, *Xenopus laevis* (the African clawed frog). In contrast to the *Rana* species, whose availability is seasonally restricted, *X. laevis* is available year round and rapidly reaches sexual maturity[2]. In the authors' experiments, donor nuclei from cells at various developmental stages, from early blastomeres to cells from tadpoles just before hatching, were transferred into *Xenopus* egg cells.

The donor nuclei were derived from a mutant stock in which each cell contained only one nucleolus (an organelle inside the nucleus) instead of the usual two. This approach provided a useful visual marker to confirm that the resulting animals obtained from nuclear transfer were indeed derived from the transferred nucleus, and not from existing material in the egg. These experiments demonstrated that normal tadpoles could be obtained from cells at stages of development up to pre-hatching tadpole stages (Fig. 1c) — much later than the developmental stage of the cells that Briggs and King had used.

Many of the tadpoles that developed from cells containing transferred nuclei underwent normal metamorphosis into frogs, which seemed to be sexually mature. The authors noted that the lone frog derived from the most-differentiated cell nucleus was "accidentally killed shortly before metamorphosis". A subsequent report[6] was free of such misadventure; it described the derivation of fertile adult frogs from the transplanted nuclei of fully differentiated cells collected from the intestines of feeding tadpoles.

Gurdon and colleagues thus demonstrated, unlike Briggs and King, that differentiated

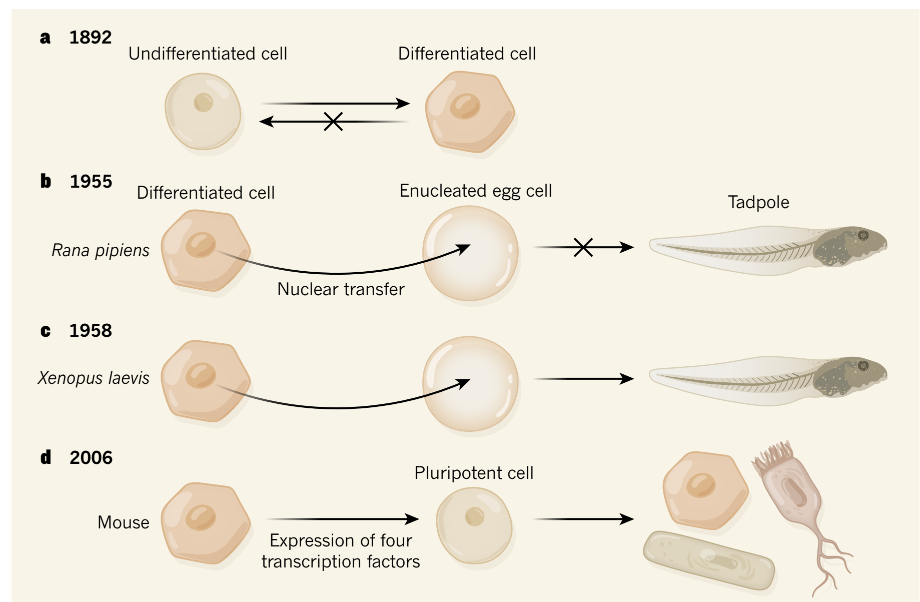

Figure 1 | Key milestones in understanding the potential of differentiated cells. **a**, In 1892, Weismann proposed that, as cells in a developing embryo differentiate, they retain only those genes required to maintain cell-type identity, rendering differentiation an irreversible process[1]. **b**, Studying the Northern leopard frog (*Rana pipiens*), Briggs and King reported[5] in 1955 that nuclei from differentiated cells that were transferred into an egg cell from which the nucleus had been removed (an enucleated egg cell) could not support normal development, in line with Weismann's thinking. **c**, In their 1958 *Nature* paper[2], Gurdon, Elsdale and Fischberg challenged the notion that development is irreversible, reporting that nuclei derived from differentiated cells of the African clawed frog (*Xenopus laevis*) could, in fact, support normal development. **d**, In 2006, Takahashi and Yamanaka[13] identified a core set of four transcription factors that reset differentiated mouse cells to a pluripotent state, capable of giving rise to any cell types in the body.

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nuclei could support successful development. Despite this discordance, both groups agreed that the advance of a nucleus through differentiation was accompanied by a reduction in its ability to support normal development. On the basis of their findings that some differentiated nuclei could support normal development (albeit with a relatively limited frequency of success), Gurdon and colleagues concluded that the differentiated cell state is not a result of irreversible genomic changes. Rather, the nuclei of differentiated cells retain the capacity to orchestrate the development of a fully functioning organism.

Almost 40 years after these amphibian experiments, transfer of the nucleus of an adult mammary epithelial cell was used to generate a cloned mammal: Dolly the sheep[7]. The first mouse to be cloned using nuclear transfer from adult cells, Cumulina, was reported shortly afterwards[8]. To prove beyond doubt that cloned animals could be produced using nuclei from fully differentiated cells (and had not previously been derived from contaminant stem cells that had broader potential), mice were derived using the nuclei of mature B cells and T cells[9]. During maturation, the genomes of both of these types of immune cell undergo DNA rearrangements, which were detected in the clones.

Together, this rich history of nuclear transfer revealed that cell differentiation can be reversed, resetting cell identity to the earliest embryonic stages. This pioneering work formed the foundations for the reprogramming field, which has the core goal of manipulating cell identity to produce any desired cell type.

In the 1980s, early work in reprogramming revealed that it is possible not only to reset cell identity to the blank slate of early embryonic development, but also to switch a cell's identity altogether. For example, one study[10] showed that fusion of a mouse muscle cell with a human amniocyte (a fetal cell that floats in the amniotic fluid) to produce a cell with both a human and a mouse nucleus resulted in the rapid expression of human muscle-specific genes. This showed that factors produced in a differentiated cell (in this case, the mouse muscle cell) can induce the expression of genes that are repressed in another type of differentiated cell (in this case, the human amniocyte). Together with the nuclear-transfer studies, these pivotal experiments established that factors produced in egg cells and differentiated cells are able to direct cell fate by regulating gene expression.

A key moment came in 1987, when a single factor capable of reprogramming cell identity was identified; the expression of a protein called MyoD (a transcription factor) was shown to convert fibroblast cells into contracting muscle cells[11]. Gurdon was somewhat pessimistic about the prospect that cell reprogramming could be quickly achieved using a defined set of factors, stating in 2006, "Looking far ahead, it may become possible to convert cells of an adult to an embryonic state without needing to use eggs"[12]. However, just a few months later, Kazutoshi Takahashi and Shinya Yamanaka reported that differentiated cells could be reset to a pluripotent state — that is, a state in which they could differentiate into multiple types of cell — through the expression of only four transcription factors[13] (Fig. 1d). In 2012, Gurdon and Yamanaka were awarded the Nobel Prize in Physiology or Medicine for their work.

Since Gurdon and colleagues' paper demonstrating that developmental potential can be reinstated in differentiated cells, cell biologists have developed the ability to reprogram cell identity by several routes. For example, we can use transcription-factor-mediated reprogramming to return cells to an embryonic state[13] and subsequently direct their differentiation to desired identities by mimicking normal developmental processes. Alternatively, embryonic states can be altogether avoided by expressing specific factors to directly convert a differentiated cell type to another cell identity[14–16]. Such strategies offer the potential to produce patient-derived cells for modelling diseases *in vitro*[17].

Moreover, cell reprogramming forms the basis of various proposed regenerative therapies, including the generation of cells that line the retina at the back of the eye to treat a disorder called age-related macular degeneration[18], a major cause of vision loss.

Gurdon and colleagues' 1950s conclusions that the developmental clock can be reset challenged the long-standing theory at that time that cell differentiation is an irreversible process. Their work now represents a cornerstone of current reprogramming technologies that aim to deliver a range of cell types for disease modelling and regenerative therapies. ■

Samantha A. Morris *is in the Departments of Developmental Biology and of Genetics, and in the Center of Regenerative Medicine, Washington University School of Medicine in St Louis, St Louis, Missouri 63110, USA.*
e-mail: s.morris@wustl.edu

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